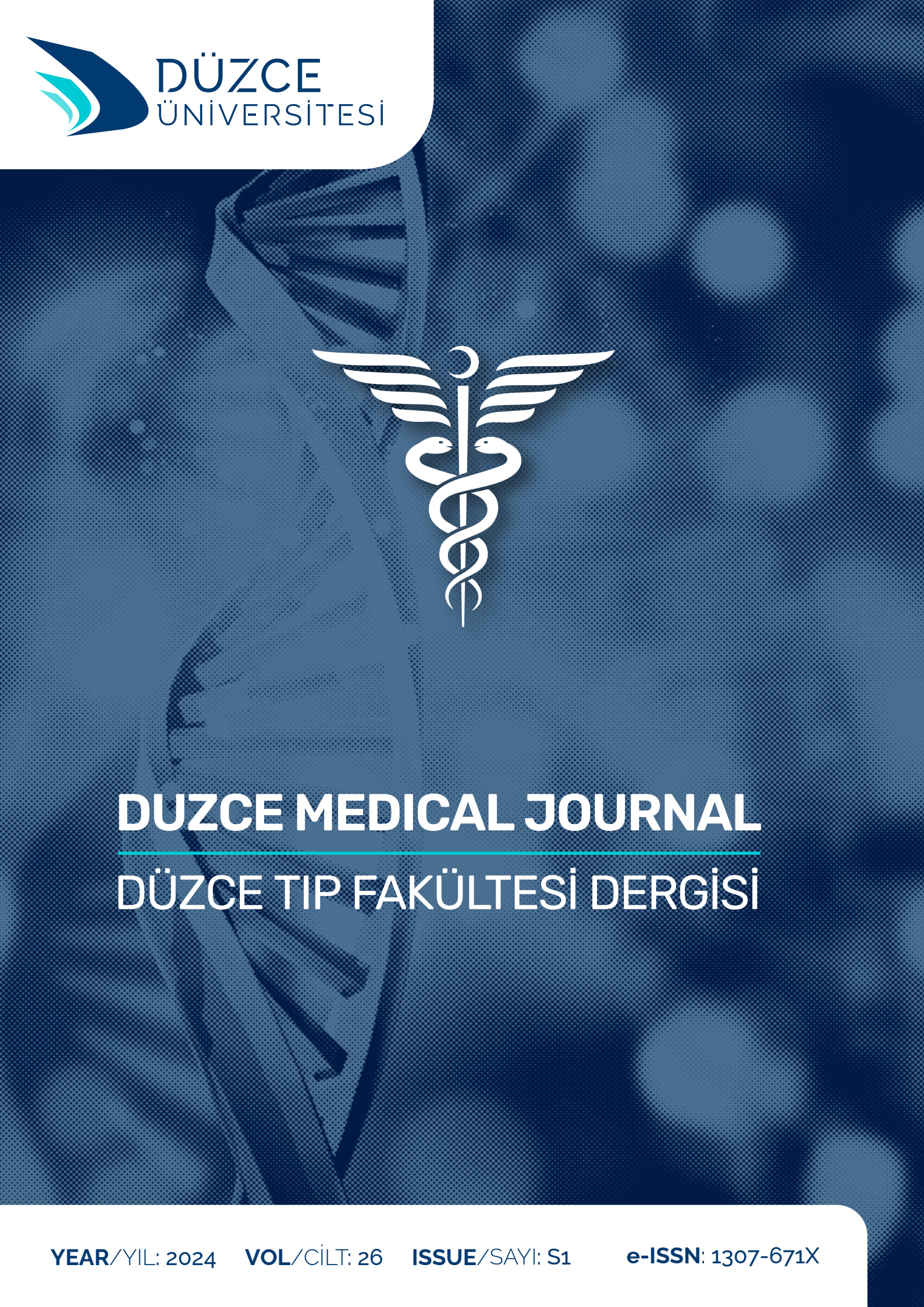

DÜZCE ÜNİVERSİTESİ

DUZCE MEDICAL JOURNAL

DÜZCE TIP FAKÜLTESİ DERGİSİ

YEAR/YIL: 2024 **VOL**/CİLT: 26 **ISSUE**/SAYI: S1 **e-ISSN**: 1307-671X

DÜZCE ÜNİVERSİTESİ

TIP FAKÜLTESİ

DUZCE MEDICAL JOURNAL

DÜZCE TIP FAKÜLTESİ DERGİSİ

Indexed In / Tarandığı İndeksler

CINAHL, CrossRef, DOAJ, EBSCO, EBSCOhost, EMBASE, ICMJE, Scopus, Türkiye Atıf Dizini, Türk Medline, ULAKBİM TR Dizin, Web of Science: E-SCI

An international peer-reviewed journal published three times a year. / Yılda üç kez yayınlanan uluslararası hakemli bir dergidir.
The authors are responsible for their articles. / Makalelerin sorumluluğu yazarlarına aittir.

YEAR/YIL: 2024 **VOL**/CİLT: 26 **ISSUE**/SAYI: S1 **e-ISSN**: 1307-671X

Duzce Medical Journal (Duzce Med J) / Düzce Tıp Fakültesi Dergisi (Düzce Tıp Fak Derg)
e-ISSN: 1307-671X

2024;26(S1)

Special Issue on In Vivo Studies

"General Principles and Modeling Techniques in Experimental Animal Studies"

The aim of this special issue is to compile qualified reviews on the experimental animal models for various diseases, and also ethical principles, statistical analyses, animal nutrition, and alternative methods with an interdisciplinary perspective.

Editors / Editörler

Pınar YILDIZ GÜLHAN, MD, Chest Diseases, Düzce University, Düzce/Türkiye

Merve ALPAY, PhD, Medical Biochemistry, Düzce University, Düzce/Türkiye

FROM THE EDITORS

Experimental animals have been the cornerstone of scientific research throughout history. Many important discoveries related to human health have been made possible through studies conducted using experimental animals. In recent years, the acceleration of in vivo studies has significantly advanced science, driven by several key factors. These include elucidating how biological processes and diseases develop and interact in a more realistic environment, operating under physiological conditions closer to those of the human body, the ease of application facilitated by evolving technologies and techniques, and the goal of planning personalized medicine approaches. Structuring and managing in vivo studies with these objectives in mind will lay the foundation for future health innovations. However, considering the ethical principles and sensitivities regarding animal welfare, the use of experimental animals should be meticulously regulated and monitored. Scientists, research institutions, policymakers, and society at large must work together to maintain this balance.

EDİTÖRLERDEN

Deney hayvanları, tarihin başından beri bilimsel araştırmaların temel taşı olmuştur. İnsan sağlığıyla ilgili birçok önemli keşif, deney hayvanları kullanılarak yapılan çalışmalar sayesinde mümkün olmuştur. Son yıllarda, in vivo çalışmaların ivmelenmesi, biyolojik süreçlerin ve hastalıkların daha gerçekçi bir ortamda nasıl geliştiğini ve etkileştiğini anlamamıza yardımcı olarak bilimi önemli ölçüde ilerletmiştir. Bu ivmenin arkasındaki faktörler arasında, insan vücuduna daha yakın fizyolojik koşullar altında çalışma imkanı, gelişen teknolojiler ve tekniklerin kolay uygulanabilirliği ve kişiselleştirilmiş tıp yaklaşımlarının planlanması yer almaktadır. Bu hedefler doğrultusunda in vivo çalışmaların yapılandırılması ve yönetilmesi, gelecekteki sağlık inovasyonlarının temelini oluşturacaktır. Ancak, hayvan refahıyla ilgili etik prensipler ve hassasiyetler göz önünde bulundurulduğunda, deney hayvanlarının kullanımı titizlikle düzenlenmeli ve izlenmelidir. Bilim insanları, araştırma kurumları, politika yapıcılar ve geniş toplum, bu dengeyi korumak için birlikte çalışmalıdır.

In this special issue, various topics will be covered, including methods used in studies with experimental animals, ethical principles, statistical analyses, animal nutrition, anesthesia practices, and experimental animal models used in cancer, chest diseases, neurological diseases, obstetrics and gynecology, heart diseases, and pharmacological research. Additionally, alternative methods to experimental animal models and senescence model theories will also be included in this issue.

The reliability and reproducibility of studies conducted with experimental animals are fundamental to scientific progress. Therefore, careful attention should be given to the selection and care of experimental animals, standardization of experimental procedures, and analysis of results. Furthermore, ensuring the welfare of experimental animals and acting in accordance with ethical rules are crucial for the ethical and legal acceptability of scientific research.

We hope that this special issue will serve as a valuable resource for researchers working with experimental animals, ethics committee members, and professionals from other relevant disciplines. We believe it will contribute to overcoming challenges encountered in studies with experimental animals, developing new methods, and better protecting the welfare of experimental animals.

Within increasing importance of experimental research in biological processes, there has arisen a need to bring together researchers in the field to develop a roadmap. We extend our thanks to Professor Dr. Nedim SÖZBİR, Rector of Duzce University, and Professor Dr. Serkan TORUN, Vice-Rector of Düzce University and Dean of the Faculty of Medicine, for their support at every stage of our special issue. Additionally, we express our gratitude to Associate Professor Dr. Mehmet Ali SUNGUR, the Chief Editor of Duzce Medical Journal, whose unwavering high motivation tirelessly brought all aspects of the process together from inception to publication. We are thankful to the distinguished scientists who bridged diverse perspectives in various fields and unified them under the theme of in vivo studies in shaping our journal issue, to the reviewers who enriched our authors' works with valuable feedback, and to all our colleagues who contributed their efforts sincerely.

Regards,

Bu özel sayıda, deney hayvanlarıyla yapılan çalışmalarda kullanılan yöntemler, etik prensipler, istatistiksel analizler, hayvan beslenmesi, anestezi uygulamaları, kanser, göğüs hastalıkları, nörolojik hastalıklar, doğum ve jinekoloji, kalp hastalıkları ve farmakolojik araştırmalarda kullanılan deney hayvanı modelleri gibi çeşitli konular ele alınacaktır. Ayrıca, deney hayvanı modellerine alternatif yöntemler ve yaşlanma modeli teorileri de bu sayıda yer alacaktır.

Deney hayvanlarıyla yapılan çalışmaların güvenilirliği ve tekrarlanabilirliği, bilimsel ilerlemenin temelidir. Bu nedenle, deney hayvanlarının seçimi ve bakımına dikkat edilmesi, deney prosedürlerinin standardizasyonu ve sonuçların analiz edilmesi büyük önem taşımaktadır. Ayrıca, deney hayvanlarının refahının sağlanması ve etik kurallara uygun hareket edilmesi, bilimsel araştırmanın etik ve yasal kabul edilebilirliği açısından hayati öneme sahiptir.

Bu özel sayının, deney hayvanlarıyla çalışan araştırmacılar, etik komite üyeleri ve ilgili diğer disiplinlerden profesyoneller için değerli bir kaynak olmasını umuyoruz. İleride deney hayvanlarıyla yapılan çalışmalarda karşılaşılan zorlukların üstesinden gelinmesine, yeni yöntemlerin geliştirilmesine ve deney hayvanlarının refahının daha iyi korunmasına katkı sağlayacağına inanıyoruz.

Deneysel araştırmaların biyolojik süreçlerdeki öneminin artmasıyla birlikte, alanında çalışan araştırmacıları bir araya getirerek bir yol haritası oluşturma ihtiyacı doğmuştur. Bize bu yolda özel sayımızın her aşamasında verdikleri destek için Düzce Üniversitesi Rektörü Prof. Dr. Nedim SÖZBİR hocamıza ve Düzce Üniversitesi Rektör Yardımcısı ve Tıp Fakültesi Dekanı Prof. Dr. Serkan TORUN hocamıza teşekkürlerimizi sunarız. Ayrıca, bir harften yola çıkıp tüm eserleri buluşturan hiç yorulmadan yüksek motivasyonu ile tüm süreçleri birlikte yürüttüğümüz Düzce Tıp Fakültesi Dergisi Baş Editörü Doç. Dr. Mehmet Ali SUNGUR hocamıza teşekkür ediyoruz. Dergi sayımızın şekillenmesinde farklı alanlardaki bakış açılarıyla in vivo temelinde birleştiren değerli bilim insanlarına, katkı veren yazarlarımızın eserlerini değerli yorumlarıyla olgunlaştıran hakemlerimize ve emek veren tüm meslektaşlarımıza içtenlikle teşekkürlerimizi arz ederiz.

Saygılarımızla,

Pınar YILDIZ GÜLHAN, MD,
Associated Professor of Chest Diseases

Merve ALPAY, PhD,
Associated Professor of Medical Biochemistry

CONTENTS / İÇİNDEKİLER

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1497730

Duzce Med J, 2024;26(S1):1-13
Düzce Tıp Fak Derg, 2024;26(S1):1-13

Ethical Principles and Rules in Experimental Animal Studies: A Comprehensive Review

Deneysel Hayvan Çalışmalarında Etik İlke ve Kurallar: Kapsamlı Bir İnceleme

Kardelen KOCAMAN KALKAN[1]
0000-0002-5749-5687
Meral KEKEÇOĞLU[2]
0000-0002-2564-8343

[1]Experimental Animals Application and Research Center, Düzce University, Düzce, Türkiye

[2]Department of Biology, Düzce University Faculty of Arts and Sciences, Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Meral KEKEÇOĞLU
meralkekecoglu@duzce.edu.tr

Received / Geliş Tarihi : 03.04.2024
Accepted / Kabul Tarihi : 14.05.2024
Available Online / Çevrimiçi Yayın Tarihi : 07.06.2024

ABSTRACT

When doing scientific research including animal experiments, it is crucial to prioritize ethical issues due to the many moral, legal, and scientific aspects involved. This study provides an in-depth analysis of the core ethical concepts and regulations that govern experimental investigations using animals. The 3R principle (replacement, reduction, refinement), which prioritizes the ethical treatment of animals especially in scientific research, is emphasized. Tracing the historical development of ethical rules sheds light on the important events that shaped the creation of important principles. The review examines the complex relationship between scientific investigation and ethical concerns, focusing on the idea of informed consent in relation to animal care. Also, this explores the ethical dilemmas that arise from experimental techniques and sheds light on how researchers ensure the well-being of the animals involved. The crucial importance of an ethical committee is emphasized in guaranteeing strict ethical standards. The examination of ethical concerns related to certain animal models, and analysis of differing perspectives among the scientific community is done. At the same time, it examines the latest developments in experimental animal research, providing insight into the future of ethical issues in this ever-evolving area. To summarize, this review not only synthesizes the main discoveries and ethical concerns in experimental animal studies but also highlights potential future paths. It supports the idea of continuing to balance scientific progress in the field of experimental animal studies with ethical obligations by suggesting additional areas of research and ethical review.
Keywords: Animal experimentation; ethics; replacement; reduction; refinement.

ÖZ

Hayvan deneyleri de dahil olmak üzere bilimsel araştırma yaparken, birçok ahlaki, hukuki ve bilimsel yön nedeniyle etik konulara öncelik vermek çok önemlidir. Bu çalışma, hayvanlar kullanılarak yapılan deneysel araştırmaları yöneten temel etik kavramların ve düzenlemelerin derinlemesine bir analizini sunmaktadır. Özellikle bilimsel araştırmalarda hayvanlara etik muameleye öncelik veren 3R ilkesi (yer değiştirme, azaltma, iyileştirme) vurgulanmaktadır. Etik kuralların tarihsel gelişiminin izini sürerek önemli ilkelerin oluşumunu şekillendiren önemli olaylara ışık tutmaktadır. Bu derleme, hayvan bakımıyla ilgili bilgilendirilmiş onam fikrine odaklanarak bilimsel araştırma ile etik kaygılar arasındaki karmaşık ilişkiyi incelemektedir. Ayrıca, deneysel tekniklerden kaynaklanan etik ikilemleri araştırarak ve araştırmacıların ilgili hayvanların refahını nasıl sağladıklarına ışık tutulmaktadır. Etik kurulların, katı etik standartların garanti edilmesindeki hayati önemi vurgulanmaktadır. Belirli hayvan modelleriyle ilgili etik kaygıları inceleyerek bilim camiasındaki farklı bakış açılarını analiz etmektedir. Aynı zamanda, deneysel hayvan araştırmalarındaki en son gelişmeleri inceleyerek sürekli gelişen bu alandaki etik konuların geleceğine dair fikir verilmektedir..
Özetlemek gerekirse, bu derleme yalnızca deneysel hayvan çalışmalarındaki ana keşifleri ve etik kaygıları sentezlemekle kalmamakta, aynı zamanda gelecekteki potansiyel yolları da vurgulamaktadır. Ek araştırma ve etik inceleme alanları önererek, deneysel hayvan çalışmaları alanındaki bilimsel ilerlemeyi etik yükümlülüklerle dengelemeye devam etme fikrini desteklemektedir.
Anahtar kelimeler: Hayvan deneyleri; etik; yer değiştirme; azaltma; iyileştirme.

INTRODUCTION

The Significance of Experimental Animal Studies in Scientific Research

Experimental animal research is crucial for improving scientific understanding and achieving medicinal advances. These investigations are crucial instruments for researchers who aim to comprehend biological processes, experiment with theories, and formulate novel therapies. Animal models serve as a bridge between basic research and therapeutic applications, enabling scientists to study intricate processes in living species before applying the findings to human situations. The utilization of experimental animals provides distinct benefits, allowing researchers to change variables, regulate surroundings, and examine physiological reactions in manners that are frequently impracticable or immoral in experiments involving humans (1). Experimental animal research plays a crucial role in advancing medical knowledge, providing significant insights into the genetic causes of illnesses, and evaluating the safety and effectiveness of proposed treatments (1,2). While recognizing the valuable contributions of animal research to scientific progress, it is imperative to also address the ethical implications involved with these experiments. Maintaining a careful equilibrium between scientific investigation and ethical duty is a constant difficulty, leading to continual debates on improving research methods and adopting other techniques. This study explores the ethical principles and regulations that govern experimental animal studies, acknowledging their importance in scientific exploration and the necessity of maintaining ethical standards in the quest for knowledge.

The Ethical Dilemmas Associated With Using Animals in Research

The use of animals in scientific study is accompanied by several ethical difficulties, which provoke extensive debates among scientists and the wider population (4). The core of these conflicts is the conflict between the indisputable scientific advantages gained from animal research and the moral need to protect the welfare of live creatures. An important ethical problem arises from the inherent worth of animal lives. Animals employed in research, being aware beings capable of experiencing pain and discomfort, give rise to ethical concerns regarding the moral grounds for their involvement in studies (4). Finding a middle ground between expanding scientific understanding and acknowledging the intrinsic worth of animal life may be a tricky balancing act (2,3,5). Another ethical quandary emerges due to the possible anguish animals may experience during experimental operations. The ethical ramifications of causing discomfort or agony for research reasons are substantial, ranging from invasive operations to exposure to potentially toxic drugs (3,5). Researchers have the challenge of balancing the need to prevent harm with the goal of attaining scientific objectives, which leads to an ongoing search for improved procedures and alternatives. When doing ethical research with people, obtaining informed permission is essential. However, applying this principle becomes more complex when dealing with animals. Due to the fact that animals are unable to provide explicit agreement, it is crucial to carefully examine their well-being. Researchers must traverse the ethical landscape of assuring the compassionate care of animals, while also addressing problems of autonomy and ethical accountability (5,6). Furthermore, the choice of animal models in itself poses an intricate ethical quandary. Selecting species that accurately reflect human physiology and illness, while also recognizing the distinct characteristics of each species, necessitates a conscientious and ethical approach (2,4,6). The inclusion of species-specific responses and the applicability of findings to human situations introduce additional ethical intricacies to the selection process. It is important to recognize the changing ethical issues in experimental animal studies as we deal with these moral difficulties. Researchers, ethicists, and regulatory agencies work together to create recommendations that aim to tackle these problems while promoting scientific progress (3,5). This study examines the ethical principles and regulations that govern experimental animal experiments, aiming to clarify the intricate relationship between scientific advancement and ethical accountability.

The Purpose and Scope of the Review

This paper aims to thoroughly examine the ethical complexities inherent in experimental animal studies within the field of scientific inquiry. The study seeks to provide a comprehensive summary of the historical progression, fundamental ethical principles, and developing patterns in this important field by combining current knowledge. Our primary focus is to analyze ethical concerns related to the utilization of animals in scientific research. This encompasses a thorough examination of issues pertaining to the well-being of animals, deliberations on obtaining informed permission, and the ethical rationales for their involvement in research. The review aims to offer valuable insights into the ethical aspects that come with scientific advancement through these research. Additionally, this research aims to assess the efficacy of oversight systems, namely the function of ethical committees, in guaranteeing compliance with ethical norms and guidelines in experimental animal experiments. The review explores current discussions and disagreements among scientists, contributing to a thorough understanding of the ethical issues related to certain animal models and experimental methods. This review has a wide range, encompassing several aspects of the ethical environment in experimental animal experiments. The review starts by examining the historical backdrop, delving into the origins and development of ethical norms. This establishes a solid foundation for a comprehensive understanding of the ethical framework that governs such investigations. A substantial part of the paper is focused on analyzing the three core ethical concepts of replacement, reduction, and refinement. This analysis seeks to clarify the use of these principles in order to reduce the impact on animals, highlighting the changing tactics used by researchers to maintain ethical standards. The issues of informed consent and animal welfare are of utmost importance within the context. The paper examines the ethical ramifications associated with the notion of informed consent in the realm of animal welfare, highlighting the researcher's duty to provide compassionate care during trials. The scope includes the assessment of supervision and compliance methods, providing insight into the procedures carried out by

committees to authorize and supervise animal experiments. The study also includes discussions on current debates and new trends in ethical concerns. This recognizes the ever-changing ethical duties in the field of experimental animal studies. The review seeks to make a relevant contribution to the continuing discussion about the ethical implications of experimental animal studies by addressing several complex factors. It aspires to provide helpful insights for researchers, ethicists, and policymakers.

HISTORICAL CONTEXT OF ETHICAL GUIDELINES

The Historical Development of Ethical Guidelines for Experimental Animal Studies

The ethical concerns pertaining to experimental animal experiments have experienced a substantial evolution throughout history. The development of ethical principles in this field demonstrates a deliberate attempt to reconcile the quest for scientific knowledge with the ethical handling of animals used in research. Centuries ago, there were documented cases of doing scientific experiments on animals without taking ethical issues into account (7). Nevertheless, with the growth of the scientific community and increased social consciousness about animal care, ethical issues started to arise. An important milestone in the historical progression of ethical norms occurred in 1959 with the release of William Russell and Rex Burch's influential paper, "The Principles of Humane Experimental Technique" (7). This revolutionary publication proposed the "3 Rs" - Replacement, Reduction, and Refinement - as fundamental principles for doing ethical research using animals. The principles promote the exploration of alternative ways to replace animal testing and lower the overall number of animals involved. They also emphasize the improvement of experimental procedures to minimize possible harm and enhance animal welfare (7). During the latter part of the 20th century, there was a significant increase in worldwide recognition of ethical concerns associated with animal research (7,10). The usage of animals in scientific research has led to the emergence of ethical principles and laws, prompting nations and institutions to build frameworks to control this practice. The National Institutes of Health (NIH) and the European Union, among other prominent institutions, have formulated recommendations that underscore the significance of ethical issues in experimental protocols (9). The release of the "Guide for the Care and Use of Laboratory Animals" by the Institute for Laboratory Animal Research (ILAR) in 1963, along with later updates, emphasized the need for ethical standards. This guide has since become a fundamental text, influencing the moral framework of experimental animal studies by offering extensive rules for the proper care, utilization, and ethical treatment of animals in study (10). Over the past few decades, there has been an increasing focus on the importance of openness, accountability, and public involvement in conversations on the ethical aspects of animal research (7-10). The progress in technology and scientific approaches has prompted a renewed emphasis on seeking alternatives to animal utilization whenever feasible (4). As we examine the historical progression of ethical principles in experimental animal research, it becomes clear that ethical issues have shifted from being a secondary concern to being a fundamental principle of competent scientific investigation. The continuous dedication to improving ethical standards demonstrates a shared recognition of the moral responsibility to responsibly care for animals while pushing the boundaries of knowledge via research (8). Having a historical background is essential for comprehending the ethical norms that govern modern experimental animal investigation.

Landmark Events or Studies That Prompted the Establishment of Ethical Principles

The development of ethical norms in animal studies has been shaped by significant events and studies that have provided insight into the treatment of animals in scientific studies. The ethical concerns surrounding the use of animals in research have received considerable attention, resulting in the creation of standards and principles designed to ensure compassionate care and reduce unnecessary pain. Below are many significant milestones in the discourse around ethical concerns in animal studies.

The Cruelty to Animals Act (1876): This was an early piece of law in the United Kingdom that aimed to regulate the treatment of animals used in studies (11). The objective was to prevent the avoidable distress of animals and mandate a permit for conducting experiments. Although considered basic by contemporary criteria, it was a major milestone in acknowledging the moral ramifications of animal experimentation.

"The Principles of Humane Experimental Technique" by William Russell and Rex Burch (1959): This landmark paper presented the ideas of "Replacement, Reduction, and Refinement" as ethical guidelines for doing animal research. The text highlights the significance of seeking substitutes for animal utilization, decreasing the number of animals employed, and improving experimental techniques to minimize possible damage (12).

The Guide for the Care and Use of Laboratory Animals (1963, undergone updates): This handbook, created by the ILAR, offers thorough suggestions for the ethical treatment and use of laboratory animals (13). It gained widespread recognition as a valuable resource for organizations and academics engaged in animal studies.

The Declaration of Helsinki (1964, revised in 1975 and 2008): Although its main focus is on human research, the Declaration of Helsinki has also had an impact on ethical issues in animal experiments. The principles of international ethical guidelines for medical research involving human participants have been taken into account in discussions addressing the ethical treatment of animals in medical and scientific investigations (14).

The Animal Welfare Act (1966): The Animal Welfare Act in the United States was a key legal measure that established guidelines for the compassionate treatment of animals employed in research, exhibition, and commerce. The implementation of the Institutional Animal Care and Use Committee (IACUC) was required to supervise and assess the ethical components of animal research methods (15).

The Phenomenon of Public Outcry and Animal Welfare Activism: Multiple instances of animal cruelty and public awareness initiatives resulted in heightened examination of animal research protocols. Notable instances, like the Silver Spring monkeys in the 1980s, sparked debates on the moral handling of animals in scientific studies and the necessity for openness.

Ethical Guidelines in the United States (1985): The IACUC in the United States was created in accordance with the Animal Welfare Act. The IACUC is tasked with ensuring that research institutes and facilities adhere to ethical principles for the treatment and use of animals in research, experimentation, and teaching (16).

Guiding Principles for the Care and Use of Animals (2011): This document is a set of principles that provide guidance on how to properly care for and use animals. Several institutions, including the NIH in the United States and the European Union, have created extensive protocols for the ethical implementation of animal research (17). The recommendations provide criteria for housing, care, and experimental methods to guarantee the welfare of animals participating in scientific research (17).

The Progress of Alternatives and Technology: Technological advancements, including the creation of in vitro models, computer simulations, and other alternative approaches, have offered researchers alternate options for employing animals in certain investigations (18). This has facilitated the improvement of ethical principles, advocating for the utilization of alternatives whenever feasible. These events and developments, along with others, have together shaped the creation and progression of ethical norms in experimental animal experiments (18). These actions demonstrate an increasing recognition of the moral obligations linked to utilizing animals in research and a continuous dedication to reducing harm and safeguarding the welfare of animals participated in scientific inquiries.

KEY ETHICAL PRINCIPLES

Within the field of scientific investigation, the ethical treatment of animals used in experiments is of utmost importance (19). This not only reflects our moral responsibilities but also ensures the integrity of the study. The core of this discussion revolves around a group of fundamental ethical principles that act as guiding principles in the planning, execution, and supervision of animal experiments (20). The ethics of compassion, respect for life, and scientific rigor serve as the basis for researchers as they traverse the intricate landscape of testing. It is crucial to comprehend and uphold these principles in order to build trust, maintain credibility, and promote progress in both knowledge and ethical standards in the field of experimental animal studies. This includes taking into account animal welfare and pursuing scientific advancement responsibly.

Principle of Replacement

The principle of replacement is a fundamental principle in ethical standards for scientific research using animals (19). It promotes the use of alternative methods that can replace or minimize the necessity for animal testing, wherever possible. This philosophy acknowledges the inherent worth and well-being of animals and strives to reduce their utilization in scientific research by pursuing alternative methodologies that can accomplish scientific goals without subjecting animals to experimental procedures (18-21). An essential approach to adopting the principle of replacement is the creation and application of alternative methodologies, such as in vitro models, computer simulations, and non-animal testing approaches (22). These techniques present feasible substitutes for conventional animal testing and can give an important understanding of biological processes, medication reactions, and disease causes without the necessity of using animals as test subjects. For instance, researchers can utilize cell cultures, organoids, and tissue engineering techniques to investigate intricate biological systems within a regulated setting (21,22). Similarly, computer simulations and mathematical models can accurately replicate physiological processes and forecast results with surprising precision (22). Moreover, the principle of replacement urges researchers to investigate novel methods that utilize advancements in technology and technique in order to decrease dependence on animal models. For example, employing cells and tissues obtained from humans, microfluidic devices, and bioinformatics tools can yield more pertinent and applicable data in comparison to conventional animal models (18-22). This enhances the scientific accuracy and ethical soundness of research projects. The principle of replacement is effectively implemented through collaboration and knowledge-sharing among members of the scientific community. Through the collaborative sharing of resources, data, and knowledge, researchers may expedite the progress and acceptance of alternative methodologies, assist the validation and standardization efforts, and encourage the general adoption of ethical and sustainable research practices. The principle of replacement demonstrates a dedication to ethical management, advancement in science, and responsible development in research (21,22). Researchers may respect ethical standards, improve animal care, and progress knowledge by adopting alternative methods and minimizing dependence on animal testing. This approach ensures scientific rigor and ethical integrity.

The Importance of Finding Alternative Methods to Replace Animal Use

It has the ability to optimize research procedures, decrease the amount of time and money needed for investigations, and alleviate issues regarding animal welfare rules and public scrutiny. Furthermore, giving priority to different methodologies improves the credibility and reliability of scientific research (23,24). Demonstrating a dedication to scientific integrity, openness, and accountability, enhances public trust in the scientific community and its efforts. Overall, the pursuit of alternative approaches to substitute animal utilization is not only a moral obligation but also a driving force for scientific advancement, effectiveness, and trustworthiness. By adopting alternative methodologies, researchers may enhance their understanding, advocate for ethical research protocols, and ultimately contribute to the improvement of both human and animal well-being (24).

Highlight Advancements in Technology and Other Methodologies

The progress in technology and other strategies has greatly increased the range of alternative ways accessible to researchers, providing inventive alternatives that can efficiently substitute or decrease the requirement for animal experiments. A significant progress may be observed in the domain of computational modeling and simulation. Scientists may use high-performance computing and advanced software algorithms to develop complex models of biological systems, accurately predicting the behavior

of molecules, cells, and organisms (25). These models allow researchers to replicate intricate physiological processes, medication interactions, and disease mechanisms, offering vital insights without the necessity of animal experiments (25). Advancements in in vitro methods have transformed biomedical research by providing meticulously controlled experimental settings that replicate some elements of human physiology. Organ-on-a-chip technologies mimic the shape and function of human organs in small devices that control the flow of fluids (26). This enables researchers to investigate drug reactions, toxicity, and disease mechanisms in an environment that closely resembles the human body (26). Compared to traditional animal models, these systems have benefits in terms of scalability, repeatability, and ethical issues. In addition, advancements in molecular and cellular biology have resulted in the creation of new procedures, including gene editing tools such as CRISPR-Cas9, stem cell technologies, and tissue engineering approaches (27,28). These techniques enable researchers to modify genes, construct tissues, and produce organoids that closely match the architecture and function of humans. Through the utilization of these methodologies, scientists are able to explore disease processes, evaluate therapeutic treatments, and examine biological phenomena with unparalleled accuracy and specificity. Furthermore, improvements in imaging technology have augmented our capacity to see and examine biological processes in real time and with great precision. Methods such as magnetic resonance imaging (MRI), positron emission tomography (PET), and multiphoton microscopy allow researchers to monitor cellular dynamics, tissue shape, and physiological changes in living creatures without causing harm or damage (29). These imaging techniques provide valuable options instead of intrusive treatments and make it easier to conduct long-term investigations, hence minimizing the necessity for animal trials that result in the animal's death (29,30). In summary, the combination of technological advancements and scientific creativity has driven the progress of alternative approaches that not only substitute animal experimentation but also provide clear benefits in terms of precision, applicability, and ethical concerns (23). By adopting these technological innovations, researchers may enhance their understanding, expedite the process of drug discovery, and advocate for ethical and conscientious research methodologies.

Principle of Reduction

The principle of reduction is a crucial ethical principle in scientific research that pertains to animals. It highlights the need of limiting the number of animals utilized in tests, while yet attaining substantial scientific results (19). This concept recognizes that although certain experimentation may be required to further knowledge and solve significant scientific inquiries, it is crucial to strive towards limiting the total number of animals engaged in order to minimize possible harm and maximize efficiency (19,20). An effective approach to using the principle of reduction entails meticulous experimental design and rigorous statistical analysis. By utilizing rigorous statistical techniques and experimental designs, researchers may guarantee that investigations have sufficient statistical power to identify significant effects with the most minimal sample size (31). These methods, such as factorial designs, randomization, and stratification, are used to enhance the effectiveness of studies and minimize the requirement for a large number of animals. In addition, researchers might employ methodologies such as longitudinal studies, which include gathering data from the same animals over a period of time, in order to minimize the number of animals required for research purposes (31). Longitudinal studies enable researchers to collect various measures from individual animals, offering vital insights into developmental processes, illness progression, and therapy effects without requiring new subjects. The principle of reduction is effectively implemented through collaboration and data exchange among members of the scientific community (32). Researchers can prevent redundant experiments and enhance their own study by exchanging data and resources, thereby using existing datasets. This not only lowers the overall quantity of animals required for experiments but also fosters transparency, efficacy, and cooperation among the scientific community (38). Technological and methodological advancements provide further possibilities for decreasing the use of animals in research. For instance, the advancement of in vitro models, computer simulations, and non-invasive imaging tools enables researchers to investigate biological processes and disease pathways without relying on animal trials. These alternative strategies provide benefits in terms of effectiveness, scalability, and ethical concerns, further reinforcing the objectives of the principle of reduction (4,18,23). To summarize, the principle of reduction emphasizes the ethical need to decrease the utilization of animals in scientific research while yet attaining significant scientific results. Researchers may respect ethical standards, enhance animal welfare, and progress knowledge in a responsible and sustainable manner by using tactics such as meticulous experimental design, teamwork, and the utilization of alternative methodologies.

Strategies to Minimize the Number of Animals Used in Experiments

Researchers utilize many ways to limit the number of animals utilized in studies while still generating dependable and significant data. Scientists continuously improve experimental procedures to enhance efficiency and reduce unpredictability (32). This encompasses the process of establishing uniform processes, strengthening the methods used to gather data, and increasing the accuracy of measurements, thereby minimizing the necessity for doing experiments many times (20,31). By employing strong statistical approaches, researchers may plan trials with suitable sample sizes, guaranteeing enough statistical power to identify significant effects. Through meticulous experimental design and rigorous data analysis, researchers can reduce the number of animals needed while still obtaining statistically meaningful findings. Carefully planned experimental design is essential for reducing the number of animals used in research. By utilizing methods like as factorial designs, randomized controlled trials, and cross-over studies, researchers are able to optimize the amount of data obtained from each animal while lowering the overall number of animals required. Collaborative endeavors to exchange and repurpose data and resources across scientists can decrease duplication and eliminate the

necessity for further research. Researchers can utilize preexisting databases and research outcomes to inform their own investigations, so eliminating redundant experimentation (18). Researchers prioritize the use of non-invasive approaches wherever feasible to collect data. Non-invasive imaging techniques, behavioral evaluations, and remote monitoring technologies allow researchers to investigate physiological processes and behaviors in animals without using intrusive methods (18,19). This approach reduces the necessity for euthanasia and minimizes animal discomfort. Embracing alternative approaches, such as in vitro models, computer simulations, and tissue engineering, provides feasible alternatives to conventional animal experiments. By employing these techniques, researchers can investigate study inquiries with a reduced or nonexistent number of animal subjects, so avoiding animal use while still progressing in scientific understanding (33). Conducting longitudinal studies enables researchers to collect many measurements over time from the same animals, hence minimizing the requirement for new volunteers. Moreover, advocating for the adoption of data-sharing procedures fosters transparency and collaboration among scientists, so enabling the more effective utilization of animal resources. By implementing these strategies and embracing a culture of responsible research practices, researchers can minimize the number of animals used in experiments while upholding scientific rigor and ethical standards.

Examples of Successful Reduction Practices

Longitudinal studies involve researchers in domains like psychology and neuroscience who observe and monitor the same group of animals for a prolonged duration. Researchers can minimize the number of animals required for their studies by gathering data from the same patients over several time intervals (34). This approach allows them to get vital knowledge on developmental processes, illness progression, and treatment effectiveness. In preclinical studies focusing on surgical operations, the improvement of surgical methods has resulted in substantial decreases in the number of animals used (34). Researchers have created less invasive surgical techniques and improved anesthetic methods to decrease post-operative pain and discomfort. This has resulted in more effective surgeries with better recovery results and lower fatality rates.

Cell culture and in vitro models are widely used in biomedical research as substitutes for animal testing. Cell-based assays, organoids, and tissue engineering techniques enable researchers to investigate biological processes, medication responses, and disease causes in a controlled setting (35). This reduces the need for animal models and provides pertinent and applicable data. Computer modeling and simulation have become effective methods for minimizing the necessity of animal experimentation in areas such as pharmacology, toxicology, and biomechanics (25). Scientists employ computer simulations to forecast drug interactions, evaluate toxicity, and replicate physiological processes, allowing them to choose potential therapeutic candidates for additional testing and minimize the need for animals in preclinical investigations. The area of biomedical research has been transformed by the progress made in non-invasive imaging methods, including MRI, PET, and ultrasound (29). These techniques enable researchers to observe anatomical features, track the development of diseases, and evaluate the effectiveness of treatments in living animals without the need for intrusive surgeries or death. This reduces animal pain and minimizes the number of animals required in studies. By integrating these reduction strategies into their research procedures, scientists may accomplish their scientific goals while limiting the utilization of animals, advocating for ethical research practices, and propelling the advancement of alternative approaches.

Principle of Refinement

The principle of refinement encompasses the moral duty to consistently improve experimental techniques and housing circumstances in order to promote the well-being of animals and reduce their suffering (36). This principle recognizes that although a certain amount of testing may be required for scientific advancement, it is crucial to minimize the pain and discomfort that animals may endure throughout the process (18-21,36). Refinement strategies refer to a diverse set of methods that try to optimize the care, management, and experimental procedures for laboratory animals in order to enhance their well-being. An essential component of refinement is the creation and execution of enrichment programs aimed at improving the overall physical and psychological well of animals. Enrichment activities encompass facilitating social contact, stimulating the environment, and promoting species-specific behaviors like nesting or foraging. Enhancing the living habitat of laboratory animals can diminish stress, ease monotony, and enhance general well-being, consequently augmenting the quality of data acquired from trials.

Moreover, refining standards prioritizes the implementation of humane endpoints and minimally intrusive strategies to mitigate pain and discomfort experienced during experimental operations. Scientists diligently observe animals for indications of pain or discomfort and take action when needed to ease their distress. Furthermore, advancements in anesthetic, analgesia, and surgical methods allow researchers to conduct surgeries with enhanced accuracy and reduce postoperative discomfort, thereby assuring that animals endure less injury. Another crucial element of refining entails optimizing experimental techniques to minimize the number of animals used for research projects. Scientists utilize statistical methodologies, experimental design strategies, and efforts to share data in order to optimize the knowledge obtained from each animal while lowering the total number of animals needed. Through meticulous experimental design and meticulous data analysis, researchers can attain scientific goals while minimizing the number of animals used, therefore diminishing the total impact on animal welfare. Essentially, the principle of refinement emphasizes the significance of consistently enhancing and refining research methods to reduce the distress experienced by laboratory animals. By using refining procedures, researchers exhibit a dedication to ethical behavior, advance the welfare of animals in their custody, and improve the caliber and reliability of scientific research.

Efforts to Improve the Welfare of Animals during Experimentation

Efforts to enhance the welfare of animals during experiments are diverse, involving several approaches that seek to reduce stress, pain, and suffering while enhancing their

general state of being (37). These endeavors demonstrate a dedication to upholding ethical standards, ensuring compassionate care, and practicing appropriate research methods in scientific investigations that include animals. One primary method for enhancing animal well-being during experiments is the adoption of refining procedures. Refinement refers to a range of methods used to improve experimental techniques, housing conditions, and care protocols in order to reduce suffering and improve the well-being of laboratory animals (38). This may involve creating stimulating surroundings that encourage natural behaviors, adjusting living conditions to assure comfort and social interaction, and employing humane endpoints and minimally invasive approaches to minimize pain and suffering during experimental operations. Moreover, progress in veterinary care and anesthetic techniques has played a crucial role in achieving notable enhancements in animal welfare during the process of experimentation (37). Researchers collaborate closely with veterinary practitioners to create customized care plans and pain management techniques that prioritize the health and well-being of animals during the whole course of research. This may entail the use of analgesics, anesthetics, and post-operative care protocols to mitigate pain and suffering linked to surgical operations and other treatments. Collaboration and knowledge-sharing among scientists are essential for enhancing animal care in experimental research. Scientists cooperate to provide optimal methods, standardized protocols, and standards for the ethical care of animals in research environments. This encompasses the exchange of resources, data, and knowledge to promote the use of improved methodology, alternative techniques, and new approaches that emphasize the well-being of animals while still accomplishing scientific goals (18). In research settings, regulatory supervision and institutional oversight committees have a crucial role in ensuring the protection of animal welfare. Ethical committees are responsible for evaluating research procedures, examining the ethical and scientific rationale for animal experimentation, and ensuring adherence to applicable rules and standards (16). These regulatory agencies play a vital role in overseeing and assessing the well-being of animals involved in research, as well as fostering ethical behavior among researchers. Efforts to enhance the well-being of animals during experiments are driven by a dedication to ethical behavior, scientific thoroughness, and empathetic treatment. Researchers may mitigate the effects on animal welfare, advance knowledge, and promote ethical research procedures by employing refining tactics, cooperating with veterinary specialists, sharing information and resources, and adhering to regulatory monitoring (37,38).

Highlight Studies that have Implemented Refinement Strategies

A published study utilized advanced surgical procedures to decrease pain and misery in rats undergoing stereotaxic surgery for neural implantation (39). The researchers enhanced anesthetic methods, utilized precise surgical techniques, and delivered post-operative care to minimize pain and guarantee the welfare of the animals during the experimental process (39). Enrichment programs were created by researchers studying monkey behavior to encourage natural behaviors and improve the welfare of the animals (40). Another research included environmental enrichment strategies such as introducing new toys, creating chances for foraging, and promoting social interaction. These measures were aimed at reducing stress and enhancing the psychological well-being of the lorises while they were in captivity (41). The utilization of in vitro models as substitutes for animal testing in toxicity research was investigated in a paper published (42). The scientists created a liver microtissue model in three dimensions using cells obtained from humans. This model was used to evaluate the way drugs are processed and their potential harm, showing that it is possible to replace animal trials with in vitro methods that are more ethical and scientifically appropriate.

Another study emphasized the need for collaborative efforts in the scientific community to share resources and data (43). The aim is to prevent redundant experiments and decrease the use of animals in research. The study facilitated efficiency, transparency, and responsible resource usage in neuroscience research by exchanging genetically modified mouse lines, research tools, and data repositories. These examples demonstrate how researchers from several disciplines have employed refining tactics to enhance the well-being of animals during experiments. Researchers may reduce the impact on animals and achieve scientific aims by giving priority to animal welfare, using improved procedures, and collaborating to share resources and expertise.

INFORMED CONSENT AND ANIMAL WELFARE

Within the field of animal studies, the notion of informed consent assumes a distinct nature when compared to research involving people, owing to the inherent disparities in cognitive capacities and communication between humans and animals. Although animals are unable to give informed permission in the same manner as people, ethical concerns surrounding their well-being and care remain of utmost importance. Researchers adhere to ethical principles and animal welfare guidelines to ensure that animals participate in experiments willingly, with the least intrusion, and with the greatest regard for their wellbeing, rather than seeking express agreement from them (45). This approach is based on the acknowledgment of animals who are entitled to ethical consideration and safeguarding against avoidable harm. The utilization of animals in research is regulated by ethical rules and regulations that prioritize the concepts of reduction, refinement, and replacement. These principles aim to reduce harm and enhance animal welfare. Ethical committees have a vital function in evaluating research procedures, evaluating the scientific and ethical reasons for animal use, and ensuring that studies are carried out in compliance with applicable laws and guidelines (44,45). Scientists utilize many methodologies to ensure the well-being of animals and reduce any potential harm throughout the process of conducting experiments. This involves applying techniques to improve the quality of care, such as introducing enrichment programs to encourage natural behaviors, ensuring suitable housing and husbandry settings, and utilizing anesthetics and analgesics to reduce pain and discomfort during experimental operations (45). In addition, researchers are increasingly acknowledging the significance of transparency and communication in relation to their study methodologies and results (49).

Researchers may promote confidence and responsibility in the scientific community and prioritize animal welfare in research procedures by providing precise information about their techniques, results, and any potential negative impacts or limits of their investigations. Although animals are unable to give informed permission in the same manner as people, it is the responsibility of researchers to prioritize their well-being and reduce their distress in study environments. Researchers may maintain principles of animal care and guarantee that research is performed in a scientifically rigorous and morally acceptable way by following ethical guidelines, employing refining tactics, and fostering transparency and communication (3,5,16).

How Researchers Ensure the Well-Being of Animals during Experiments

Scientists utilize a range of tactics to guarantee the welfare of animals during studies, prioritizing ethical concerns, compassionate care, and the goals of reducing, refining, and replacing animal use:

Optimized Housing and Husbandry: Animals are given housing circumstances that are specifically designed to meet their species-specific requirements, which include adequate space, environmental enrichment, and availability of food and water (46). Researchers guarantee that housing facilities are hygienic, properly cared for, and devoid of environmental factors that cause stress in order to enhance the physical and psychological welfare of animals.

Minimization of Stress and Discomfort: Researchers utilize refining tactics to decrease stress and pain in animals during experiments, aiming to reduce their negative experiences. This may entail familiarizing animals with experimental methods through habituation and training, employing positive reinforcement approaches to alleviate anxiety, and reducing handling and constraint to prevent undue stress (47).

Implementation of Enrichment Programs: Enrichment programs are put into action with the aim of fostering innate behaviors and providing cognitive stimulation for laboratory animals. This may include providing toys, environmental enrichment devices, and social housing to enhance the quality of life and prevent boredom and stereotypic behaviors in animals housed in captivity (41).

Use of Minimally Invasive Techniques: Researchers utilize minimally invasive techniques and procedures to reduce pain and distress in animals during experimentation (18,19,29). This may involve the use of anesthesia and analgesia to alleviate pain, sedation to minimize anxiety, and minimally invasive surgical techniques to reduce tissue trauma and facilitate recovery.

Regular Monitoring and Health Checks: Continuous monitoring and health assessments are conducted on animals during the whole length of trials to evaluate their physical condition and overall welfare (48). Researchers do regular health assessments, track the consumption of food and drink, and study behavioral signs of stress or illness to promptly identify and address any potential health problems.

Adherence to Ethical Guidelines and Regulations: Researchers comply with ethical norms and laws that control the utilization of animals in research, in order to guarantee the compassionate care and well-being of animals. Ethical committees evaluate research procedures, analyze the scientific and ethical reasons for animal utilization, and guarantee that studies are carried out in compliance with applicable laws and norms (16).

Transparency and Reporting: Researchers uphold transparency and accountability by precisely documenting their methodologies, findings, and any possible detrimental consequences or constraints of their investigations (49). Through the act of sharing data and findings with the scientific community, researchers enable the process of peer review, replication, and validation of study findings. This ensures that animal welfare issues continue to be a primary focus in research procedures.

In order to guarantee the welfare of animals during studies, it is necessary to use a holistic strategy that gives priority to ethical concerns, humane treatment, and the adoption of refining tactics aimed at reducing harm and promoting animal welfare. By embracing these concepts and implementing these procedures, researchers may maintain ethical standards, advocate for the conscientious utilization of animals in research, and progress scientific understanding in a way that is both scientifically rigorous and morally sound.

Challenges and Ongoing Debates Regarding Animal Consent and Welfare

The challenges and ongoing arguments around animal consent and welfare in research settings are a result of many ethical, scientific, and societal factors. An obstacle arises when attempting to analyze animal behavior and evaluate their well-being in research environments. Although researchers make efforts to reduce stress and pain, precisely assessing an animal's subjective experience and well-being can be challenging. Controversies emerge over the sufficiency of existing techniques for evaluating animal welfare and the necessity for additional impartial criteria to guarantee the ethical treatment of animals. The pursuit of scientific progress frequently clashes with the imperative to safeguard animal welfare throughout research endeavors. Scientists encounter ethical quandaries while devising research that may entail potentially detrimental methods or treatments. Ensuring a balance between scientific goals and animal welfare necessitates a meticulous evaluation of the possible advantages and disadvantages of research projects. The ethical use of animals in research is a subject of controversy, namely concerning the reasons for their usage and the ethical consequences of experimentation. There are concerns regarding the moral standing of animals, their ability to feel pain and suffering, and the ethical obligations of researchers and organizations towards them (18). These disputes have an impact on discussions on the ethical guidelines that govern animal research and the necessity for stricter ethical standards and oversight procedures. There is a continuous discussion about the creation and acceptance of alternate approaches to animal research. Although alternatives such as in vitro models, computer simulations, and tissue engineering show potential for decreasing the usage of animals, concerns persist over their accuracy, dependability, and capacity to be applied to human biology (4,18,23). Controversies can emerge over the ethical ramifications of employing alternative techniques and the degree to which they may substitute established animal models (50). Regulatory oversight and enforcement involve the task of ensuring that ethical norms and rules for the use of animals in research are

followed. This task is accompanied by continuous problems. Although there are legislative frameworks in place to safeguard animal welfare and uphold ethical behavior, the methods of enforcement might differ throughout jurisdictions, resulting in variations in the supervision and implementation of these regulations. The debates revolve around the sufficiency of existing rules, the necessity for more rigorous enforcement methods, and the function of regulatory agencies in advancing ethical research procedures. The opinions and involvement of the general public on animal research and welfare have a significant impact on policy choices, funding priorities, and discussions on ethical matters. Discussions on the ethical treatment of animals in research frequently mirror wider societal values, cultural views, and ethical frameworks. It is crucial to involve the public in conversations on animal welfare, ethical issues, and the use of animals in research in order to foster openness, accountability, and responsible research methodologies (50). To summarize, the issues and current discussions around animal consent and welfare in research settings underscore the many ethical, scientific, and societal factors involved in utilizing animals for research purposes. To tackle these issues, it is necessary to adopt a multidisciplinary strategy that gives importance to ethical concerns, scientific rigor, and stakeholder participation. This method ensures that animals are treated humanely and research is conducted responsibly.

OVERSIGHT AND COMPLIANCE OF THE ANIMAL STUDIES

Effective oversight and compliance systems are essential for guaranteeing the ethical conduct of animal research. These systems are specifically created to protect the well-being of animals, ensure the honesty and accuracy of scientific work, and maintain high ethical standards in research environments (50). Effective monitoring and compliance in animal research are influenced by several crucial components. Institutional animal ethics committees have the responsibility of evaluating and authorizing research methods that involve animals to guarantee adherence to ethical principles and regulations (16,50). These committees comprise scientists, veterinarians, ethicists, and community representatives who appraise the scientific and ethical rationales for animal utilization, evaluate the potential hazards and advantages of research studies, and guarantee that experiments are carried out in compliance with applicable laws and guidelines. Ethical principles and regulations establish the structure for ethical behavior and supervision in the field of animal research. The guidelines, such as the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act, provide a framework for the concepts of reduction, refinement, and replacement (13,15,17). They also provide criteria for the housing, care, pain management, and death of animals used in research. To add, researchers must provide comprehensive study protocols to these committees for evaluation and authorization prior to undertaking animal experiments. The protocol review process entails assessing the scientific justification, experimental methodology, methods, and strategies for minimizing pain and discomfort in animals. The committee evaluates the possible hazards and advantages of research studies and guarantees that experiments comply with ethical principles and regulatory obligations.

Institutions have the responsibility of delivering training and educational programs to researchers, animal care workers, and committee members in order to guarantee their proficiency in animal handling, husbandry, and welfare methods (51). The training sessions encompass subjects such as appropriate animal care and handling, ethical issues in animal research, and adherence to regulatory compliance standards. Institutions guarantee that staff engaged in animal research possess a comprehensive understanding of ethical norms and regulatory requirements by actively promoting education and training. Moreover, regular monitoring and inspections of animal facilities and research laboratories are mandatory for institutions to guarantee adherence to ethical principles and legislation. Inspections are carried out by internal compliance officers, external regulatory authorities, or accrediting bodies to evaluate the state of animal facilities, the health and well-being of animals, and the execution of appropriate husbandry and care methods. Organizations must keep comprehensive documentation of animal care and utilization endeavors, encompassing research procedures, records of animal housing and care, veterinarian care records, and personnel training records. These records function as proof of adherence to ethical norms and regulatory requirements and are susceptible to scrutiny by regulatory bodies during inspections and audits (16,50,51). Essential for guaranteeing the ethical conduct of research with animals is adequate supervision and compliance methods. Institutions may ensure ethical standards, protect animal welfare, and encourage responsible research procedures by implementing strong monitoring systems, following ethical rules and legislation, offering training and education programs, and encouraging openness and responsibility.

The Role of Ethical Committees in Ensuring Ethical Standards

Ethical committees have a crucial function in guaranteeing ethical standards and the compassionate care of animals in research environments. These committees have the responsibility of evaluating, authorizing, and supervising animal research plans. They play a crucial role in ensuring the well-being of animals, maintaining scientific standards, and ensuring compliance with regulations at research institutes. The main duty of them is to assess planned animal research to ensure it complies with ethical standards, and regulatory obligations, and minimizes harm to animals. This entails a comprehensive examination of study protocols, encompassing the scientific justification, experimental structure, techniques, and strategies to mitigate pain and suffering in animals. These committees evaluate the possible dangers and advantages of research studies during the review process, taking into account variables such as the need for animal usage, the suitability of experimental techniques, and the effectiveness of efforts to reduce potential damage. The committee also assesses the credentials and training of researchers and animal care workers engaged in the study to guarantee proficiency in animal handling and welfare protocols (38). After a research plan is authorized, it maintains oversight of the project to guarantee continuing adherence to ethical norms and regulatory requirements. The ethical committee not

only has regulatory powers but also acts as a valuable resource and support system for researchers. It offers advice on ethical issues, animal care procedures, and regulatory compliance needs. The committee also has a vital role in educating the research community about ethical norms and fostering a culture of responsible conduct in animal research (16). In summary, regulatory organizations play a crucial role in guaranteeing the ethical execution of animal research. These committees play a crucial role in preserving public confidence in the honesty of scientific research and protecting the well-being of research animals by adhering to ethical standards, advocating for animal welfare, and enforcing regulatory compliance.

CURRENT ISSUES AND DEBATES

Contemporary ethical concerns in experimental animal research involve a variety of intricate and multidimensional factors that mirror the changing scientific, ethical, and societal viewpoints. These problems elicit discussion and examination among scientists, ethical discussions, and circles involved in crafting policies.

Debates Surrounding the Use of Specific Animal Models

The debates about the utilization of certain animal models in research are intricate and nuanced, encompassing a wide range of viewpoints, ethical concerns, and scientific goals (53). Various animal species are employed in research for a range of objectives, including fundamental biology study, disease modeling, and medication development. Several crucial discussions revolve on the use of certain animal models, encompassing. One of the main topics of discussion is the choice of suitable animal species for research purposes. When selecting animal models, researchers must take into account criteria such as the degree of evolutionary relatedness to humans, similarities in physiology, ease of genetic manipulation, availability, and ethical issues (54). Controversies emerge over the appropriateness of certain species for specific scientific inquiries, the transferability of discoveries to humans, and the ethical ramifications of employing certain species in study. A significant point of disagreement revolves over the degree to which conclusions drawn from animal research may be applied to people. Critics contend that variations in physiology, anatomy, metabolism, and genetics between people and animals might restrict the applicability of study findings, resulting in possible inconsistencies and inefficiencies in the creation of drugs and their translation to clinical use (53,54). Advocates of animal models argue that, despite their limitations, studies conducted on animals offer essential knowledge about biological systems, disease processes, and therapeutic treatments that may be applied to human health. The ethical discussions over the utilization of particular animal models revolve around concerns related to animal welfare, the experience of suffering, and the moral standing of animals. Critics express apprehensions over the ethical ramifications of using sentient creatures in research, especially when it involves invasive or detrimental operations (55). The ethical rationale behind utilizing specific animals, such as non-human primates, dogs, or pigs, in research is being closely examined, with demands for increased attention to alternative options, improvement measures, and ethical supervision. The advancement and acceptance of alternative techniques for animal testing question the dependence on particular animal models in scientific investigation. Controversies emerged regarding the verification, dependability, and significance of alternative techniques in comparison to animal models, along with the moral need to promote non-animal procedures wherever possible (53-56). Issues around certain animal models are influenced by concerns over the scientific validity and repeatability of animal experiments. Factors such as differences in animal strains, environmental conditions, experimental procedures, and biased publication practices might affect the dependability and replicability of study results. The objective of advocating for enhanced experimental design, reporting standards, and replication studies is to strengthen the rigor and reliability of animal research. Discussions about the utilization of particular animal models in research are marked by intricate ethical, scientific, and practical factors. To address these arguments, it is necessary to carefully analyze the selection of species, the capacity to translate findings to humans, the ethical implications, alternative approaches, and the scientific validity (21). Researchers may traverse these controversies and make well-informed judgments concerning the use of animal models in research by participating in discourse, fostering openness, and incorporating ethical and scientific principles.

Advancing Ethical Standards in Animal Testing: Identifying Gaps, Unanswered Questions, and Future Directions

Divergent viewpoints, beliefs, and interests give rise to conflicting opinions about the ethical aspects of different research methods. In order to address these conflicts, active engagement in discussions, thoughtful deliberation, and inclusion of relevant stakeholders are essential to negotiate complex moral dilemmas, promote ethical decision-making, and uphold the principles of benevolence, personal autonomy, justice, and respect for all research participants (53-56). The ethical considerations surrounding animal experimentation are intricate and diverse, with several areas of uncertainty and unresolved inquiries that necessitate more investigation. Although progress has been made in adopting alternative approaches and minimizing animal experimentation, there is still a want for stronger frameworks and norms to guarantee the ethical treatment of animals in scientific research. There is a significant lack of information on the lasting impacts and dependability of alternative testing techniques in comparison to conventional animal models (56). Although organoids, computer simulations, and in vitro models hold potential, further study is required to verify their effectiveness, consistency, and applicability to human physiology (18,23). Furthermore, it is crucial to carefully contemplate the ethical ramifications of employing these alternatives, taking into account their influence on study results and regulatory determinations. Moreover, there is a need for additional investigation into the ethical quandaries associated with the utilization of genetically modified animals in scientific studies. Concerns emerge over the well-being of these animals, the possibility of unforeseen outcomes, and the ethical obligations of researchers and regulatory entities. It is crucial to address these matters in order to establish ethical principles that effectively reconcile scientific advancement with the well-being of animals. Another aspect that deserves consideration is the

influence of public opinion and stakeholder participation on the development of animal testing policies (55). Gaining insight into social views, beliefs, and concerns about animal research is essential for promoting openness, responsibility, and public confidence. Future studies should prioritize the evaluation of public views, investigation of stakeholder viewpoints, and facilitation of conversation to enhance the ethical decision-making processes. To effectively address these gaps and unsolved problems, it is crucial to foster multidisciplinary collaboration, engage in ethical thought, and maintain continuing communication among scientists, politicians, ethicists, and the public. By adopting a comprehensive strategy that places importance on the well-being of animals, rigorous scientific methods, and the values of society, we may work towards implementing more ethical and sustainable practices in the field of biomedical research.

CONCLUSION

Ultimately, ethical norms and regulations in experimental animal studies are essential elements of conscientious scientific investigation and compassionate care for research animals. This thorough examination has investigated fundamental ethical factors, such as the concepts of replacement, reduction, and refinement. These principles highlight the significance of decreasing the utilization of animals, diminishing their suffering, and improving their well-being in research procedures. We have analyzed the function of ethical committees, in guaranteeing adherence to ethical norms and regulatory obligations. Additionally, we have explored methods to enhance openness, accountability, and stakeholder involvement in animal research. Furthermore, this study has emphasized current ethical concerns, discussions, and opposing perspectives about the utilization of certain animal models, alternative techniques, genetic manipulation, and clinical trials, emphasizing the intricate nature of ethical decision-making in research. To overcome ethical difficulties, encourage responsible behavior in animal research, and protect scientific integrity, animal welfare, and ethical practice, researchers, policymakers, and stakeholders must address these ethical considerations. To advance ethical principles and cultivate a culture of compassion, respect, and ethical stewardship in experimental animal research, it is crucial to engage in constant discourse, collaboration, and ethical reflection.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: MK; Design: KKK; Data Collection/Processing: KKK; Analysis/Interpretation: KKK, MK; Literature Review: KKK; Drafting/Writing: KKK, MK; Critical Review: MK.

REFERENCES

1. Domínguez-Oliva A, Hernández-Ávalos I, Martínez-Burnes J, Olmos-Hernández A, Verduzco-Mendoza A, Mota-Rojas D. The importance of animal models in biomedical research: current insights and applications. Animals (Basel). 2023;13(7):1223.
2. Houvenaghel A. Animal experiments: legal, scientific and ethical aspects. Verh K Acad Geneeskd Belg. 2000;62(2):103-17. Dutch.
3. Kiani AK, Pheby D, Henehan G, Brown R, Sieving P, Sykora P, et al.; International Bioethics Study Group. Ethical considerations regarding animal experimentation. J Prev Med Hyg. 2022;63(2 Suppl 3):E255-66.
4. Fernandes MR, Pedroso AR. Animal experimentation: A look into ethics, welfare and alternative methods. Rev Assoc Med Bras (1992). 2017;63(11):923-8.
5. Monsó S, Benz-Schwarzburg J, Bremhorst A. Animal morality: what it means and why it matters. J Ethics. 2018;22(3):283-310.
6. Gluck JP, Bell J. Ethical issues in the use of animals in biomedical and psychopharmocological research. Psychopharmacology (Berl). 2003;171(1):6-12.
7. Petetta F, Ciccocioppo R. Public perception of laboratory animal testing: Historical, philosophical, and ethical view. Addict Biol. 2021;26(6):e12991.
8. Franco NH. Animal experiments in biomedical research: a historical perspective. Animals (Basel). 2013;3(1):238-73.
9. National Research Council (US) Committee to Update Science, Medicine, and Animals. Science, medicine, and animals. Washington (DC): National Academies Press (US); 2004.
10. Arnason G. The emergence and development of animal research ethics: a review with a focus on nonhuman primates. Sci Eng Ethics. 2020;26(4):2277-93.
11. Finn MA, Stark JF. Medical science and the Cruelty to Animals Act 1876: A re-examination of anti-vivisectionism in provincial Britain. Stud Hist Philos Biol Biomed Sci. 2015;49:12-23.
12. Tannenbaum J, Bennett BT. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. J Am Assoc Lab Anim Sci. 2015;54(2):120-32.
13. Albus U. Guide for the care and use of laboratory animals (8th edn). Lab Anim. 2012;46(3):267-8.
14. Goodyear MD, Krleza-Jeric K, Lemmens T. The declaration of Helsinki. BMJ. 2007;335(7621):624-5.
15. Cardon AD, Bailey MR, Bennett BT. The animal welfare act: from enactment to enforcement. J Am Assoc Lab Anim Sci. 2012;51(3):301-5.
16. Prentice ED, Blackburn ME, Dixon RS. Fostering collaborative roles and responsibilities for members of an IACUC or oversight body. In: Weichbrod RH, Thompson GA, Norton JN, editors. Management of animal care and use programs in research, education, and testing. 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; 2018. Chapter 3.

17. Institute of Medicine (US); National Research Council (US). International animal research regulations: impact on neuroscience research: workshop summary. Washington (DC): National Academies Press (US); 2012. 7, core principles for the care and use of animals in research.
18. Mahalmani V, Prakash A, Medhi B. Do alternatives to animal experimentation replace preclinical research? Indian J Pharmacol. 2023;55(2):71-5.
19. Richmond J. Refinement, reduction, and replacement of animal use for regulatory testing: future improvements and implementation within the regulatory framework. ILAR J. 2002;43 Suppl:S63-8.
20. Sneddon LU, Halsey LG, Bury NR. Considering aspects of the 3Rs principles within experimental animal biology. J Exp Biol. 2017;220(Pt 17):3007-16.
21. Díaz L, Zambrano E, Flores ME, Contreras M, Crispín JC, Alemán G, et al. Ethical considerations in animal research: the principle of 3R's. Rev Invest Clin. 2020;73(4):199-209.
22. Hubrecht RC, Carter E. The 3Rs and humane experimental technique: implementing change. Animals (Basel). 2019;9(10):754.
23. Husain A, Meenakshi DU, Ahmad A, Shrivastava N, Khan SA. A review on alternative methods to experimental animals in biological testing: recent advancement and current strategies. J Pharm Bioallied Sci. 2023;15(4):165-71.
24. Doke SK, Dhawale SC. Alternatives to animal testing: A review. Saudi Pharm J. 2015;23(3):223-9.
25. Badyal DK, Modgill V, Kaur J. Computer simulation models are implementable as replacements for animal experiments. Altern Lab Anim. 2009;37(2):191-5.
26. Ingber DE. Human organs-on-chips for disease modelling, drug development and personalized medicine. Nat Rev Genet. 2022;23(8):467-91.
27. Lee H, Yoon DE, Kim K. Genome editing methods in animal models. Anim Cells Syst (Seoul). 2020;24(1):8-16.
28. Arora T, Mehta AK, Joshi V, Mehta KD, Rathor N, Mediratta PK, et al. Substitute of animals in drug research: an approach towards fulfillment of 4R's. Indian J Pharm Sci. 2011;73(1):1-6.
29. Ohno Y, Koyama H, Lee HY, Yoshikawa T, Sugimura K. Magnetic resonance imaging (MRI) and positron emission tomography (PET)/MRI for lung cancer staging. J Thorac Imaging. 2016;31(4):215-27.
30. Catana C. Principles of simultaneous PET/MR imaging. Magn Reson Imaging Clin N Am. 2017;25(2):231-43.
31. Flecknell P. Replacement, reduction and refinement. ALTEX. 2002;19(2):73-8.
32. Lewis DI. Animal experimentation: implementation and application of the 3Rs. Emerg Top Life Sci. 2019;3(6):675-9.
33. Tannenbaum J, Bennett BT. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. J Am Assoc Lab Anim Sci. 2015;54(2):120-32.
34. de Boo J, Hendriksen C. Reduction strategies in animal research: a review of scientific approaches at the intra-experimental, supra-experimental and extra-experimental levels. Altern Lab Anim. 2005;33(4):369-77.
35. Goldberg L. Charting a course for cell culture alternatives to animal testing. Fundam Appl Toxicol. 1986;6(4):607-17.
36. Musk GC. Refinements to animal models for biomedical research. Animals (Basel). 2020;10(12):2425.
37. Clutton RE. An anglocentric history of anaesthetics and analgesics in the refinement of animal experiments. Animals (Basel). 2020;10(10):1933.
38. Prescott MJ, Lidster K. Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Anim (NY). 2017;46(4):152-6.
39. Ferry B, Gervasoni D. Improving stereotaxic neurosurgery techniques and procedures greatly reduces the number of rats used per experimental group-A practice report. Animals (Basel). 2021;11(9):2662.
40. Coleman K, Novak MA. Environmental enrichment in the 21st century. ILAR J. 2017;58(2):295-307.
41. Alejandro J, Yamanashi Y, Nemoto K, Mouri K, Huang CW, Bercovitch FB, et al. Reducing stress and stereotypic behaviors in captive female pygmy slow lorises (Nycticebus pygmeaus). Am J Primatol. 2023;85(7):e23495.
42. Movia D, Bruni-Favier S, Prina-Mello A. In vitro alternatives to acute inhalation toxicity studies in animal models-a perspective. Front Bioeng Biotechnol. 2020;8:549.
43. NIMH Collaborative Data Synthesis for Adolescent Depression Trials Study Team including; Perrino T, Howe G, Sperling A, Beardslee W, Sandler I, et al. Advancing science through collaborative data sharing and synthesis. Perspect Psychol Sci. 2013;8(4):433-44.
44. Mohan S, Huneke R. The role of IACUCs in responsible animal research. ILAR J. 2019;60(1):43-9.
45. Budda ML, Pritt SL. Evaluating IACUCs: Previous research and future directions. J Am Assoc Lab Anim Sci. 2020;59(6):656-64.
46. Prager EM, Bergstrom HC, Grunberg NE, Johnson LR. The importance of reporting housing and husbandry in rat research. Front Behav Neurosci. 2011;5:38.
47. National Research Council (US) Committee on Recognition and Alleviation of Distress in Laboratory Animals. Recognition and alleviation of distress in laboratory animals. Washington (DC): National Academies Press (US); 2008. 4, Avoiding, minimizing, and alleviating distress.
48. FELASA working group on revision of guidelines for health monitoring of rodents and rabbits; Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, et al. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim. 2014;48(3):178-92.
49. Carbone L. Open transparent communication about animals in laboratories: dialog for multiple voices and multiple audiences. Animals (Basel). 2021;11(2):368.
50. King WW, Paramastri YA, GuillÁ©n J. Compliance and regulatory programs. In: Weichbrod RH, Thompson GA, Norton JN, editors. Management of animal care and use programs in research, education, and testing. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2018. Chapter 7.

51. Wege-Rost T. Medical-ethical decision-making-contribution of the clinical ethics committee by ethics consultation and development of ethical guidelines. Med Klin Intensivmed Notfmed. 2023;118(3):175-9. German.
52. Hansen LA. Institution animal care and use committees need greater ethical diversity. J Med Ethics. 2013;39(3):188-90.
53. Ashall V, Millar K, Hobson-West P. Informed consent in veterinary medicine: Ethical implications for the profession and the animal 'patient'. Food Ethics. 2018;1(3):247-58.
54. Rollin BE. Animal research: a moral science. Talking point on the use of animals in scientific research. EMBO Rep. 2007;8(6):521-5.
55. Saraf SK, Kumaraswamy V. Basic research: Issues with animal experimentations. Indian J Orthop. 2013;47(1):6-9.
56. Ferdowsian HR, Beck N. Ethical and scientific considerations regarding animal testing and research. PLoS One. 2011;6(9):e24059.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1481602

Duzce Med J, 2024;26(S1):14-18
Düzce Tıp Fak Derg, 2024;26(S1):14-18

General Principles, Designs, and Statistical Analyses in Experimental Animal Studies

Deney Hayvanı Çalışmalarında Genel Prensipler, Tasarımlar ve İstatistiksel Analizler

Şengül CANGÜR
0000-0002-0732-8952

Department of Biostatistics and Medical Informatics, Düzce University Faculty of Medicine, Düzce, Türkiye

ABSTRACT

Research using animals contributes significantly to many research and development studies, especially in the biomedical field. Within the scope of the study, conducting animal experiments in accordance with scientific principles and ensuring the ethical use and welfare of animals are issues that should be taken into consideration. In this context, the scientific contribution to be achieved by conducting these studies in line with scientific and ethical principles will be directly proportional. In many studies, while investigating the biological significance, it is seen that the effects of many factors are ignored, the answer to the biological question is investigated with simple experimental designs, or the accurate statistical analyses are not chosen. Therefore, in this study, the principles that a researcher planning an animal study should follow within the scope of the research (animal ethics, 3R, and other R rules, determination of sample size, randomization, and blinding) are briefly mentioned. Then, completely randomized design, regression design, split-unit design, hierarchical (nested) design, mixed effects design, and appropriate statistical analyses for these designs, which are thought to be useful in these studies, are discussed. It is thought that this review will be useful as it contains important summative information that will guide all researchers in planning animal studies accurately and quickly.
Keywords: Animal study; experimental designs; 3R rules, 12R's.

ÖZ

Hayvanların kullanıldığı araştırmalar, özellikle biyomedikal alanda birçok araştırma ve geliştirme çalışmalarına önemli düzeyde katkı sağlamaktadır. Çalışma kapsamında hayvan deneylerinin bilimsel prensiplere uygun yürütülmesi, hayvanların etik kullanımı ve refahının sağlanması dikkate alınması gereken hususlardır. Bu bağlamda bu çalışmaların bilimsel ve etik ilkelere uygun yürütülmesiyle elde edilecek bilimsel katkı doğru orantılı olacaktır. Birçok çalışmada biyolojik önemliliğinin veya sorunun araştırılmasında bazı faktörlerin etkisinin göz ardı edilerek basit deney tasarımlarıyla biyolojik cevabın araştırıldığı ya da doğru istatistiksel analizlerin seçilmediği görülmektedir. Bu nedenle bu çalışmada hayvan çalışması planlayan bir araştırmacının araştırma kapsamında uyması gereken prensiplerden (hayvan etiği, 3R ve diğer R kuralları, örneklem genişliğinin belirlenmesi, randomizasyon ve körleme yaklaşımları) kısaca bahsedilmiştir. Ardından da bu araştırmalarda faydalı olduğu düşünülen tamamen rasgele tasarım, regresyon tasarımı, split-unit tasarım, hiyerarşik (iç içe) tasarım, karma etkili tasarım ve bu tasarımlara uygulanacak istatistiksel analizler üzerinde durulmuştur. Bu derlemenin hayvan çalışmaları planlayan tüm araştırmacıları doğru ve hızlı yönlendirecek önemli özetleyici bilgiler içermesi nedeniyle faydalı olacağı düşünülmektedir.
Anahtar kelimeler: Hayvan çalışması; deney tasarımları; 3R kuralı; 12R kuralı.

Corresponding Author
Sorumlu Yazar
Şengül CANGÜR
sengulcangur@duzce.edu.tr

Received / Geliş Tarihi : 23.02.2024
Accepted / Kabul Tarihi : 02.04.2024
Available Online /
Çevrimiçi Yayın Tarihi : 10.05.2024

INTRODUCTION

Experiments using animals have contributed significantly to many research and development studies, especially in the biomedical field throughout the history of science. Although the results obtained from animal experiments cannot be directly translated to humans (1), they provide important information and clues about the possible behavioral attributes of pharmaceutical agents and therapeutic modalities tested in humans and other species (2).

Conducting animal experiments in accordance with scientific principles and ensuring the ethical use and welfare of animals are matters that must be taken into consideration. In many countries, the 3R rules (Replacement, Reduction, and Refinement) are recommended as an ethical approach in legislation regarding animal research (3,4). Researchers are further advised to adhere to guidelines such as Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE), Design and Execution of Protocols for Animal Research and Treatment (DEPART), and Animal Research: Reporting of In Vivo Experiments (ARRIVE) when conducting animal experiments (3-6).

In research planned within the framework of scientific principles, the researcher formulates one or more hypotheses to seek the appropriate answer to the biological question at hand. The selection of the appropriate experimental design must be made to test these hypotheses. There are many experimental designs used in animal research. However, the selection of the appropriate experimental design, considering the constraints and limitations of the study, will guide the researcher toward obtaining an accurate answer to the biological question. The choice of statistical analyses varies according to the selected experimental designs. Statistical analysis serves as a crucial tool for examining data and determining whether observed differences stem from sample variations or genuine disparities within the underlying population (2,7-10).

In numerous studies, experimental designs are often formulated without adequately accounting for the effects of multiple factors when exploring biological significance or addressing problems. Instead of employing complex designs to investigate the biological problem comprehensively, researchers may opt for simpler experimental designs or fail to select an appropriate statistical analysis for the experimental design, potentially compromising the rigor and validity of the findings.

For these reasons, this study has mentioned the principles that a researcher should adhere to when planning an animal study within the scope of the research. Then, some special experimental designs including completely randomized design, regression design, split-unit design, hierarchical (nested) design, and mixed effects design are explained, and the statistical analyses that can be applied to these experimental designs are emphasized.

GENERAL PRINCIPLES FOR ANIMAL EXPERIMENTS

Animal Ethics

Animal ethics, a subset of bioethics, delineates the boundaries of permissible actions within the realms of human and animal sciences, particularly concerning the use of animals in research. It encompasses universal principles governing attitudes and behaviors towards animals, setting forth ethical guidelines and standards to ensure their welfare and humane treatment. Compliance with animal ethics enables the fulfillment of several objectives, including providing justifications for research and training studies involving experimental animals, organizing, and conducting experiments guided by scientific purposes and ethical principles, as well as safeguarding animal rights and preventing harm to animals (11,12).

Extending the Framework from 3Rs to 12Rs

All animal experiments should adhere to the principles of *Replacement*, *Reduction*, and *Refinement* (3Rs) within the framework of bioethics. *Replacement*, as one of these principles, involves prioritizing alternative methods such as in vitro experiments utilizing cell and/or tissue cultures whenever feasible, along with employing phylogenetically lower species (e.g., insects or other invertebrates), and utilizing methods such as computer simulation and inanimate systems whenever possible. *Reduction* entails minimizing the number of subjects by carefully selecting the appropriate number of groups, employing the most suitable experimental design, and conducting accurate statistical analyses. *Refinement* focuses on mitigating the adverse aspects of the method, such as unnecessary pain, suffering, and distress experienced by the animals, while simultaneously enhancing efficiency (9,12).

In the literature, it is evident that in addition to the traditional 3R rules, new guidelines have been introduced and expanded upon, leading to the development of the 12R rules. The initial 3Rs primarily concern animal welfare.

Apart from this, *Respect*, among the R's related to social value, refers to the respect shown to the animal's dignity (care of the animal), its welfare, as well as the rights and privacy of the animal owner or the community. *Responsibility* means the responsibility of the researcher and *Regulations* refers to compliance with applicable national legislation and regulations, as well as directives or standards, legal and/or professional registers. *Reproducibility*, among the R's associated with scientific integrity, pertains to utilizing a robust study design that effectively addresses research inquiries, employs appropriate animal numbers, and employs rigorous statistical analyses to ensure statistical validity. Transparency in reporting and sharing information is also crucial to facilitate reproducibility and enhance the generalizability of data. Moreover, *Relevance* underscores the justification or added value of the research, considering its potential benefits to animals, human health, society, and scientific advancement. *Transferability/Translatability* concerns the applicability and relevance of experimental models, simulations, or representations to real-world scenarios. *Righteousness* represents the intersection of animal welfare and social values in the effort to be fair, good, and worthy scientists and members of society and therefore respectable science. *Reliability* is about the robustness, quality, reliability, applicability, and generalizability of the data produced and the conclusions drawn, embodied in the culture of scientific quality and integrity. *Reckoning* refers to accountability for precautions to be taken during the planning and execution of an animal study and after its conclusion (13).

Determination of Sample Size

When determining the appropriate number of subjects for animal experiments, it is imperative to consider both scientific factors and ethical considerations such as financial constraints, study objectives, data structure, experimental design, and the characteristics of the experimental subjects. Hence, at this stage, it is advisable to adopt the 3Rs, insights gleaned from prior studies or pilot studies, power analysis, sequential sampling, resource equation method, or other strategies informed by practical experience (9,14-17).

Randomization and Blinding

Randomization is a vital procedure in animal studies that guarantees the complete random assignment of animals to experimental groups or conditions. This process can be facilitated using various tools such as computer software programs (e.g., Excel, SPSS, Minitab) or random number generators. Blinding is to ensure that information regarding specific treatments remains confidential to all relevant parties in the experiment who may be consciously or unconsciously influenced by this information. Thus, through the utilization of blinding and randomization, the potential for any conscious or unconscious interferences that could disrupt the experiment can be effectively minimized (2,15).

EXPERIMENTAL DESIGNS AND STATISTICAL ANALYSES

Completely Randomized Design

In a completely randomized design, treatments are allocated entirely at random, ensuring that each experimental unit has an equal opportunity to receive any treatment. Within such designs, any variances observed between experimental units subjected to the same treatment are regarded as experimental errors. This uncomplicated design represents the fundamental framework upon which other experimental designs are built. Noise variables are assumed to affect all treatment groups equally (2,10). In studies involving more than two treatment groups, the model for the completely randomized design is shown in Equation [1]:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad [1]$$

y_{ij} refers to the outcome variable value of the *j*th animal in the *i*th group, μ denotes the grand mean, α_i refers to the main effect of the treatment, and ε_{ij} states the random error term. Classic analysis of variance (ANOVA) can be used to estimate this model (2).

For instance, consider a study aimed at investigating the effect of a drug on blood cortisol levels in rats with Cushing's syndrome. In this study, groups are established with three different doses of the drug, and animals are randomly allocated to each group. It can be asserted that a completely randomized design is well-suited for this investigation.

If a covariate exists within the design, impacting the outcome variable, it is incorporated into the experiment, and techniques for noise reduction are employed. The relevant model is given in Equation [2].

$$y_{ij} = \mu + \alpha_i + \beta\left(x_{ij} - \bar{x}\right) + \varepsilon_{ij} \quad [2]$$

y_{ij} is the outcome variable value of the *j*th animal in the *i*th group, μ is the overall mean, α_i is the main effect of the treatment, β is the slope of the regression line between the covariate and the outcome variable, that is, the effect of the covariate, $\bar{x}$ is the covariate mean, x_{ij} is the covariate value of the *j*th animal in the *i*th group, and ε_{ij} denotes the random error term (2).

For example, in a Cushing's syndrome rat study, the plasma adrenocorticotropic hormone (ACTH) levels of the rats measured before the experiment or the body weights of the animals can be taken as a covariate in the experimental design. Thus, the completely randomized design is transformed into a completely randomized design with covariate.

Furthermore, a covariate can be added to the experiment to remove a confounder whose effect cannot be eliminated with some approaches (standardization or randomization). Thus, the power of the experiment can be increased. When a covariate is removed from the experiment, the amount of unexplained variation will increase, reducing power. In such instances, the covariate included in the experiment can be kept under control by using some statistical analysis such as analysis of covariance (10,18,19). Furthermore, Wang et al. (20) demonstrated the importance of including covariate in experiment to increase statistical power.

Regression Design

Regression design can be used when it comes to examining many different values of a variable, that is, different doses of a drug. By adding a generic f(x) function to the ANOVA linear model, the Equation [3] is obtained (2).

$$y_{ij} = f(D_i) = \beta_0 + \beta_1 D_i + \beta_2 D_i^2 + \varepsilon_{ij} \quad [3]$$

In the regression model, D_i indicates the procedure applied to the *i*th animal, β_0, β_1, and β_2 show the regression coefficients, and ε_{ij} represents the random error term. Regression analysis is used in modeling the outcome variable y_{ij}.

For example, consider a study to investigate the impact of various doses of a drug on cholesterol levels. In this experiment conducted on rats, groups are established with 10 different doses of the drug, with 6 animals randomly allocated to each group. Regression design is suitable for this experiment.

Comparatively, regression design offers several advantages over a completely randomized design. Firstly, it entails lower costs due to the utilization of fewer animals in the experiment. Secondly, the estimated regression model enables the estimation of outcome variable values for intermediate doses, thereby providing valuable insights into dose-response relationships. Thirdly, it can be checked whether each of the estimated regression coefficients is significant (2).

Split-Unit Designs

Split-unit designs are typified by the random assignment of at least two treatment factors to distinct nested unit factors. Usually, one factor may be applied to larger experimental units, while others may be applied to a smaller subset of these units. Experiments incorporating repeated measurements are categorized within this design framework. These include experiments in which an animal is measured multiple times across multiple areas of its

body or across multiple tasks (2,21). The model for a basic split-unit design with two factors is given in Equation [4].

$$y_{ijk} = \mu + \left(\gamma_i + \alpha_j^{(P)} + \eta_{ij}\right) + \alpha_k^{(Q)} + \alpha_{jk}^{(PQ)} + \varepsilon_{ijk} \quad [4]$$

Here, *P* indicates the factor applied to large units (*j*=1,…,*j*), and *Q* indicates the factor applied to small units (*k*=1,…,*k*). y_{ijk} is the outcome variable representing the *j*th *P* treatment and the *k*th *Q* treatment in the *i*th block. This model shows that the measurement is affected by block γ_i, the treatment applied to the large unit ($\alpha_j^{(P)}$), and some noise (η_{ij}) effective at the level of large units. All terms in parentheses indicate contribution to the larger experimental unit. $\alpha_k^{(Q)}$ shows the contribution of split-units given by the *Q* treatment, $\alpha_{jk}^{(PQ)}$ indicates the contribution of the interaction between *P* and *Q* factors, and ε_{ijk} shows the contribution of some noise at the split-unit level. A linear mixed modeling approach can be employed for the analysis of these models (2).

For example, assume an animal experiment to examine the effects of two different diets (high-fat, low-fat) and a drug administered at different doses (D1, D2, D3, D4) on the enzyme. In this experiment, there are 6 cages and 4 mice in each cage. Each mouse within each cage will receive a different dose, while all mice within the same cage will receive the same diet. Thus, each cage serves as a block for different doses and as an experimental unit for diets. Split-unit design can be used for this study.

Hierarchical or Nested Designs

These designs, which are similar to the split-unit design, may not include all possible combinations between factors. It encompasses repeated measurements, wherein the same animal undergoes measurement multiple times, often involving multiple assessments taken from the liver. Additionally, there may be specific time intervals between these measurements (0, 2, 6, 12, 24 hours). Thus, the animal becomes the factor hard to change in the experiment and the animal cannot be given more than one treatment. It is important to define the animal as the block variable in these designs because the animal can greatly influence the results and samples from the same animal are not independent. The model to be created differs depending on the number of factors in the experiment (2). The nested design model with three factors (A, B, C) is given in Equation [5].

$$y_{ijkl} = \mu + \alpha_i + \beta_{j(i)} + \gamma_{l(ij)} + \varepsilon_{ijkl} \quad [5]$$

y_{ijkl} is the *l*th observation value at the *k*th level of factor *C* nested at the *j*th level of factor *B* and the *i*th level of factor *A*. μ is the general average, α_i is the *i*th level of factor *A*, $\beta_{j(i)}$ represents the effect of the *j*th level of factor *B* nested at the *i*th level of factor *A*, $\gamma_{l(ij)}$ indicates the effects of the *i*th level of factor *A*, the *k*th level of factor *C* nested at the *j*th level of factor *B*. ε_{ijkl} represents the random error term. ANOVA can be used to analyze these models.

For example, consider a study examining the impact of a drug on the concentration of a specific protein in the liver. In this study, conducted by two different technicians, there are two distinct groups: experimental and control. Multiple measurements (3 times) will be taken from the liver of each animal, with only one technician examining each animal. Consequently, the technician and the treatment are nested within the animal. A hierarchical design is appropriate for conducting this study.

Mixed Effects Design

The model for this design includes at least one fixed and at least one random effect factors and the interactions between these factors. Fixed effect factors are the type of animal (wild type vs. transgenic), age group of the animal (2 months vs. 6 months vs. 1 year), time of the experiment, diet, supplier, and experimenter performing the observations or operations. Conversely, random effects refer to processes where the specific level of the process is expected to come from a larger population. In such cases, the focus is on understanding the variability of processes rather than their individual contributions. The mixed effects model can be given as in Equation [6] (2).

$$y_{ijk} = \mu + \alpha_i^{(A)} + \alpha_j^{(B)} + \alpha_{ij}^{(AB)} + \varepsilon_{ijk} \quad [6]$$

In this two-way mixed effects model, it is assumed that $\alpha_i^{(A)}$ is normally distributed with a random effect, $\alpha_j^{(B)}$ is a fixed effect, and the interaction $\alpha_{ij}^{(AB)}$ is assumed to be randomly distributed and normally distributed. Linear mixed effects modeling approach is used to analyze these designs.

For example, assume an animal study investigating the effects of different levels of neuro-exercise (no exercise, moderate and intense) and gender (female and male) on neurogenesis. The results of histopathological examination of tissues taken from four different regions of each mouse's brain will be used for evaluation. In this design, neuro-exercise and gender are fixed effects, while the mouse and the sections are random effects, so the results can be examined by creating a mixed-effects design model.

CONCLUSION

The aim is to attain more effective, cheaper, and more reliable results in animal studies. This necessitates planning, executing, and reporting studies within the framework of scientific and ethical principles. During the planning phase, the establishment of hypotheses can be achieved by selecting the appropriate experimental design and statistical analyses. Hence, this review offers valuable insights into the scientific and ethical principles fundamental to animal studies, along with details on various experimental designs and statistical analyses. As such, it is recommended for researchers, as it provides important summative information to guide the effective planning of animal studies.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: ŞC; Design: ŞC; Data Collection/Processing: ŞC; Analysis/Interpretation: ŞC; Literature Review: ŞC; Drafting/Writing: ŞC; Critical Review: ŞC.

REFERENCES

1. Leist M, Hartung T. Inflammatory findings on species extrapolations: humans are definitely no 70-kg mice. Arch Toxicol. 2013;87(4):563-7.
2. Sorzano COS. Statistical experiment design for animal research. OSF Preprints e9s25, Center for Open Science; 2023. doi: 10.31219/osf.io/e9s25.
3. Smith MM, Clarke EC, Little CB. Considerations for the design and execution of protocols for animal research and treatment to improve reproducibility and standardization: DEPART well-prepared and ARRIVE safely. Osteoarthritis Cartilage. 2017;25(3):354-63.
4. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. Lab Anim. 2018;52(2):135-41.
5. Smith AJ. Guidelines for planning and conducting high-quality research and testing on animals. Lab Anim Res. 2020;36:21.
6. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. BMC Vet Res. 2020;16(1):242.
7. de Aguilar-Nascimento JE. Fundamental steps in experimental design for animal studies. Acta Cir Bras. 2005;20(1):2-8.
8. Festing MFW. Experimental design and statistical analysis. In: Hau J, Schapiro SJ, editors. Handbook of laboratory animal science. Volume 1: Essential principles and practices. 3rd ed. Boca Raton: CRC Press; 2011. p.369-400.
9. Cangur S, Ankarali H, Ankarali S. Special experimental designs using laboratory animals and sample size: Review. Turkiye Klinikleri J Biostat. 2013;5(2):75-88. Turkish.
10. Karp NA, Fry D. What is the optimum design for my animal experiment? BMJ Open Sci. 2021;5(1):e100126.
11. Beauchamp TL, Frey RG, editors. The Oxford handbook of animal ethics. New York: Oxford University Press; 2011.
12. Shim J, Kim J. Considerations for experimental animal ethics in the research planning and evaluation process. Kosin Med J. 2022;37(4):271-7.
13. Brink CB, Lewis DI. The 12 Rs framework as a comprehensive, unifying construct for principles guiding animal research ethics. Animals (Basel). 2023;13(7):1128.
14. Festing MF, Baumans V, Combes RD, Halder M, Hendriksen CF, Howard BR, et al. Reducing the use of laboratory animals in biomedical research: Problems and possible solutions. Altern Lab Anim. 1998;26(3):283-301.
15. Festing MFW. How to reduce the number of animals used in research by improving experimental design and statistics. Australian & New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) Fact Sheet 2011;T10:1-11.
16. Ankaralı H, Ankaralı S. Experimental designs and sample sizes for increased efficiency in animal experimentation. Anatol Clin. 2019;24(3):248-59. Turkish.
17. Doğan İ, Doğan N. Estimation of sample size with resource equation method in experimental animal studies. Turkiye Klinikleri J Biostat. 2020;12(2):211-7. Turkish.
18. Ankarali H, Cangur S, Ankarali S. A new Seyhan's approach in case of heterogeneity of regression slopes in ANCOVA. Interdiscip Sci. 2018;10(2):282-90.
19. Cangur S, Sungur MA, Ankaralı H. The methods used in nonparametric covariance analysis. Duzce Med J. 2018;20(1):1-6.
20. Wang YA, Sparks J, Gonzales JE, Hess YD, Ledgerwood A. Using independent covariates in experimental designs: quantifying the trade-off between power boost and type I error inflation. J Exp Soc Psychol. 2017;72:118-24.
21. Kaltenbach HM. Statistical design and analysis of biological experiments. Cham: Springer; 2021.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1504050

Duzce Med J, 2024;26(S1):19-23
Düzce Tıp Fak Derg, 2024;26(S1):19-23

Animal Experiments and Laboratory Safety within the Scope of Occupational Health and Biosafety

İş Sağlığı ve Biyogüvenlik Kapsamında Hayvan Deneyleri Çalışmaları ve Laboratuvar Güvenliği

Münir UÇAK[1,2]
0000-0003-1538-6711

[1]Department of Herbal and Animal Production, Düzce University Düzce Vocational School, Düzce, Türkiye

[2]Beekeeping Research, Development and Application Center, Düzce University, Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Münir UÇAK
ucak.munir01@gmail.com

Received / Geliş Tarihi : 14.05.2024
Accepted / Kabul Tarihi : 14.06.2024
Available Online / Çevrimiçi Yayın Tarihi : 24.06.2024

ABSTRACT

Occupational health and biosecurity terms include scientific approaches regarding ethical values, reliable methods, and a series of precautions to be taken against risks and hazards for employees and the living material being studied. Research and development activities carried out within the scope of biotechnology involve many risks under laboratory conditions, which are their own physical area. Many precautions and practices have been defined to analyze these risks and take precautions before they occur, and laboratory guides have been created in line with these definitions. During studies carried out with experimental animals under laboratory conditions, safe working rules may be violated due to physical conditions, equipment and materials, treatments applied to the subjects, negligence, or faulty practices caused by the researcher or expert. Biosafety in animal experiments consists of a set of ethical conditions and practices declared in the guidelines of specialized laboratories at their own level, protecting employees, subjects, and life outside the laboratory. In this review, possible risks and dangers that may arise at different biosafety levels and the precautions and practices that can be taken against them are evaluated. In this way, it was aimed to contribute to the development of laboratory and biosafety criteria in animal experiments.
Keywords: Occupational health and safety; biosecurity; experimental animals.

ÖZ

İş sağlığı ve biyogüvenlik terimleri çalışanların ve üzerinde çalışma yapılan canlı materyalin etik değerler, güvenilir yöntemler, risk ve tehlikelere karşı alınacak bir dizi önlemlere dair bilimsel yaklaşımları içermektedir. Biyoteknoloji kapsamında gerçekleştirilen araştırma ve geliştirme faaliyetleri, kendi fiziki alanı olan laboratuvar koşullarında pek çok riski bünyesinde barındırmaktadır. Bu risklerin analiz edilerek, oluşmadan önlem alınmasına yönelik birçok önlem ve uygulama tanımlanmış ve bu tanımlar doğrultusunda laboratuvar rehberleri oluşturulmuştur. Laboratuvar koşullarında deney hayvanları ile yapılan çalışmalar sırasında fiziki koşullar, ekipman ve materyaller, deneklere uygulanan tedaviler, ihmaller veya araştırmacı veya uzmandan kaynaklanan hatalı uygulamalar nedeniyle güvenli çalışma kuralları ihlal edilebilmektedir. Hayvan deneyleri çalışmalarında biyogüvenlik ise kendi seviyesinde özelleşmiş laboratuvarların kılavuzlarında bildirilen, çalışanları, denekleri ve laboratuvar dışındaki yaşamı koruma altına alan bir dizi etik şart ve uygulamalarından ibarettir. Bu derlemede genel olarak farklı biyogüvenlik seviyelerinde ortaya çıkabilecek olası risk ve tehlikeler ile bunlara karşı alınabilecek önlem ve uygulamalar değerlendirilmiştir. Bu sayede, hayvan deneyleri çalışmalarında laboratuvar ve biyogüvenlik kriterlerinin geliştirilmesine katkı sağlaması amaçlanmıştır.
Anahtar kelimeler: İş sağlığı ve güvenliği; biyogüvenlik; deney hayvanları.

INTRODUCTION

As a social living being, humans have been carrying out many activities to meet their individual and community needs since their existence on earth. These activities reveal scientific and technological specializations according to the needs of the period, and this creates the basis for job and employee differentiation according to the power and technical capacities of individuals. However, these resulting specializations and differentiations have caused tragic work accidents, occupational diseases, poisoning, and deaths since the early periods of humanity.

The concept of occupational health and safety (OHS) comes from Hippocrates (460-370 BC), who detected lead poisoning in workers: "The cost of obtaining precious metals such as gold and silver; all substances are poison". There is no substance that is not poisonous. It emerged until Paracelsus (1493-1541) with his words "The appropriate dose reveals the difference between poison and medicine", and until Ramazzini (1), the founder of OHS, with his approach "Ask patients about their profession". Following the Industrial Revolution, there were historical cases such as diseases seen in workers, anatomical and physiological damages, necrotic wounds caused by chemical contamination, chimney sweep child workers, and London fires (2-4). These cases and the legal processes experienced during the period paved the way for the formation of today's occupational and worker health rules and organizations in the fields of health and work on an international scale World Health Organization (WHO), International Labor Organization (ILO) (5-7).

Today, OHS has begun to find a place for itself in every field by specializing in various branches of science in parallel with developments in technique and technology. Among the most prominent of these fields is biotechnology. "Biosafety" rules have been established to ensure the safety of researchers and technical personnel working in this field, as well as to keep the risk and stress factors of experimental animals used in research under control. In this context, OHS is a systematic and scientific study carried out to protect against conditions that may harm health arising from various reasons during the execution of work in the workplace. Biosecurity is defined as control principles, technologies, and practices applied to prevent exposure to biological agents for any reason or their uncontrolled release (8). Within the framework of both definitions, the field of study of the term biosecurity refers to all measures aimed at detecting the possible risks that the biological agents, living materials, and applied techniques used in modern biotechnology applications may pose to humans, animals, and other living and inanimate environments, and solving or controlling the problem at its source.

Research and development activities carried out within the scope of biotechnology involve many risks under laboratory conditions, which are their own physical area. Many precautions and practices have been defined to analyze these risks and take precautions before they occur, and laboratory guides have been created in line with these definitions.

This review aims to evaluate possible risks and dangers that may arise at different biosafety levels and the precautions and practices that can be taken against them with the purpose of contributing to the development of laboratory and biosafety criteria in animal experiments.

BIOSAFETY LEVELS AND RISK GROUPS

WHO (9) and US Centers for Disease Control and Prevention (CDC) animal biosafety level (ABSL) guide was created and possible risks were grouped under four groups.

Risk Group 1

In this group, there is no individual or social risk or the probability of occurrence is very low. This level of microorganism is unlikely to cause human or animal diseases.

Risk Group 2

In this group, there is a moderate individual risk and a low social risk. A pathogen that can cause human or animal disease but is unlikely to pose a serious hazard to laboratory workers, the public, livestock, or the environment. Laboratory exposures can cause serious infection, but effective treatment and preventive measures are available and the risk of spreading the infection is limited.

Risk Group 3

In this group, there is high individual risk and low social risk. A pathogen that usually causes serious human or animal disease but does not normally spread can be transmitted from one infected person to another. Effective treatment and preventive measures are available.

Risk Group 4

There is a high individual and social risk in this group. An easily cleared pathogen that usually causes serious human or animal disease is transferred directly or indirectly from one person to another. Effective treatment and preventive measures are often not available (Table 1).

Table 1. Characteristics of animal facilities and laboratory safety practices (9)

Risk Group	Biosafety Level	Laboratory Practices and Safety Equipment
1	ABSL-1	Restricted entry, protective clothing, and gloves
2	ABSL-2	In addition to ABSL-1 applications, danger warning signs should be used. Class 1 or 2 BSC should be used for activities that generate aerosols. Waste and cages should be decontaminated before washing.
3	ABSL-3	In addition to ABSL-2 applications, controlled entry. Special protective clothing must be worn for BSCs and all activities.
4	ABSL-4	In addition to ABSL-3 applications, full limited entry. Change of clothes before entering. Class 3 BSCs or positive pressure suits. You must take a shower upon exit. Decontamination of all waste before removal from the facility.

ABSL: animal biosafety level, BSC: biological safety cabinet

PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) includes wearable equipment worn by personnel to provide an additional barrier between personnel and biological agents under investigation, reducing the risk of possible exposure (10-13). Laboratory coats, gloves, various laboratory masks, caps, shoe covers, etc. materials are among the basic PPE. Basic protective equipment varies and specializes according to the job and risk definitions of the employees, laboratory safety levels, and the biological material to be used in the experiment (14,15).

It is an enclosed, ventilated work area designed to provide protection to the user, laboratory environment, and/or work materials during aerosol hazard activities. Containment is achieved by separating the work from the main area of the laboratory and/or by using controlled, directional airflow mechanisms. Exhaust air is passed through a high-efficiency particulate air filter (HEPA) before being recirculated to the laboratory or building's heating, ventilation, and air conditioning system. There are different types (Type I, II, and III) of biological safety cabinets (BSCs) that provide different levels of protection.

BSC Type I

They are open-fronted cabinets with inward airflow, designed to protect the user and the environment from infectious aerosols generated during operation. In the cabin that uses room air, the air passing through a HEPA filter is released back into the environment.

BSC Type II

These cabinets, which have a more complex structure than BSC Type I cabinets, are open at the front and are in contact with the room air. However, differently, the air taken into the cabin first passes through the HEPA filter, and the air in the cabin is released into the environment by passing through a second HEPA filter before being released to the outside. This cabin, which is used especially in cell culture studies, is important in terms of protecting the health of employees and the environment. It can be affected by environmental factors such as its orientation in the room in which it is located, air flow in the room, and pressure changes.

BSC Type III

Unlike other cabins, the front is closed and there is a separation between the user and the research environment and material. The researcher works with arm-length gloves made of rubber integrated into the system. Airflow is provided by a special exhaust system outside the cabin. This system keeps the interior of the cabin under negative pressure compared to the surrounding area. The air taken from the HEPA filter passes into the working environment and passes through a second HEPA filter before being released (8,16,17).

SAFE WORKING WITH EXPERIMENTAL ANIMALS

Laboratory animals live under the influence of environmental factors such as temperature, humidity, airflow, ventilation degree, suspended dust particles in the air, and noise under the environmental conditions they live in. In addition, experimental animals are under the influence of many stress factors, such as breeding, transportation, and care. In order to obtain reliable and sustainable results, genetically and microbiologically defined pedigrees are needed, as well as having a good laboratory animal laboratory. Moreover, infectious diseases in experimental animals must be kept under control and safe conditions must be provided for researchers and other employees (18-22).

Biological risk assessment is a key factor in safe laboratory work. Risk assessment requires careful decision-making and is an important responsibility for managers of microbiology and biomedical laboratories and principal investigators. Institutional structures such as Institutional Biosafety Committees or equivalent units, animal care and use committees, biological safety specialists, occupational health personnel, and laboratory animal veterinarians share responsibility. When assessing the risk, previous incidents should be taken into consideration, and criteria regarding trust conditions should be determined. Biosafety guides that include risk management should be created under the responsibility of the laboratory manager in line with the determined criteria. Creating these guidelines alone is not sufficient; it is of great importance to implement them and transmit them as a biosafety culture among working generations.

The main risks that may arise when safe conditions are partially or completely eliminated are classified as follows (21,23-25):

- Direct skin, eye, or mucosal membrane exposure to an agent
- Bites from a contaminated laboratory sharps instrument or infected animals and arthropod vectors
- Ingestion of a liquid suspension of an infectious agent or exposure from contaminated hand-to-mouth
- Inhalation of infectious aerosols

The dangers arising from these phenomena, called risks, can be grouped under five main headings (21,26,27):

- **Biting and scratching:** Acting outside the rules of working with experimental animals or dangers arising from an unforeseen reason.
- **Allergenic effect:** Body fluids and secretions of laboratory workers' experimental animals, hair, feathers, hides, etc. their sensitivity to the material.
- **Hazards specific to the experiments:** Piercing-cutting, contamination-toxic, etc. used in the experiments. Hazards arising from the material.
- **Natural hazards:** These are the dangers arising from the violation of rules and carelessness of employees / falling, ergonomic deficiencies, noise, etc.
- **Zoonoses:** Diseases that can pass between humans and animals/hantavirus, Salmonella, etc.

SAFE WORKING WITH ARTHROPODS

As with vertebrates, the biosecurity level of the animal facility where research on arthropods is conducted is determined by taking into account the biosecurity element and risk groups subject to research. However, additional precautions can be taken for some arthropods that have the ability to fly. The basic precautions that must be taken for safe work in laboratories where arthropod experiments are carried out can be listed as follows (12,25,28-30):

- Separate chambers should be provided for infected and non-infected invertebrates.
- The rooms must have sufficient technology to seal the environment against fumigation.
- Insecticide sprays should be available.

- "Cooling" facilities should be available to reduce invertebrate activity when necessary.
- Access should be provided through a room entrance equipped with insect traps and arthropod-proof curtains on the doors.
- All ventilation ducts and openable windows must have arthropod-proof surfaces.
- Waste catchers in sinks and weirs should not be allowed to dry out.
- All waste should be purified by autoclaving, as some invertebrates may be resistant to disinfectants.
- The numbers of larval and adult forms of flying, crawling, and jumping arthropods should be checked.
- Live storage containers used for ticks and mites should be placed in oil trays.
- Infected or potentially infected arthropods and flying insects should be kept in double-mesh cages.
- Infected or potentially infected arthropods should be handled in biological safety cabinets or isolators.
- Infected or potentially infected arthropods may be restricted in their movement on the arthropod cooling tray.

CONCLUSION

During studies carried out with experimental animals under laboratory conditions, safe working rules may be violated due to physical conditions, equipment and materials, treatments applied to the subjects, negligence, or faulty practices caused by the researcher or expert. "Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees" was published in the Official Gazette dated 15 February 2014 and numbered 28914, in order to prevent these negligence and violations, to establish the basis of practices based on ethical principles on employee safety and laboratory animals, and to determine their standards. In this regulation, the central ethics committee for animal experiments and local ethics committees for animal experiments were defined, criteria for safe working with laboratory animals were determined, and the way was opened for organizing certified training through laboratory animal use courses. Correct studies are to be applied to experimental animals in courses carried out in accredited laboratories that have permission to work with laboratory animals.

In the courses held in accredited laboratories that have permission to work with experimental animals, the correct working methods to be applied to laboratory animals (gavage, disease models, etc.), as well as the risks and dangers that these methods may carry within the framework of OHS rules, are evaluated.

The data obtained from all these studies constitute the infrastructure of biosafety laboratory manuals according to their levels. However, this infrastructure is not stable and is open to rapid changes under the influence of modern techniques and scientific schools. For this reason, it is inevitable that more advanced occupational health and biosafety criteria and practices, which can develop and change rapidly in the light of science and are specialized in different fields, will be needed in the near future.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: MU; Design: MU; Data Collection/Processing: MU; Analysis/Interpretation: MU; Literature Review: MU; Drafting/Writing: MU; Critical Review: MU.

REFERENCES

1. Ramazzini B. Opera omnia medica et physiologica. Neapoli: Expensis Josephi Ponzelli, P. & I. Vaillant; 1739.
2. Wood JR. Removal of the entire lower jaw. New York; Royal College of Surgeons of England; 1857.
3. Garrioch D. 1666 and London's fire history: A re-evaluation. Hist J. 2016;59(2):319-38.
4. Zengin M. William Blake's "London": A Resistance to Power and Authority. AÜ DTCF Derg. 2014;54(1):117-48.
5. International Labour Organization. Guide to international labour standards and rights at work concerning young people. Geneva: International Labour Organization; 2017.
6. Horozoğlu, K. The analysis of work accident in terms of workplace safety. Karabük Üniversitesi Sosyal Bilimler Enstitüsü Dergisi, 2017;7(1):265-81. Turkish.
7. Nurdoğan AK. The International Labor Organization (ILO) centenary and Turkey relations. BEÜ AİD. 2018;3(4):78-95. Turkish.
8. World Health Organization. Laboratory biosafety manual, 4th ed. Geneva: World Health Organization; 2020.
9. World Health Organization. Laboratory biosafety manual, 3rd ed. Geneva: World Health Organization; 2004.
10. Barry M, Russi M, Armstrong L, Geller D, Tesh R, Dembry L, et al. Brief report: treatment of a laboratory-acquired Sabiá virus infection. N Engl J Med. 1995;333(5):294-6.
11. World Health Organization. Personal protective equipment. Geneva: World Health Organization; 2020.
12. World Health Organization. Biological safety cabinets and other primary containment devices. Geneva: World Health Organization; 2020.
13. Sejvar JJ, Johnson D, Popovic T, Miller JM, Downes F, Somsel P, et al. Assessing the risk of laboratory-acquired meningococcal disease. J Clin Microbiol. 2005;43(9):4811-4.
14. Karaman M. Basic laboratory safety and general survey of the Turkish laboratory safety status. J Clin Anal Med. 2011;2(3):130-4. Turkish.
15. Pace D. Quadrivalent meningococcal ACYW-135 glycoconjugate vaccine for broader protection from infancy. Expert Rev Vaccines. 2009;8(5):529-42.

16. Kruse RH, Puckett WH, Richardson JH. Biological safety cabinetry. Clin Microbiol Rev. 1991;4(2):207-41.
17. Centers for Disease Control and Prevention. Primary contaminment for biohazards: Selection, installation and use of biological safety cabinets, 2nd ed. U.S. Department of Health and Human Services; 2000.
18. Yamauchi C. Studies on the environmental control of laboratory animals. Exp Anim. 1995;44(1):9-21.
19. Gürkan EH. Sustainable laboratory safety culture. CBU-SBED. 2018;5(4):224-30. Turkish.
20. Ergün Y. Ethics in animal experiments. Arşiv. 2010;19(4):220-35. Turkish.
21. Luneva AV, Lysenko YA, Gneush AN, Shantyz AY, Simonov AN, Verevkina MN, et al. Assessment of the biosafety of microorganisms and their joint composition. Pharmacophore. 2021;12(3):42-8.
22. Gül Y, İssi M, Gül Baykalır B. Eradication of medical waste and zoonotic diseases, biosafety in research laboratories. Atatürk Üniversitesi Vet Bil Derg. 2013;8(1):81-96. Turkish.
23. Siegel JD, Rhinehart E, Jackson M, Chiarello L, the Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings. Centers for Disease Control and Prevention; 2007.
24. Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories, 5th ed. U.S. Department of Health and Human Services; 2009.
25. Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories, 6th ed. U.S. Department of Health and Human Services; 2020.
26. World Health Organization. Laboratory biosafety manual, 1st ed. Geneva: World Health Organization; 1983.
27. Wurtz N, Papa A, Hukic M, Di Caro A, Leparc-Goffart I, Leroy E, et al. Survey of laboratory-acquired infections around the world in biosafety level 3 and 4 laboratories. Eur J Clin Microbiol Infect Dis. 2016;35(8):1247-58.
28. Akşit A, Erdoğan E, Karaca S, Özkan B, Gürgel A, Yürük M, et al. Biosafety principles in parasitology laboratories. J Immunol Clin Microbiol. 2019;4(4);117-51.
29. Furr AK. CRC handbook of laboratory safety, 5th ed. Boca Raton, FL: CRC Press; 2000.
30. World Health Organization. Laboratory biosafety manual, 2nd ed. Geneva: World Health Organization; 1993.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1504013

Duzce Med J, 2024;26(S1):24-29
Düzce Tıp Fak Derg, 2024;26(S1):24-29

Technological Processes Applied to Laboratory Animal Feeds and New Feeding Approaches

Laboratuvar Hayvanı Yemlerine Uygulanan Teknolojik İşlemler ve Yeni Besleme Yaklaşımları

Atakan TEPE
0000-0001-5497-6234
Tolga ALTAŞ
0000-0001-6267-2118

Department of Veterinary, Düzce University Çilimli Vocational School, Düzce, Türkiye

ABSTRACT

Laboratory animal nutrition plays a crucial role in ensuring the health, welfare, and scientific validity of research studies involving animals. Technological advancements in feed processing have provided researchers with innovative tools and techniques to enhance the nutritional quality and stability of laboratory animal diets. Recent developments in feed processing technology have focused on improving the precision, consistency, and nutrient bioavailability of laboratory animal diets. Techniques such as pelleting, extrusion, and coating have been utilized to create homogeneous feed formulations with controlled nutrient profiles. In addition to advances in feed processing, new feeding approaches have emerged to address the specific nutritional requirements of laboratory animals. Precision feeding technologies, incorporating real-time monitoring systems and data analytics, allow for the customization of feed formulations based on individual animal needs. Overall, the integration of technological processes and new feeding approaches in laboratory animal nutrition represents a promising avenue for advancing animal welfare, research quality, and scientific outcomes in preclinical and biomedical research. By leveraging cutting-edge feed processing techniques and tailored feeding strategies, researchers can ensure the optimal nutrition, health, and well-being of laboratory animals, fostering both ethical research practices and robust scientific results. This review provides an overview of the technological processes applied to laboratory animal feeds and introduces new feeding approaches aimed at optimizing animal health and research outcomes.
Keywords: Laboratory animal feeding; technological processes; feeding models.

ÖZ

Laboratuvar hayvanlarının beslenmesi, hayvanları içeren araştırma çalışmalarının sağlık, refah ve bilimsel geçerliliğinin sağlanmasında çok önemli bir rol oynamaktadır. Yem işlemedeki teknolojik gelişmeler, araştırmacılara laboratuvar hayvanı diyetlerinin besin kalitesini ve stabilitesini artırmak için yenilikçi araçlar ve teknikler sağlar. Yem işleme teknolojisindeki son gelişmeler, laboratuvar hayvanı diyetlerinin hassasiyetini, tutarlılığını ve besin biyoyararlılığını artırmaya odaklanmıştır. Kontrollü besin profillerine sahip homojen yem formülasyonları oluşturmak için peletleme, ekstrüzyon ve kaplama gibi teknikler yaygın olarak kullanılmaktadır. Yem işlemedeki ilerlemelere ek olarak laboratuvar hayvanlarının özel beslenme gereksinimlerini karşılamak için yeni besleme yaklaşımları ortaya çıkmıştır. Gerçek zamanlı takip sistemlerini ve veri analitiğini birleştiren hassas besleme teknolojileri, yem formülasyonlarının bireysel hayvan ihtiyaçlarına göre özelleştirilmesine olanak tanımaktadır. Genel olarak, laboratuvar hayvanlarının beslenmesinde teknolojik süreçlerin ve yeni besleme yaklaşımlarının entegrasyonu, klinik öncesi ve biyomedikal araştırmalarda hayvan refahını, araştırma kalitesini ve bilimsel sonuçları geliştirmek için umut verici bir yolu temsil etmektedir. Araştırmacılar, en ileri yem işleme tekniklerinden ve özel beslenme stratejilerinden yararlanarak, hem etik araştırma uygulamalarını hem de doğru bilimsel sonuçları destekleyerek laboratuvar hayvanlarının beslenmesini, sağlığını ve refahını sağlayabilmektedirler. Bu derleme, laboratuvar hayvan yemlerine uygulanan teknolojik işlemlere genel bir bakış sunmakta ve hayvan sağlığını ve araştırma sonuçlarını optimize etmeyi amaçlayan yeni besleme yaklaşımlarını ele almaktadır.
Anahtar kelimeler: Laboratuvar hayvanı besleme; teknolojik işlemler; besleme modelleri.

Corresponding Author
Sorumlu Yazar
Tolga ALTAŞ
tolgaaltas@duzce.edu.tr

Received / Geliş Tarihi : 03.05.2024
Accepted / Kabul Tarihi : 06.06.2024
Available Online /
Çevrimiçi Yayın Tarihi : 24.06.2024

INTRODUCTION

Laboratory animal feeds play a crucial role in providing essential nutrients for the health and well-being of animals used in research settings. These specialized feeds are carefully formulated to meet the specific nutritional requirements of different species of laboratory animals, ensuring their growth, reproduction, and overall health are adequately supported (1).

Laboratory animals have unique dietary needs based on their species, age, weight, and health status. Properly formulated feeds provide the necessary balance of proteins, carbohydrates, fats, vitamins, minerals, and other nutrients to meet these requirements (1). Feed manufacturers adhere to strict quality control measures to ensure the consistency and safety of laboratory animal feeds. This includes sourcing high-quality ingredients, monitoring production processes, and conducting quality assurance tests (2).

Different species of laboratory animals, such as rodents, rabbits, and non-human primates, require feeds with specific nutrient compositions tailored to their physiological and metabolic characteristics. Specialized formulations may include ingredients like amino acids, fiber, and micronutrients (1,2).

Laboratory animal feeds are essential components in biomedical research and play a crucial role in maintaining the health, well-being, and scientific integrity of laboratory animals (2). Proper attention to the formulation, quality control, and specific nutritional requirements of these feeds is vital to ensure the reliability and validity of research outcomes.

This review aims to provide an overview of the technological processes applied to laboratory animal feeds and introduces new feeding approaches aimed at optimizing animal health and research outcomes.

IMPORTANCE OF TECHNOLOGICAL PROCESSES IN ENHANCING ANIMAL FEED QUALITY

Technological processes play a significant role in the production of high-quality animal feeds by improving digestibility, nutrient absorption, and overall feed efficiency. These processes encompass various methods and techniques, such as grinding, mixing, pelleting, and coating, that contribute to enhancing the nutritional value and palatability of the feeds (1,3).

Technological processes such as grinding and pelleting break down feed ingredients into smaller particles, increasing the surface area available for enzymatic action and improving nutrient absorption in animals. Coating and flavoring processes can improve the taste and aroma of feeds, making them more appealing to animals and encouraging adequate feed intake, which is crucial for meeting their nutritional requirement (4). Precision in feed processing techniques, such as accurate mixing and pelleting, can reduce feed wastage and ensure that animals consume a balanced diet without selective feeding.

Technological processes in feed production are essential for optimizing feed quality and ensuring that animals receive the necessary nutrients for growth, health, and performance. By implementing advanced processing techniques, feed manufacturers can enhance the nutritional value, consistency, and palatability of animal feeds, leading to better overall feed efficiency and animal well-being (4,5).

TECHNOLOGICAL PROCESSES IN PRODUCTION

Grinding and mixing are essential processes in animal feed production that impact feed quality, nutrient availability, and animal performance. The efficiency and precision of these techniques can significantly influence the digestibility, palatability, and overall effectiveness of the feed in meeting the nutritional requirements of animals (6).

Grinding Techniques

Grinding is a mechanical process used to reduce feed ingredients into smaller particles, thereby increasing surface area for enzymatic action and improving nutrient accessibility. Various grinding methods, such as hammer mills, roller mills, and attrition mills, are employed in feed production to achieve the desired particle size distribution and consistency (1).

Mixing Processes

Mixing involves blending different feed ingredients to create a uniform and balanced feed formulation. Proper mixing ensures that nutrients are distributed evenly throughout the feed, minimizing nutrient segregation and reducing the risk of animals selectively consuming certain components of the feed. Techniques like horizontal and vertical mixers are commonly used in feed mills to achieve thorough mixing (1).

Efficient grinding and mixing processes are crucial for maintaining feed quality and optimizing nutrient utilization by animals. Properly ground feed particles and uniform feed mixtures contribute to improved feed intake, digestion, and absorption of nutrients. In contrast, inadequate grinding or mixing can lead to feed wastage, reduced feed efficiency, and compromised animal health (4,6). Grinding and mixing techniques are fundamental processes in animal feed production, influencing feed quality, nutrient availability, and animal performance. By adopting effective grinding methods and thorough mixing practices, feed manufacturers can optimize feed formulation, enhance nutrient utilization, and support the health and well-being of animals. Continued research and innovation in grinding and mixing technology are essential for advancing animal nutrition (7).

Pelleting and Extrusion Methods

Pelleting and extrusion are advanced processing techniques used in animal feed production to enhance feed quality, digestibility, and overall performance of animals. These methods involve the compression, heating, and shaping of feed ingredients to create pellets or extruded products that offer numerous benefits in terms of nutrient preservation, feed efficiency, and animal health (8).

Pelleting Process

Pelleting is a mechanical process that involves compressing feed ingredients into cylindrical pellets, often using heat, moisture, and pressure to facilitate binding and shaping. Pelleted feeds are known for their improved palatability, reduced dustiness, and enhanced nutrient bioavailability compared to mash feeds. The pelleting process can result in increased feed intake, digestive efficiency, and growth performance in animals due to the optimized nutrient delivery and physical characteristics of the pellets (1).

Extrusion Technique

Extrusion is a thermal and mechanical process that involves passing feed ingredients through a high-pressure extruder, where they undergo heat, pressure, and shear forces to form expanded, cooked pellets or kibbles. Extruded feeds are distinguished by their increased

digestibility, deactivation of anti-nutritional factors, and improved starch gelatinization, making them highly digestible and bioavailable to animals. Extrusion can enhance the nutritional value and functional properties of feed ingredients, promoting better growth, feed conversion, and nutrient utilization in animals (1).

Pelleting and extrusion methods offer several advantages in animal feed production, including improved feed digestibility, reduced ingredient segregation, enhanced pellet durability, and decreased microbial contamination. By processing feed ingredients through pelleting or extrusion, feed manufacturers can achieve greater feed consistency, nutrient retention, and performance benefits for animals, contributing to overall feed efficiency and health outcomes (9,10).

Coating and Encapsulation Processes

Coating and encapsulation are advanced techniques used in animal feed production to improve feed quality, palatability, nutrient retention, and targeted delivery of bioactive compounds (11). These processes involve applying protective coatings or encapsulating active ingredients around feed particles to enhance stability, bioavailability, and performance benefits for animals.

Coating Techniques

Coating in feed production involves applying a protective layer of encapsulating material around feed particles to enhance stability, reduce nutrient degradation, and improve palatability. Coatings can be made from various materials, including lipids, polysaccharides, proteins, and synthetic polymers, to provide a barrier against moisture, oxidation, and other destabilizing factors. Coating feed ingredients can help mask undesirable tastes or odors, increase feed acceptance, and ensure the targeted release of nutrients in the digestive tract (11,12).

Encapsulation Methods

Encapsulation is a process that involves encapsulating active ingredients, such as vitamins, minerals, probiotics, or enzymes, within a protective shell or matrix to enhance their stability, bioavailability, and targeted delivery. Encapsulated ingredients are protected from environmental factors, such as heat, moisture, and pH fluctuations, ensuring their efficacy and controlled release in the digestive system. Encapsulation can improve the performance of sensitive nutrients and functional additives in animal feeds, leading to enhanced nutrient utilization and health benefits for animals (12).

Coating and encapsulation processes offer several advantages in animal feed production, including improved nutrient retention, reduced nutrient interaction, enhanced stability of sensitive compounds, and controlled release properties. By utilizing coating and encapsulation techniques, feed manufacturers can tailor feed formulations to meet specific nutritional requirements, enhance feed efficiency, and optimize animal performance (13). Coated and encapsulated feeds can provide targeted nutrition, support gut health, and improve nutrient absorption in animals.

INNOVATIONS IN LABORATORY ANIMAL FEED TECHNOLOGY

Several advancements have been made in laboratory animal feed technology to enhance the nutritional quality, palatability, and digestibility of feeds. Formulations are tailored to meet the specific requirements of different species, strains, and research purposes. Microencapsulation techniques have been utilized to protect sensitive nutrients and ensure their stability during storage (14). Additionally, the use of probiotics and prebiotics in animal feeds has gained popularity to promote gut health and improve overall well-being. In recent years, efforts have been made to reduce the environmental impact of animal feed production by incorporating sustainable and alternative protein sources. These include insect-based proteins, algae-based ingredients, and plant-based alternatives, which not only provide a sustainable source of nutrients but also offer health benefits to laboratory animals (15). Furthermore, advancements in precision nutrition have enabled researchers to customize feed formulations based on individual animal requirements and research goals. This personalized approach ensures that laboratory animals receive optimal nutrition for their specific needs, leading to improved research outcomes and animal welfare (15,16).

Innovations in laboratory animal feed technology have significantly improved the nutritional quality, sustainability, and personalized approach to feeding laboratory animals. These advancements play a crucial role in ensuring the health, well-being, and research reliability of laboratory animals (16). Continued research and development in this field are essential to further optimize animal nutrition and welfare in scientific research settings.

NEW STRATEGIES IN THE NUTRITION OF LABORATORY ANIMALS

In the nutrition of laboratory animals, the content and quality of feeds, and appropriate feeding programs are very important and directly affect animal health and welfare. Accordingly, with good and proper nutrition, the metabolism and physiological systems of animals will function normally and the desired real data can be obtained in experimental studies. Otherwise, the findings obtained as a result of the experiments will be erroneous and the reliability of the study will decrease. For almost 40 years, the scientific community has taken action to control environmental factors that contribute to variation, and because of the contribution of laboratory animals to scientific studies, the nutrition of laboratory animals has been recognized as an important element. Over the past years, various individuals and scientific institutions have developed various guidelines. These guidelines aimed to improve the quality of research by standardizing the selection, use, and reporting of diets used for research animals. One of these is the work of Knapka et al. (17), a laboratory animal nutritionist, who, in the early 1970s, initiated a standardization program and formulated the first "open formula" formula, aiming to achieve standardization in laboratory animal diets.

As a result of these and other studies, the Central Laboratory Animal Diet Advisory Committee supported the use of 'standard reference diets' in biomedical research as an idea to improve the ability to replicate research. As a result, fixed-formula laboratory animal diets (AIN76) were formulated. In 1993, the AIN93 Growth and AIN93 Maintenance diets were subsequently formulated (18). During this period, the AIN93 re-emphasized the need to standardize experimental laboratory animal diets so that intrinsic variation could be reduced.

Around the same time, laboratory animal nutritionists began formulating open-formula, natural-ingredient experimental animal diets to meet the need for standardized laboratory animal diets. With the development of open-formula diets, the fixed-formula, fixed-nutrient-concentration, closed-formula, and natural-ingredient diets were developed to reduce the potential variation that diet could cause in research.

Open Formula Diets

In open-formula diets, the concentrations of all ingredients are publicly available. Open-formula diets also enable a retrospective analysis of possible diets. New open-formula diets are being formulated and made available as needed. For example, the NIH31 open formula diet is an autoclavable rodent diet, formulated in response to requests from the NIH Animal Research Center. However, when the diet is autoclaved, minimal starch gelatinization occurs, so problems with pellet clumping and increased hardness can occur.

Closed Formula Diets

Commercial diets manufactured and marketed under seller trade names are typically 'closed formula diets' and proprietary products. While ingredients are listed, quantitative ingredient formulation is not specified. Therefore, ingredient composition may vary. Differences in formulation may occur feed manufacturers prefer closed formula diets to achieve the 'least cost' formulation. Least-cost strategy refers to formulating diets that maximize profit by using the least costly ingredients.

Fixed Formula Diets

In a fixed-formula diet, the quantitative ingredient formulation does not change. For open-formula diets, the terms fixed-formula and open-formula are mistakenly thought to be synonymous, as quantitative formulations do not change. Both fixed- and open-formula diets may occasionally require changes in nutrient composition or formulation to meet changing nutrient requirements. However, while changes to the quantitative ingredient formulation are publicly disclosed when open-formula diets are changed, information on changes to the fixed closed formula is diet-specific and therefore not publicly disclosed.

Constant Nutrition Diets

Constant nutrition is a trademarked expression of PMI Nutrition International and describes laboratory animal diets (LabDiets) for which the concentrations of known nutrients and ingredient groups remain constant. However, the quantitative content of the formulations of constant nutrition diets may be changed without public disclosure. Changes in diet formulation may alter undefined nutrients or dietary components such as fat.

The ability to replicate research is essential for science. One key to replicating research is to control all variables, i.e. to reduce variation. To meet this requirement, microbiological and genetic characterization of laboratory animals has become increasingly well-defined over the years. Health status and environmental factors (e.g. feeding, bedding, light cycles, noise, humidity, temperature, and staff interaction with animals) are factors that can affect research. They should be controlled as far as possible. Diet in laboratory animals is an important environmental factor affecting reproduction, growth, disease, and experimental manipulation.

A new and comprehensive approach to biological research is also developing, namely systems biology. This "systems biology" refers to how all relevant components of a biological drug interact functionally over time and under changing conditions. New technologies such as genomics, proteomics, metabolomics, and nutrigenomics are emerging and being used to advance the systems biology approach (19). These technologies are influenced by both intrinsic and environmental factors.

Progress in the battle against human disease and suffering is accelerating with the availability of genomic information for humans, mice, and other organisms. The techniques and knowledge emerging from these genome projects have reinvigorated the process of locating and identifying genes involved in disease. To date, approximately 1,000 human disease genes have been identified and partially characterized, 97% of which are known to cause monogenic diseases (20). However, most cases of obesity, cardiovascular disease, diabetes, cancer, and other chronic diseases are caused by complex interactions between various genes and environmental factors. It is therefore not surprising that strategies to characterize and identify monogenic diseases fail when applied to chronic diseases. Despite more than 600 association studies published since 2002, the molecular basis of chronic diseases is still not understood. Such results have led to the development of the "common disease/common variant hypothesis", which states that chronic diseases are caused by clusters of gene variants that collectively contribute to disease onset and development (19).

Nutrition is the most important environmental factor affecting the health and productivity of animals. Traditional research in the field of animal nutrition has concentrated on the components that, in excess or deficiency, affect the health and productivity of animals. In recent years, due to the advances in molecular genetics, increasing knowledge about the composition and functions of genomes has begun to be transferred to practice. These advances have allowed us to understand how nutrients alter gene and protein expression and how they affect cell and organismal metabolism (21). The term "nutrigenomics" was first used by DellaPenna (22) as a branch of science dealing with the role of nutrients in gene expression. Nutrigenomics or nutritional genomics, which was later defined by various researchers, can be considered as a combination of molecular genetics and genomics in the fields of health, nutrition, and genomics.

The basic approach of this new field of research is that common food chemicals may directly or indirectly affect the genome, altering gene expression or structure, that nutrition may be a risk factor in some individuals under certain conditions, and that genes regulated by nutrition are likely to influence the onset, impact and progression of various chronic diseases, the magnitude of the effect of nutrition on the balance of health and disease states depends on the genetic makeup of the individual, and that nutrition based on information about nutrient needs, nutritional level and genotype (i.e. individualized nutrition) can be used to prevent, mitigate and treat chronic diseases (19). In line with these assumptions, the goal of nutrigenomics is to find nutritional practices that are appropriate for each individual's genetic profile to optimize their health and productivity (23).

A number of new dietary models are emerging in the modeling phase of feeding experimental animals;

Obesity diets and high-fat diets; are 24% fat, 35% fat, and 45% fat diets. Diets in which vegetable or animal fats are used as fat sources according to the choice of model.

Metabolic disorder diet; also known as purified-purified diet.

Diabetes-forming diets; which are also obesity diets, are diets that are lower in protein and higher in carbohydrates than high-fat diets.

Atherosclerosis diets; are diets that appropriately raise blood cholesterol and other blood fats, unlike high-fat diets, also known as hyperlipidemia, diets with the addition of cholesterol and cholic acid.

Diets that cause fatty liver disease; are methionine- and choline-poor diets and contain animal fat. Variations of high-fat diets and methionine- and choline-deficient diets are used to study different stages of NAFLD/NASH, depending on the type of diet and feeding duration.

High-carbohydrate diets; diets high in carbohydrates along with fat, are the closest dietary group to the human diet. Such diets cause metabolic disorders in rats and mice. They are diets with a carbohydrate ratio of 25-70%.

Ketogenic diets; are diets high in fat and low in carbohydrates. Both fats and oils can be used in these diets.

Research initiation and synchronization diets; are diets used as microbial diets during the adaptation phase of the animals in order to provide proper results for the experiments.

As a result, there are traditionally closed and open formula diet feeds and recently emerging nutrigenomics, individualized, and tailored feeding models, and various feeding models used in modeling with diet, especially in the modeling phase.

CONCLUSION

The integration of technological processes in feed processing and the implementation of novel feeding approaches represent significant advancements in enhancing laboratory animal nutrition. The utilization of cutting-edge techniques such as pelleting, extrusion, and coating has revolutionized the formulation of laboratory animal diets, leading to improved digestibility, nutrient bioavailability, and overall diet quality. These technological processes have enabled researchers to create standardized and tailored feed formulations that meet the specific nutritional requirements of laboratory animals, ultimately contributing to the health, welfare, and research outcomes of these animals. Moreover, the adoption of new feeding approaches, including precision feeding technologies and behavioral enrichment strategies, has further optimized the nutritional management and well-being of laboratory animals. Precision feeding algorithms personalized to individual animal needs have enhanced nutrient utilization and growth performance, while enrichment programs and diversified diets have promoted behavioral enrichment and stress alleviation in research animals. By addressing the diverse nutritional and behavioral needs of laboratory animals through innovative feeding approaches, researchers can ensure the ethical treatment, welfare, and scientific validity of research studies involving animals. Moving forward, continued research and innovation in laboratory animal nutrition are essential to furthering our understanding of the complex interplay between diet, health, and research outcomes in laboratory settings. By leveraging technological advancements and embracing novel feeding approaches, the scientific community can strive towards promoting optimal animal welfare, research reproducibility, and ethical standards in laboratory animal research. Ultimately, the combination of advanced technological processes and new feeding strategies holds promise for advancing the field of laboratory animal nutrition and fostering improved health and well-being outcomes for research animals.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: AT, TA; Design: AT, TA; Data Collection/Processing: AT, TA; Analysis/Interpretation: AT, TA; Literature Review: AT, TA; Drafting/Writing: AT, TA; Critical Review: AT, TA.

REFERENCES

1. National Research Council (US) Subcommittee on Laboratory Animal Nutrition. Nutrient requirements of laboratory animals; 4th ed. Washington, DC: National Academies Press (US); 1995.
2. Weiss WP. Predicting energy values of feeds. J Dairy Sci. 1993;76(6):1802-11.
3. Rojas OJ, Vinyeta E, Stein HH. Effects of pelleting, extrusion, or extrusion and pelleting on energy and nutrient digestibility in diets containing different levels of fiber and fed to growing pigs. J Anim Sci. 2016;94(5):1951-60.
4. Tobin G, Schuhmacher A. Laboratory animal nutrition in routine husbandry and experimental and regulatory studies. In: Hau J, Schapiro SJ, editors. Handbook of laboratory animal science, 4th ed. Boca Raton, FL: CRC Press; 2021. p.269-312.
5. Laurinen P, Siljander-Rasi H, Karhunen J, Alaviuhkola T, Näsi M, Tuppi K. Effects of different grinding methods and particle size of barley and wheat on pig performance and digestibility. Anim Feed Sci Technol. 2000;83(1):1-16.
6. Acosta JA, Petry AL, Gould SA, Jones CK, Stark CR, Fahrenholz A, et al. Effects of grinding method and particle size of wheat grain on energy and nutrient digestibility in growing and finishing pigs. Transl Anim Sci. 2020;4(2):682-93.
7. Stark CR. Feed processing to maximize feed efficiency. In: Patience JF, editor. Feed efficiency in swine. Wageningen: Wageningen Academic Publishers; 2012. p.131-51.
8. Amornthewaphat N, Attamangkune S. Extrusion and animal performance effects of extruded maize quality on digestibility and growth performance in rats and nursery pigs. Anim Feed Sci Technol. 2008;144(3-4):292-305.

9. Hancock JD, Behnke KC. Use of ingredient and diet processing technologies (grinding, mixing, pelleting, and extruding) to produce quality feeds for pigs. In: Lewis AJ, Southern LL, editors. Swine nutrition, 2nd ed. Boca Raton, FL: CRC Press; 2000. p.489-518.
10. Berrocoso JD, Cámara L, Rebollar PG, Guzmán P, Mateos GG. Influence of source and micronization of soya bean meal on growth performance, nutrient digestibility and ileal mucosal morphology of Iberian piglets. Animal. 2014;8(4):555-64.
11. Li YO, Dueik González VP, Diosady LL. Microencapsulation of vitamins, minerals, and nutraceuticals for food applications. In: Sobel R, editor. Microencapsulation in the food industry, 2nd ed. Elsevire: Academic Press; 2023. p.507-28.
12. İpçak HH, Alçiçek A, Denli M. Dietary encapsulated fennel seed (Foeniculum vulgare Mill.) essential oil supplementation improves performance, modifies the intestinal microflora, morphology, and transcriptome profile of broiler chickens. J Anim Sci. 2024;102:skae035.
13. Aloui H, Khwaldia K. Natural antimicrobial edible coatings for microbial safety and food quality enhancement. Compr Rev Food Sci Food Saf. 2016;15(6):1080-103.
14. Sauer N, Mosenthin R, Bauer E. The role of dietary nucleotides in single-stomached animals. Nutr Res Rev. 2011;24(1):46-59.
15. Celi P, Cowieson AJ, Fru-Nji F, Steinert RE, Kluenter AM, Verlhac V. Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. Anim Feed Sci Technol. 2017;234:88-100.
16. Pluske JR, Kim JC, Black JL. Manipulating the immune system for pigs to optimise performance. Anim Prod Sci. 2018;58(4):666-80.
17. Bomford M, Redhead T. A field experiment to examine the effects of food quality and population density on reproduction of wild house mice. Oikos. 1987;48:304-11.
18. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A rodent diet. J Nutr. 1993;123(11):1939-51.
19. Kaput J, Rodriguez RL. Nutritional genomics: the next frontier in the postgenomic era. Physiol Genomics. 2004;16(2):166-77.
20. Jimenez-Sanchez G, Childs B, Valle D. Human disease genes. Nature. 2001;409(6822):853-5.
21. DellaPenna D. Nutritional genomics: manipulating plant micronutrients to improve human health. Science. 1999;285(5426):375-9.
22. Afman L, Müller M. Nutrigenomics: from molecular nutrition to prevention of disease. J Am Diet Assoc. 2006;106(4):569-56.
23. Müller M, Kersten S. Nutrigenomics: goals and strategies. Nat Rev Genet. 2003;4(4):315-22.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1504037

Duzce Med J, 2024;26(S1):30-35
Düzce Tıp Fak Derg, 2024;26(S1):30-35

Anesthesia Applications in Experimental Neurological Disease Modeling

Deneysel Nörolojik Hastalık Modellemelerinde Anestezi Uygulamaları

Ayhan ÇETİNKAYA[1]
0000-0002-8212-7149
Hümeyra ÇELİK[2]
0000-0002-3394-2438

[1]Department of Physiology, Bolu Abant İzzet Baysal University Faculty of Medicine, Bolu, Türkiye
[2]Department of Physiology, Alanya Alaaddin Keykubat University Faculty of Medicine, Antalya, Türkiye

Corresponding Author
Sorumlu Yazar
Ayhan ÇETİNKAYA
cetinkayaayhan@hotmail.com

Received / Geliş Tarihi : 06.05.2024
Accepted / Kabul Tarihi : 11.06.2024
Available Online / Çevrimiçi Yayın Tarihi : 24.06.2024

ABSTRACT

Neuroscience, which covers the molecular mechanisms employed by the brain to cause neurological diseases and how they can be treated, remains current as the subject of high-budget investigations to develop early diagnosis and new treatment modalities with multidisciplinary approaches. For this purpose, creating the appropriate model with the correct modality and anesthesia in neurological in vivo experimental models is the most important phase to obtain accurate results and ensure animal welfare. To manage anesthesia in rodents, knowing the physiological characteristics of anesthetics and their risks will strengthen experimental procedures. Among the injectable anesthetics, ketamine, xylazine, and pentobarbital are the most frequently preferred agents for general anesthesia in short surgical procedures. Isoflurane and sevoflurane are inhaler anesthetics that are administered through a vaporizer because they are liquids. Important advantages of inhaled anesthetics, such as rapid induction and rapid withdrawal, make inhaled anesthetics stand out in neuroscience studies. In this review, the properties of frequently used anesthetic agents in rodents, their usage methods, and which model they are preferred will be discussed. For this purpose, the selection of appropriate anesthetics in animal models such as epilepsy, Alzheimer's disease, ischemia-reperfusion injury, traumatic brain injury, ischemic stroke, experimental autoimmune encephalomyelitis, and ophthalmic surgical procedures and their side effects will be reviewed.
Keywords: Anesthesia; neuroscience; in vivo models.

ÖZ

Beynin hangi moleküler mekanizmalar kullanarak nörolojik hastalıkların oluştuğu ve nasıl tedavi edilebileceği kapsamındaki sinir bilim, multidisipliner yaklaşımlar ile erken tanı ve yeni tedavi geliştirmek üzere yüksek bütçeli araştırmaların konusu olarak güncelliğini korumaktadır. Bu amaçla nörolojik in vivo deneysel modellerde uygun modeli doğru metot ve anestezi ile oluşturmak doğru sonuçları elde etmenin ve hayvan refahını sağlamanın en önemli basamaklarıdır. Kemirgenlerde anesteziyi yönetmek adına anesteziklerin fizyolojik özelliklerini tanımak ve risklerine hakim olmak deneysel prosedürleri güçlendirecektir. Enjekte edilebilir anesteziklerden ketamin, ksilazin ve pentobarbital genel anestezi için kısa cerrahi prosedürlerde en sık tercih edilen ajanlardır. İzofluran ve sevofluran sıvı olduklarından bir vaporizer ile verilen inhaler anesteziklerdendir. İnhale anesteziklerin hızlı indüksiyon ve hızlı çekilme gibi önemli avantajları sinirbilim çalışmalarında inhaler anestezikleri öne çıkarmaktadır. Bu derlemede kemirgenlerde sık kullanılan anestezik ajanların özellikleri, kullanım şekilleri ve hangi modelde tercih edildiğinden bahsedilecektir. Bu amaçla epilepsi, Alzeimer hastalığı, iskemi-reperfüzyon hasarı, travmatik beyin hasarı, iskemik inme, deneysel otoimmun ensefalomyelitis, oftalmik cerrahi prosedürler ve yan etkileri gibi hayvan modellerinde uygun anesteziklerin seçimi gözden geçirilecektir.
Anahtar kelimeler: Anestezi; sinirbilim; in vivo modeller.

INTRODUCTION

The brain is the most important organ in living things and remains largely unknown compared to the present century. How the brain works and remembers, what molecular mechanisms it employs to make decisions, how neurological diseases occur, and how they can be treated are the subjects of neuroscience (1). Recently, neuroscience has become a research subject not only in the field of medicine but also in many other branches of science such as engineering, chemistry, physics, computer science, philosophy, and psychology. This multidisciplinary approach reveals large-budget research for early diagnosis and new treatment modalities of neurodegenerative diseases, which are increasing in the entire world (2). For this purpose, experimental models in neurological in vivo and in vitro studies remain up-to-date.

Creating the appropriate model with the accurate method and choosing the appropriate anesthesia according to the model to be studied is a must in experimental research to obtain accurate results and ensure animal welfare (3). Managing anesthesia and analgesia in rodents is challenging because of their small size and varying sensitivity to anesthetics and analgesics. Also, the characteristics of the preoperative and postoperative periods may be overlooked if the characteristics of the anesthetic are not known because the focus is only on the experimental procedure to be performed. For this reason, knowing the physiological characteristics of various anesthetics and knowing their risks is important to strengthen experimental procedures (4).

This review aims to discuss the properties of frequently used anesthetic agents in rodents, their usage methods, and the selection of appropriate anesthetics in animal models for neurological diseases and their side effects.

INJECTABLE ANESTHETICS IN RODENTS

Mice and rats require higher doses of anesthetic to reach full anesthesia depth because of their higher metabolic rates than larger animals with lower metabolic rates. Although the duration of anesthesia in rodents is short, on average, 20-30 minutes, respiratory depression, hypothermia, and dehydration might occur because of high doses (5).

Injectable anesthetics allow short surgical procedures to be performed by anesthetizing multiple mice or rats simultaneously or serially. They are easy to use without starving the animals because these anesthetics do not require special equipment (6). Whichever injectable anesthetic is used, it must be dosed according to the size of the animal (5). If a repeated dose is required, this should be approximately 10-25% of the initial dose, but it should be noted that this might increase the risk of mortality.

When observing the depth of general anesthesia in animals, when the tail is pinched, the animal's pawing movement in response or the movement of the head towards the stimulus as a positive response to the stimulus is evaluated to tell us that the anesthesia is not deep enough and it is too early to start the procedure.

As a benzodiazepine, pentobarbital provides sedation between 80-95 minutes in rats (40-50 mg/kg) and 10-300 minutes in mice (40-70 mg/kg) with intraperitoneal (IP) administration (7,8). It depresses the central nervous system by potentiating gamma aminobutyric acid (GABA) receptors (9).

The most commonly used agents are ketamine and xylazine in rats (40-90 mg/kg ketamine / 5-10 mg/kg xylazine), and in mice (60-100 mg/kg ketamine / 5-10 mg/kg xylazine). Ketamine and xylazine, which can be administered IP and intramuscularly (IM), provide 45-90 minutes of sedation in rats and 30-45 minutes of sedation in mice (7,8). Ketamine provides anesthetic effects by providing N-methyl-D-aspartate (NMDA) receptor antagonism and agonistic effects on GABA receptors (10). As an analgesic and muscle relaxant, xylazine has sedative effects as an alpha 2 receptor agonist (11). Ketamine and xylazine can be injected simultaneously in rats and mice, and this procedure is slightly different in rabbits (Table 1). First xylazine (5 mg/kg) injection is administered IM, and 10 minutes later ketamine (30 mg/kg) is administered IM in rabbits (12,13).

Table 1. Anesthetic administration for short surgical procedures

	Injectable anesthesia
Rats	40-90 mg/kg ketamine / 5-10 mg/kg xylazine, IP or IM
Mice	60-100 mg/kg ketamine / 5-10 mg/kg xylazine, IP or IM
Rabbits	30 mg/kg ketamine (after) / 5 mg/kg xylazine (before), IM

IP: intraperitoneal, IM: intramuscular

Locally administered bupivacaine (0.25% solution) and lidocaine (1% solution) are local anesthetics and can be used for short procedures in the incision area in mice and rats. Effect times can last from 1-15 minutes to 4-12 hours for bupivacaine, and can range from 1-5 minutes to 1.5-2 hours for lidocaine (7,8). They prevent ion transfer by stabilizing the neuron membrane and exert their effects by inhibiting voltage-gated sodium channels (14).

INHALED ANESTHETICS IN RODENTS

The most consistent anesthesia protocol is inhaler anesthesia for rodents (15). It is already known that inhalant agents activate GABA and glycine receptors while inhibiting the NMDA receptors. They prolong the duration of synaptic inhibition by increasing the response to endogenous GABA (16).

With its effects on multiple receptors, they allow almost 100% of animals to reach the desired surgical plane. Also, inhalant agents have low blood solubility, facilitating rapid drug uptake from the alveoli and effective distribution across the blood-brain barrier, which results in rapid anesthetic induction and rapid removal of inhalant agents from the central nervous system at the end of the procedure and the ability to fine-tune anesthetic depth during the procedure (17,18). Side effects of inhaled anesthetics include the use of excessive equipment to create the appropriate depth of anesthesia. Volatile anesthetics (nitrous oxide, halothane, isoflurane, desflurane, and sevoflurane) are liquid at room temperature and require the use of vaporizers for inhalational use (18).

Isoflurane is the most commonly used inhaled anesthetic. An inhaler effect of 1-3% must be applied with a mixture of air and oxygen or 100% oxygen (18).

Sevoflurane can be used at 3% concentration with 6 lt/min of 100% oxygen or 50% nitric oxide and 50% oxygen (19). Sedation is first provided with an inhaler anesthetic drug in a box in inhaler anesthetic drug administration, then it is removed from the box and the same drug is administered with a mask (20). Since the blood gas solubility coefficient of sevoflurane is lower than isoflurane, anesthesia induction and recovery from anesthesia are faster than isoflurane (21,22).

ANESTHETICS USED IN NEUROSCIENCE STUDIES

In animal models created in neuroscience studies, choosing the appropriate anesthetic is as important as animal modeling. If the anesthetic used is not appropriate, cerebral hypoperfusion because of developing hypotension affects cognitive functions (23,24) independent of the disease, causing unexpected effects on the experimental results (25). The fact that anesthesia is good for the disease model created is also a dilemma. In such a case, the anesthetic agent overshadows the effects of the test substance or treatment method itself and for this reason, choosing the appropriate anesthetic with therapeutic and minimized harmful effects must be considered the most appropriate approach. Also, depending on the experimental setup, the effects of the test substance can be combined with the anesthesia method, which has positive effects. In this review, information will be shared about the selection of appropriate anesthetics in neuroscience animal models.

Epilepsy

Epilepsy is a chronic brain disease caused by high synchronization of abnormal neuronal discharges (26) and can be affected by the anesthesia protocol and cause seizure interference. For this reason, understanding the pro- and anticonvulsant characteristics of the drugs employed for anesthesia in epilepsy studies must aim to minimize the risks of intra- and postoperative seizure activity (27).

Isoflurane or ketamine/xylazine can be used to provide intraoperative and postoperative analgesia in epilepsy studies in which stereotaxic examination will be performed primarily (28,29). Also, jump block can be prevented by applying 1% lidocaine to the periosteum before drilling the skull (30). Since isoflurane has no channel activity, it is known as a good agent for creating an epilepsy model and is recommended in intrahippocampal seizure models induced by kainic acid or penicillin (29). Also, the ketamine/xylazine combination does not seem very appropriate to use because it might delay the onset of seizures due to its blocking effect on NMDA receptors (29). Here, the approach must be chosen according to the experimental design.

Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disease causing cognitive decline with the onset of progressive dementia with pathological symptoms such as senile plaque and neurofibrillary tangle formation in the brain (31). According to the studies on the information that inhaled anesthetics increase neuroinflammation in Alzheimer's patients (32), it has been observed that sevoflurane increases neurotoxicity in mice in transgenic AD (33). Also, isoflurane was found to increase amyloid pathology in AD model mice (34). However, isoflurane anesthesia was shown to be safe in AD created in the Tg2576 mice model and did not affect experimental results by not interacting with vital parameters (35).

Ischemia-Reperfusion Injury

Ischemic stroke refers to the necrosis of the brain tissue because of insufficient cerebral blood flow (36). Although reperfusion following ischemia improves the situation, cerebral reperfusion might increase brain tissue damage by worsening oxidative stress and protein damage (37). Ischemia-reperfusion injury has extremely high mortality and morbidity rates (38) and is a model in which the test substance and treatment modality are intensively studied experimentally.

It is already known that ketamine and barbiturates have neuroprotective effects and might interfere with investigating the effect of the test substance in stroke models (39). Pentobarbital anesthesia was shown to be superior to inhaled anesthetics in cerebral ischemia-reperfusion injury because it has been shown to reduce the infarct area (40). In a study that compared isoflurane and barbiturates (thiopental), it was shown that isoflurane reduced cerebral perfusion, and the ameliorative effects of barbiturate use on ischemia-reperfusion injury were reported (41). It was shown that pentobarbital anesthesia reduces the infarct area in cerebral ischemia-reperfusion injury more than inhaled anesthetics (40). For this reason, the choice of inhalation anesthesia seems more appropriate to reveal the effects of the test substance in the induction of experimental stroke models more clearly (42).

Experimental Autoimmune Encephalomyelitis

Modeled on multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) is a disease in which myelin damage occurs based on T cell-mediated neuroinflammation. Also, since (R)-ketamine was shown to improve the clinical score of EAE by reducing the pathological findings in the spinal cord in the EAE model, it does not seem appropriate to use ketamine as an anesthetic in the EAE model to demonstrate the effects of the test agent (43). Although an EAE study reported that sevoflurane, which is an inhaled anesthetic, suppressed neuroinflammation by reducing T-cell functions (44), it was reported that isoflurane is generally preferred in the EAE model (43).

Traumatic Brain Injury

Traumatic brain injury affects approximately 50 million people worldwide every year with a high morbidity and constitutes a large portion of young adult deaths worldwide (45). In a study conducted on rats, 50 mg/kg ketamine and 0.5 mg/kg chlorpromazine, an antipsychotic, were used to induce traumatic brain injury (46). It has been shown that the narcotic analgesic fentanyl, an anesthetic used in the management of traumatic brain injury, causes more hyperglycolysis and death of hippocampal CA1 neurons than the inhaled sedative agent isoflurane (47). In a study comparing inhaled isoflurane (5%) and fentanyl, it was emphasized that isoflurane has neuroprotective effects and this should not be overlooked when evaluating the test substance (48). In a study comparing propofol and isoflurane with hypothermia in traumatic brain injury in rats, it was observed that isoflurane reduced cerebral perfusion pressure and intracranial pressure more than propofol, and it was emphasized that propofol should be

preferred (49). It has also been observed that pentobarbital injection (50 mg/kg) IP is preferred when inducing traumatic brain injury (50).

Ischemic Stroke

It is a clinical condition characterized by sudden onset of focal neurological deficits in a vascular region of the brain, retina, or medulla spinalis, resulting from underlying cerebrovascular pathologies. It is a cause of high morbidity and mortality (51). Research on anesthetic drugs in ischemic stroke is difficult because there are many comorbidities depending on the patient's history. For this reason, experimental ischemic stroke models are important for anesthetic drug research (52). Isoflurane, a volatile anesthetic, is frequently preferred because it provides neuroprotection against excitotoxicity, ease of use, and rapid recovery (53,54). However, these results caused by volatile anesthetics used in experimental studies probably lead to great confusion in the investigation of stroke mechanisms and consequences (52). Among injectable anesthetics, ketamine/xylazine, 80/20 mg/kg, (55) and pentobarbital, 30 mg/kg, (56,57) are frequently used. The therapeutic effects of ketamine (58) on ischemia should not be forgotten when evaluating the results.

Ophthalmic Surgery

In models where ophthalmic surgical procedures will be performed, ketamine/xylazine anesthesia is applied during intravitreal implant injection (59). However, it should not be forgotten that ketamine/xylazine anesthesia may increase intraocular pressure (60) and cause hyperopia (61). It is also known that IP injections cause corneal scarring (62). Ketamine-methodimine is used for enucleation (63). Isoflurane anesthesia is used to perform retroorbital injection into the venous sinuses (64).

CONCLUSION

Failure to choose appropriate anesthetic drugs, failure to determine the depth of anesthesia, and surgical intervention at an inappropriate time might affect the experimental results and cause unexpected results. For this reason, during the experimental planning phase, which anesthetic must be selected as well as the information of the test substance must be considered. In this respect, it must not be overlooked that the choice of the most appropriate anesthetic might vary depending on the subject of the study. Future studies to be conducted to determine the appropriate anesthetic will make it easier for clinicians to reach more accurate experimental results.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: AÇ; Design: AÇ; Data Collection/Processing: HÇ; Analysis/Interpretation: HÇ, AÇ; Literature Review: HÇ; Drafting/Writing: HÇ; Critical Review: AÇ.

REFERENCES

1. Kumar J, Patel T, Sugandh F, Dev J, Kumar U, Adeeb M, et al. Innovative approaches and therapies to enhance neuroplasticity and promote recovery in patients with neurological disorders: a narrative review. Cureus. 2023;15(7):e41914.
2. Amunts K, Axer M, Banerjee S, Bitsch L, Bjaalie JG, Brauner P, et al. The coming decade of digital brain research: A vision for neuroscience at the intersection of technology and computing. Imaging Neurosci. 2024;2:1-35.
3. Kiani AK, Pheby D, Henehan G, Brown R, Sieving P, Sykora P, et al. Ethical considerations regarding animal experimentation. J Prev Med Hyg. 2022;63(2 Suppl 3):E255-66.
4. Oh SS, Narver HL. Mouse and rat anesthesia and analgesia. Curr Protoc. 2024;4(2):e995.
5. Davis JA. Mouse and rat anesthesia and analgesia. Curr Protoc Neurosci. 2008;Appendix 4:Appendix 4B.
6. Jaber SM, Hankenson FC, Heng K, McKinstry-Wu A, Kelz MB, Marx JO. Dose regimens, variability, and complications associated with using repeat-bolus dosing to extend a surgical plane of anesthesia in laboratory mice. J Am Assoc Lab Anim Sci. 2014;53(6):684-91.
7. Bennett K, Lewis K. Sedation and anesthesia in rodents. Vet Clin North Am Exot Anim Pract. 2022;25(1):211-55.
8. Navarro KL, Huss M, Smith JC, Sharp P, Marx JO, Pacharinsak C. Mouse anesthesia: the art and science. ILAR J. 2021;62(1-2):238-73.
9. Johnson AB, Sadiq NM. Pentobarbital. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
10. Li L, Vlisides PE. Ketamine: 50 years of modulating the mind. Front Hum Neurosci. 2016;10:612.
11. Papudesi BN, Malayala SV, Regina AC. Xylazine toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
12. Akter MA, Yesmin N, Talukder MBA, Alam MM. Evaluation of anaesthesia with xylazine-ketamine and xylazine-fentanyl-ketamine in rabbits: A comparative study. J Adv VetBio Sci Tech. 2023;8(1):38-46.
13. Yilmaz A, Hösükler E, Kaymaz A, Üçgül AY, Erkol ZZ. Evaluation of eye and serum findings in different waters in rabbits by drowning and submersion modeling. Turk J Med Sci. 2023;54(1):42-51.
14. Karnina R, Arif SK, Hatta M, Bukhari A. Molecular mechanisms of lidocaine. Ann Med Surg (Lond). 2021;69:102733.
15. Hohlbaum K, Bert B, Dietze S, Palme R, Fink H, Thöne-Reineke C. Severity classification of repeated isoflurane anesthesia in C57BL/6JRj mice-Assessing the degree of distress. PLoS One. 2017;12(6):e0179588.
16. Garcia PS, Kolesky SE, Jenkins A. General anesthetic actions on GABA(A) receptors. Curr Neuropharmacol. 2010;8(1):2-9.
17. Sahinovic MM, Struys MMRF, Absalom AR. Clinical pharmacokinetics and pharmacodynamics of propofol. Clin Pharmacokinet. 2018;57(12):1539-58.
18. Miller AL, Theodore D, Widrich J. Inhalational anesthetic. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.

19. Özer AB, Özer S. Effects of lycopene supplementation on morphological changes induced by sevoflurane in rats. FÜ Sağ Bil Tıp Derg. 2007;21(3):103-8. Turkish.
20. Hunter CL, Yang P, Renner DM, Kennedy L. Effects of isoflurane anesthesia on C57BL/6J pups after cervical dislocation of dams. J Am Assoc Lab Anim Sci. 2023;62(5):449-52.
21. Gauthier A, Girard F, Boudreault D, Ruel M, Todorov A. Sevoflurane provides faster recovery and postoperative neurological assessment than isoflurane in long-duration neurosurgical cases. Anesth Analg. 2002;95(5):1384-8.
22. Godet G, Watremez C, El Kettani C, Soriano C, Coriat P. A comparison of sevoflurane, target-controlled infusion propofol, and propofol/isoflurane anesthesia in patients undergoing carotid surgery: a quality of anesthesia and recovery profile. Anesth Analg. 2001;93(3):560-5.
23. Sprung J, Warner DO, Knopman DS, Petersen RC, Mielke MM, Jack CR Jr, et al. Exposure to surgery with general anaesthesia during adult life is not associated with increased brain amyloid deposition in older adults. Br J Anaesth. 2020;124(5):594-602.
24. Patel D, Lunn A, Smith AD, Lehmann DJ, Dorrington KL. Cognitive decline in the elderly after surgery and anaesthesia: Results from the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort. Anaesthesia. 2016;71(10):1144-52.
25. Berger M, Schenning KJ, Brown CH 4th, Deiner SG, Whittington RA, Eckenhoff RG, et al. Best practices for postoperative brain health: Recommendations from the fifth international perioperative neurotoxicity working group. Anesth Analg. 2018;127(6):1406-13.
26. Zhao X, Wang X. Anesthesia-induced epilepsy: causes and treatment. Expert Rev Neurother. 2014;14(9):1099-113.
27. Maranhão MV, Gomes EA, de Carvalho PE. Epilepsy and anesthesia. Rev Bras Anestesiol. 2011;61(2):232-41.
28. Cho C, Michailidis V, Lecker I, Collymore C, Hanwell D, Loka M, et al. Evaluating analgesic efficacy and administration route following craniotomy in mice using the grimace scale. Sci Rep. 2019;9(1):359.
29. Bielefeld P, Sierra A, Encinas JM, Maletic-Savatic M, Anderson A, Fitzsimons CP. A standardized protocol for stereotaxic intrahippocampal administration of kainic acid combined with electroencephalographic seizure monitoring in mice. Front Neurosci. 2017;11:160.
30. Mostany R, Portera-Cailliau C. A craniotomy surgery procedure for chronic brain imaging. J Vis Exp. 2008;12:680.
31. Zhang Y, Chen H, Li R, Sterling K, Song W. Amyloid β-based therapy for Alzheimer's disease: challenges, successes and future. Signal Transduct Target Ther. 2023;8(1):248.
32. Eckenhoff RG, Johansson JS, Wei H, Carnini A, Kang B, Wei W, et al. Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity. Anesthesiology. 2004;101(3):703-9.
33. Lu Y, Wu X, Dong Y, Xu Z, Zhang Y, Xie Z. Anesthetic sevoflurane causes neurotoxicity differently in neonatal naive and Alzheimer disease transgenic mice. Anesthesiology. 2010;112(6):1404-16.
34. Perucho J, Rubio I, Casarejos MJ, Gomez A, Rodriguez-Navarro JA, Solano RM, et al. Anesthesia with isoflurane increases amyloid pathology in mice models of Alzheimer's disease. J Alzheimers Dis. 2010;19(4):1245-57.
35. Bratke S, Schmid S, Ulm B, Jungwirth B, Blobner M, Borgstedt L. Genotype- and sex-specific changes in vital parameters during isoflurane anesthesia in a mouse model of Alzheimer's disease. Front Med (Lausanne). 2024;11:1342752.
36. Chen J, Yang Y, Shen L, Ding W, Chen X, Wu E, et al. Hypoxic preconditioning augments the therapeutic efficacy of bone marrow stromal cells in a rat ischemic stroke model. Cell Mol Neurobiol. 2017;37(6):1115-29.
37. Gong L, Tang Y, An R, Lin M, Chen L, Du J. RTN1-C mediates cerebral ischemia/reperfusion injury via ER stress and mitochondria-associated apoptosis pathways. Cell Death Dis. 2017;8(10):e3080.
38. Kraft P, De Meyer SF, Kleinschnitz C. Next-generation antithrombotics in ischemic stroke: preclinical perspective on 'bleeding-free antithrombosis'. J Cereb Blood Flow Metab. 2012;32(10):1831-40.
39. Ahmadi-Noorbakhsh S, Farajli Abbasi M, Ghasemi M, Bayat G, Davoodian N, Sharif-Paghaleh E, et al. Anesthesia and analgesia for common research models of adult mice. Lab Anim Res. 2022;38(1):40.
40. Chi OZ, Mellender SJ, Kiss GK, Liu X, Weiss HR. Blood -brain barrier disruption was less under isoflurane than pentobarbital anesthesia via a PI3K/Akt pathway in early cerebral ischemia. Brain Res Bull. 2017;131:1-6.
41. Drummond JC, Cole DJ, Patel PM, Reynolds LW. Focal cerebral ischemia during anesthesia with etomidate, isoflurane, or thiopental: a comparison of the extent of cerebral injury. Neurosurgery. 1995;37(4):742-8; discussion 748-9.
42. Braeuninger S, Kleinschnitz C. Rodent models of focal cerebral ischemia: procedural pitfalls and translational problems. Exp Trans Stroke Med. 2009;1:8.
43. Wang X, Chang L, Tan Y, Qu Y, Shan J, Hashimoto K. (R)-ketamine ameliorates the progression of experimental autoimmune encephalomyelitis in mice. Brain Res Bull. 2021;177:316-23.
44. Polak PE, Dull RO, Kalinin S, Sharp AJ, Ripper R, Weinberg G, et al. Sevoflurane reduces clinical disease in a mouse model of multiple sclerosis. J Neuroinflammation. 2012;9:272.
45. Maas AIR, Menon DK, Adelson PD, Andelic N, Bell MJ, Belli A, et al. Traumatic brain injury: Integrated approaches to improve prevention, clinical care, and research. Lancet Neurol. 2017;16(12):987-1048.
46. Ozaydin D, Bektasoglu PK, Koyuncuoglu T, Ozkaya SC, Koroglu AK, Akakin D, et al. Anti-inflammatory, antioxidant and neuroprotective effects of niacin on mild traumatic brain injury in rats. Turk Neurosurg. 2023;33(6):1028-37.
47. Statler KD, Janesko KL, Melick JA, Clark RS, Jenkins LW, Kochanek PM. Hyperglycolysis is exacerbated after traumatic brain injury with fentanyl vs. isoflurane anesthesia in rats. Brain Res. 2003;994(1):37-43.
48. Statler KD, Alexander H, Vagni V, Holubkov R, Dixon CE, Clark RS, et al. Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. Brain Res. 2006;1076(1):216-24.

49. Kahveci FS, Kahveci N, Alkan T, Goren B, Korfali E, Ozluk K. Propofol versus isoflurane anesthesia under hypothermic conditions: effects on intracranial pressure and local cerebral blood flow after diffuse traumatic brain injury in the rat. Surg Neurol. 2001;56(3):206-14.
50. He H, Liu W, Zhou Y, Liu Y, Weng P, Li Y, et al. Sevoflurane post-conditioning attenuates traumatic brain injury-induced neuronal apoptosis by promoting autophagy via the PI3K/AKT signaling pathway. Drug Des Devel Ther. 2018;12:629-38.
51. Hui C, Tadi P, Khan Suheb MZ, Patti L. Ischemic stroke. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
52. Hoffmann U, Sheng H, Ayata C, Warner DS. Anesthesia in experimental stroke research. Transl Stroke Res. 2016;7(5):358-67.
53. Kudo M, Aono M, Lee Y, Massey G, Pearlstein RD, Warner DS. Effects of volatile anesthetics on N-methyl-D-aspartate excitotoxicity in primary rat neuronal-glial cultures. Anesthesiology. 2001;95(3):756-65.
54. Kimbro JR, Kelly PJ, Drummond JC, Cole DJ, Patel PM. Isoflurane and pentobarbital reduce AMPA toxicity in vivo in the rat cerebral cortex. Anesthesiology. 2000;92(3):806-12.
55. Tang H, Gamdzyk M, Huang L, Gao L, Lenahan C, Kang R, et al. Delayed recanalization after MCAO ameliorates ischemic stroke by inhibiting apoptosis via HGF/c-Met/STAT3/Bcl-2 pathway in rats. Exp Neurol. 2020;330:113359.
56. Li Y, Li J, Yu Q, Ji L, Peng B. METTL14 regulates microglia/macrophage polarization and NLRP3 inflammasome activation after ischemic stroke by the KAT3B-STING axis. Neurobiol Dis. 2023;185:106253.
57. Li Y, Liu B, Zhao T, Quan X, Han Y, Cheng Y, et al. Comparative study of extracellular vesicles derived from mesenchymal stem cells and brain endothelial cells attenuating blood-brain barrier permeability via regulating Caveolin-1-dependent ZO-1 and Claudin-5 endocytosis in acute ischemic stroke. J Nanobiotechnology. 2023;21(1):70.
58. Xiong Z, Chang L, Qu Y, Pu Y, Wang S, Fujita Y, et al. Neuronal brain injury after cerebral ischemic stroke is ameliorated after subsequent administration of (R)-ketamine, but not (S)-ketamine. Pharmacol Biochem Behav. 2020;191:172904.
59. Sun YJ, Lin CH, Wu MR, Lee SH, Yang J, Kunchur CR, et al. An intravitreal implant injection method for sustained drug delivery into mouse eyes. Cell Rep Methods. 2021;1(8):100125.
60. Qiu Y, Yang H, Lei B. Effects of three commonly used anesthetics on intraocular pressure in mouse. Curr Eye Res. 2014;39(4):365-9.
61. Tkatchenko TV, Tkatchenko AV. Ketamine-xylazine anesthesia causes hyperopic refractive shift in mice. J Neurosci Methods. 2010;193(1):67-71.
62. Turner PV, Albassam MA. Susceptibility of rats to corneal lesions after injectable anesthesia. Comp Med. 2005;55(2):175-82.
63. Aerts J, Nys J, Arckens L. A highly reproducible and straightforward method to perform in vivo ocular enucleation in the mouse after eye opening. J Vis Exp. 2014;92:e51936.
64. Yardeni T, Eckhaus M, Morris HD, Huizing M, Hoogstraten-Miller S. Retro-orbital injections in mice. Lab Anim (NY). 2011;40(5):155-60.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1496879

Duzce Med J, 2024;26(S1):36-46
Düzce Tıp Fak Derg, 2024;26(S1):36-46

Traditional Approaches and Innovative Strategies in Laboratory Animal Models for Cancer Research: A Comprehensive Review

Kanser Araştırmalarında Laboratuvar Hayvanı Modellerinde Geleneksel Yaklaşımlar ve Yenilikçi Stratejiler: Kapsamlı Bir İnceleme

Mümin Alper ERDOĞAN
0000-0003-0048-444X

Department of Physiology, İzmir Katip Çelebi University Faculty of Medicine, İzmir, Türkiye

ABSTRACT

Cancer remains one of the foremost challenges in medical research, necessitating diverse and sophisticated models to understand its complexity and develop effective treatments. This review explores the evolution and utility of experimental cancer models, highlighting their pivotal role in bridging the gap between basic research and clinical application. From the traditional use of xenografts, which provide a direct avenue for studying tumor growth and drug response in a living organism, to the innovative approaches of genetically engineered mouse models (GEMMs) that replicate human cancer's genetic and phenotypic traits, each model offers unique insights into cancer biology. Recent advances have introduced organoid models, offering a three-dimensional perspective that closely mimics the tumor's microenvironment, and computational models, which leverage patient-specific data to predict disease progression and treatment outcomes. These models enhance our understanding of cancer's molecular drivers, facilitate the development of targeted therapies, and underscore the importance of personalized medicine in oncology. Despite the diversity and potential of these experimental models, challenges remain, including the replication of the tumor's complexity and the integration of immune system interactions. Future research is directed toward refining these models, improving their predictive accuracy, and combining their strengths to offer a holistic view of cancer biology and treatment.

Keywords: Cancer experimental research; xenograft; genetically engineered mouse models; organoid model; computational models; personalized medicine; tumor microenvironment.

ÖZ

Kanser, tıbbi araştırmalarda önemli bir zorluk olarak kalmaya devam etmektedir ve karmaşıklığını anlamak ve etkili tedaviler geliştirmek için çeşitli ve karmaşık modellere ihtiyaç duyulmaktadır. Bu derlemenin amacı, deneysel kanser modellerinin evrimini ve faydasını incelemek ve temel araştırma ile klinik uygulama arasındaki boşluğu kapatmada önemli rol oynadıklarını vurgulamaktır. Tümör büyümesini ve ilaç yanıtını bir canlı organizmada çalışmak için doğrudan bir yol sağlayan ksenograftların geleneksel kullanımından, insan kanserinin genetik ve fenotipik özelliklerini kopyalayan genetiği değiştirilmiş fare modellerinin (genetically engineered mouse models, GEMMs) yenilikçi yaklaşımlarına kadar, her model kanser biyolojisine benzersiz bir bakış sunmaktadır. Son dönemdeki ilerlemeler, tümörün mikroçevresini yakından taklit eden üç boyutlu bir perspektif sunan organoid modellerini ve hastalık ilerlemesini ve tedavi sonuçlarını tahmin etmek için hastaya özgü verileri kullanan hesaplama modellerini tanıtmıştır. Bu modeller, kanserin moleküler etkenlerinin anlaşılmasına yardımcı olmakta, hedefe yönelik tedavilerin geliştirilmesini kolaylaştırmakta ve onkolojide kişiselleştirilmiş tıbbın önemini vurgulamaktadır. Bu deneysel modellerin çeşitliliği ve potansiyeline rağmen, tümörün karmaşıklığının kopyalanması ve bağışıklık sistemi etkileşimlerinin entegrasyonu gibi zorluklar devam etmektedir. Gelecekteki araştırmalar, tahmin doğruluklarının artırılmasına ve güçlü yanlarının birleştirilmesine odaklanarak kanser biyolojisi ve tedavisine bütünsel bir bakış açısı sunmak için bu modellerin iyileştirilmesine yöneliktir.

Anahtar kelimeler: Kanser deneysel araştırma; ksenograft; genetiği değiştirilmiş fare modelleri; organoid model; hesaplamalı modeller; kişiselleştirilmiş tıp; tümör mikroçevre.

Corresponding Author
Sorumlu Yazar
Mümin Alper ERDOĞAN
muminalper.erdogan@gmail.com

Received / Geliş Tarihi : 18.03.2024
Accepted / Kabul Tarihi : 13.05.2024
Available Online / Çevrimiçi Yayın Tarihi : 06.06.2024

INTRODUCTION

In cancer research, in vitro cell cultures are frequently utilized to reveal the biological behaviors of cancer cells (1). However, studies conducted in cell cultures fall short in addressing the impacts of cancer on human metabolism. On the contrary, examining the local or systemic effects of metabolism, the immune system response, angiogenesis, and the effects of drugs to be used in the treatment of cancer, the microenvironment, and other systems can only be accomplished through animal models. The type of animal to be used and the cancer models to be established have a wide spectrum. Since the biology of each animal to be used is different from one another, each subject can also be considered as a control group in its own right. For this reason, the number of animals to be used is kept to a minimum.

Mouse models, which can mimic many of the significant characteristics of human tumors, are generally very suitable models for cancer. In cancers developed in mice, mechanisms similar to those in humans are observed in terms of tumor behaviors such as metastasis development or treatment response (2). Thanks to these small laboratory animal models, the in vivo functions of cancer can be provided based on quantitative data on both normal and tumor tissue. In this way, the natural progression of the disease and the effectiveness of treatment are monitored.

Creating cancer models in laboratory animals has certain advantages and disadvantages. To discuss the general advantages; the costs of frequently used small-sized animals, such as mice or rats, are low and they are easy to maintain. They reproduce and grow quickly, and their life span can be monitored. Manipulations during the experiment are easy. The complete genetic sequence and characterization of the mouse are possible, and manipulation of the genome is relatively easy. The physiology of mice is similar to that of humans, thus enabling studies on drug pharmacodynamics and pharmacokinetics. In contrast, the small size of tumors in these animals can cause limitations in procedures such as imaging methods. Their high metabolic rates can affect the absorption and elimination of drugs and molecules.

The general ethical approach is to conduct experiments in cell environments if possible, and in laboratory animals if not, while working with the minimum possible number of animals in regard to animal rights. Various biodistribution methods make it possible to conduct an experiment with as few animals as possible. Another critical issue is whether the developed model accurately reflects the disease in humans, highlighting the importance of selecting the appropriate model.

Cancer biology varies from organ to organ and individual to individual, making it impossible to address all cancers with a single model. Therefore, different models must be developed for each type of cancer. These models will have various advantages and disadvantages, so it is crucial to select suitable models for confirming the thesis. This review aimed to explore the evolution and utility of experimental cancer models, highlighting their pivotal role in bridging the gap between basic research and clinical application.

XENOGRAFT MODELS

This model involves the transplantation of cancer cells or tissues from humans into experimental animals. According to some researchers, these models are considered a step beyond tissue culture, described as "animal culture." Nude mice are generally used in these models. These mice are immunodeficient animals with defective thymic epithelial cells and a limited number of functional T and B cells. Their lack of fur makes it easy to visualize or measure tumors subcutaneously. Tumor implantation can be done intraperitoneally, subcutaneously, intravenously, intrathecally, into tissue, or into the tissue from which the tumor originates. Xenograft models are simple and frequently used models. Tumor cells are usually transplanted into a region of the origin organ, providing a suitable microenvironment for the growth and development of the tumor cells. Since xenograft models use human-origin tumor cells, they cannot be used to assess the initial stages of the disease. The requirement for immunodeficient animals means that aspects such as the immune system's effect on cancer development and treatment response are overlooked. The creation of immunosuppressed models necessitates the isolation of these animals, thus xenograft models come with disadvantages such as production difficulty and high costs (3). These models are ideal for testing new and personalized cancer therapeutics. These models are divided into two categories based on how the tumor sample from humans is obtained.

Orthotopic Xenograft Models

The impact of the microenvironment on tumor biology has long been a subject of focus. In cancer treatment, researching the effects, toxicities of drugs, or the response of the cancerous organ to treatment necessitates the replication of the microenvironment in animal models. These models are created by the percutaneous implantation of a human cancer tissue sample into the animal. The implantation is done in the organ from which the tumor originates, thus creating the most appropriate microenvironment. This model is quite effective for developing personalized treatments (4). However, there is a high possibility of the human tumor cells being rejected by the host. To eliminate this possibility, animals with suppressed immune systems are used, which means the response of the immune system to the tumor tissue is ignored.

Xenograft Models with Circulating Tumor Cells

In these models, instead of taking a tissue sample from cancer patients, a blood sample is obtained. With the sample, circulating tumor cells or circulating cancer DNA is acquired and transferred to an immunodeficient animal. The formation of these models does not require an invasive procedure to obtain tumor samples, thus reducing the potential for harming the patient. Simple blood sampling allows for samples to be taken from the patient at different times for replication. This way, the evaluation of the tumor in humans at various developmental stages is possible (5).

SYNGENEIC MODELS

These models are created by transferring immunologically compatible cancer cells to immunologically compatible animals. They can be applied among beings of the same species and with the same genetics. The creation of these models is quite challenging, costly, and limited. A general advantage of these models is the low probability of immunological rejection of the injected cells by the experimental animal's immune system due to compatibility. This allows for more detailed studies related to the microenvironment compared to other models.

TRANSGENIC MODELS

In some experimental models, genetic modifications have been made to animals to induce spontaneous neoplastic growth. In these models, genes that initiate neoplasia are transferred into the pronucleus of the animal zygote DNA by microinjection. These genetically modified models allow for the investigation of the effects of genetic abnormalities on cancer development and progression. In transgenic models, the transferred genes can be passed on to the offspring. From the initial stage of the tumor, all development processes can be monitored. These models provide the opportunity to examine the pathogenesis of the disease within a natural stroma and in the presence of a natural immune system. They can be used to assess the effect of hormones on the disease and the response to chemotherapeutic agents.

GENETICALLY MODIFIED MODELS

These models use genetically modified animals designed to mimic many pathophysiological characteristics related to human cancers. With these models, chains of molecular events causing cancer can be examined and replicated as needed. For this purpose, methods such as physically removing a gene from the genome (knock-out), blocking its function with various agents, or physically adding a gene into the genome (knock-in) can be used (6,7).

MODELS INDUCED BY CARCINOGENIC AGENTS

Cancer models in experimental animals can be created using physical, biological, or chemical agents. Chemically induced animal models are those created using environmental factors effective in carcinogenesis in the human population (8).

VARIOUS EXPERIMENTAL CANCER MODELS

Leukemia Models

From 1955 to 1970, many experiments induced leukemia by administering 3-methylcholanthrene intraperitoneally at 20 mg/kg to DBA-2 mice. However, the use of these models has declined over time. One reason is that the leukemia induced in experimental mice by these substances is not phenotypically completely compatible with human leukemia. Another reason is that disease development occurs in only a small number of animals after a long delay when exposed to the carcinogen. Whole-body irradiation with high doses of gamma radiation or X-rays at once, or prolonged low doses, can be used to induce leukemia or lymphoma development in mice (9). Murine leukemia virus (MuLV) is a virus frequently used to induce leukemia (10).

Lung Cancer Models

To create a xenograft lung cancer model in experimental animals, tumor cells in suspension can be inoculated into the right bronchial stump of the right lung under anesthesia. The optimal number of tumor cells to be transplanted ranges between 106-107, and the time required for tumor development varies from 1 to 8 weeks (11). Cell lines such as A549, H1975, HCC4006, and HCC827 can be used to create lung adenocarcinoma. For large cell carcinoma, NCI-H460, and for squamous cell carcinoma, NCI-H226 cell lines are utilized (12).

Thyroid Cancer Models

Studies related to thyroid cancer are frequently conducted using xenograft models, where tumor cells are injected into immunodeficient mice. These models include cell-derived xenograft models, patient-derived xenograft (PDX) models, and genetically modified models (13). In cell-derived thyroid cancer xenograft models, tumor cells developed from cell lines such as 8505C, TPC-1, and FTC133 are transplanted subcutaneously, orthotopically, or metastatically into the animal (13). These models allow for the assessment of cancer cell properties such as invasion, metastasis, or angiogenesis. Instead of subcutaneous injection, delivering 30,000 thyroid cancer cells intravenously or intraventricularly to the mouse can rapidly create metastasis models in bone and lungs (14). However, models created using immunosuppressive mice lack microenvironment effects such as tumor stroma relations and the impact of the immune system on the tumor.

Patient-derived thyroid cancer xenograft models are created by transplanting tissue or cells from human tumors into immunosuppressed mice, creating a stroma-based tumor environment. These models often use NOD/Shi-scid/IL-2Rγnull and NOD.Cg-PrkdcscidIl-2rgtm1Wjl/SzJ mice (15). Models that establish the microenvironment are useful for researching new cancer drugs. Preclinical studies of drugs like Obatoclax, LOXO-292, Sorafenib, Lenvatinib, PLX51107, PD0325901, and Cabozantinib for thyroid cancer treatment have been conducted using these models (13). Genetically modified animal models are increasingly used in thyroid cancer research to investigate the roles of gene mutations, amplifications, deletions, and translocations in tumor etiopathogenesis.

Papillary Thyroid Cancer Models

BRAFV600E mutations are observed in a significant portion of human papillary thyroid cancer (PTC) cases and are indicators of aggressive tumor behavior and poor prognosis. Several mouse models have been created with BRAF activation, with the simplest being transgenic models targeting the BRAFV600E gene using the bovine thyroglobulin (Tg) promoter (16). Using this method, more than 90% of the animals can develop PTC within 12 weeks.

RET-PTC1 tumor models are generated by delivering the RET-PTC1 transgene to the animal using the bovine (Tg) promoter. In these animals, malignant thyroid overgrowth develops from the 18th day of the embryo (17). RET-PTC3 tumor models, like other transgenic models, result in thyroid hypercellularity in 69% of the animals within 3 months, creating solid PTC formations similar to those in humans (18). About 10% of PTCs have RAS mutations, and mouse models have been developed by transgenically activating Ras gene isoforms in the thyroid gland (19).

Follicular Thyroid Cancer Models

Models developed to induce follicular thyroid cancer using single transgenes like Ras-Rap1, PPFP, PTEN knockout, Prkar1a knockout, thyroid hormone receptor β, and phospho-inositide-3-kinase activation have not successfully induced thyroid carcinogenesis. However, DUAL-HIT models combining some of these genes have achieved a higher rate of carcinogenesis. Notably, combining PTEN knockout with the PPFP model has led to the development of an aggressive phenotype of follicular thyroid cancer within 5 months (20).

Medullary Thyroid Cancer Models

In this model, excessive expression of the p25 gene activates Cdk5 kinase, leading to phosphorylation and inactivation of the Rb gene, resulting in the development of medullary thyroid cancer in the animal within 16 weeks (21).

Anaplastic Thyroid Cancer Models

Deletion of the PTEN gene and inactivation of the P53 gene in mice leads to the development of undifferentiated thyroid cancer within 9 months from existing follicular hyperplasia in 75% of the animals (22).

Breast Cancer Models

Chemically Induced Breast Cancer Models

N-methyl-N-nitrosourea (MNU) directly causes DNA alkylation, disrupting DNA synthesis and repair. Intraperitoneal, subcutaneous, or intravenous injections in 4-7 week-old rats can lead to the development of ER+/PR+ tumors (23). Studies have reported obtaining breast cancer with single or double doses of 50-70 mg/kg.

2-amino-1-methyl-6-phenylimidazo [4,5-B] pyridine (PHIP) is a heterocyclic amine containing methanol and dimethyl sulfoxide (DMSO), found especially in fried foods, meat, fish, or cigarettes. It affects the colon, prostate, and breast tissues. Experimentally, breast cancer models can be created by administering 80-100 mg/kg PHIP through gavage four times a week for two weeks (24).

3-methylcholanthrene, a polycyclic aromatic hydrocarbon compound, can induce breast cancer in rats after being given 3-6 times through gavage at 10 mg/kg over 44-52 days (25).

7,12-dimethylbenz(a)anthracene (DMBA) is a polycyclic hydrocarbon, and its single-dose application can lead to breast cancer. DMBA is typically mixed with sesame oil, olive oil, or directly into the stomach. Cancer develops approximately 40 weeks after administering 50 mg/kg DMBA in olive oil through gavage to rats. BALB/c 53-P hemizygote mice can develop breast cancer 3-7 weeks after being given 1 mg/kg DMBA in flaxseed oil for six weeks (26).

Breast Cancer Model with Tumor Cell Xenografts

4T1 breast cancer cells, first isolated by Fred Miller and colleagues, are transplantable cancer cells that can grow in BALB/c hemizygote mice and tissue culture. Breast cancers developed with this model possess highly tumorigenic characteristics and unlike other tumor models, can metastasize from the primary breast tissue to distant organs such as lymph nodes, blood, liver, brain, lungs, and bones, thus resembling human breast cancer in its properties (27).

Radiation-Induced Breast Cancer Model

Breast tissue is sensitive to radiation. Calaf and Hei (28) demonstrated that 30 cGy of radiation can lead to tumor development in thymus-less mice.

Transgenic Breast Cancer Models

Mouse mammary tumor virus (MMTV) is among the primary promoters used in transgenic breast tumors in mice. This virus enters the mammary tissue with lymphocytes, infects the mammary gland epithelial cells, and thus initiates tumorigenesis (24).

Hepatocellular Carcinoma (HCC) Models

Experimental animals often utilize chemically induced models, genetically engineered models, and transplantation models to create HCC.

Chemically Induced HCC Models

Diethylnitrosamine (DEN) is the most commonly used genotoxic chemical in HCC models. Besides the liver, these substances can also induce cancer development in the gastrointestinal system. A single intraperitoneal dose of 5-25 μg/g DEN in 12-15 day-old B6C3F1 mice can induce HCC. In older mice, hepatocarcinogenesis can only be initiated with a co-carcinogen such as 2-AAF, phenobarbital, N-nitrosomorpholine, or carbon tetrachloride (CCL4) (29,30).

Carbon tetrachloride is a potent hepatotoxin that works in two ways: it directly increases oxidative damage in hepatocytes and disrupts cell membrane integrity, leading to inflammation. The inflammation causes Kupffer cells and stellate cells to secrete cytokines and chemokines. Liver damage resulting from these processes continues with tumorigenesis. Typically, liver fibrosis development is observed 4-6 weeks after two weekly intraperitoneal injections of 0.5-2 mL/kg (CCL4). After this process, some animals only show fibrosis and cirrhosis, while a significant portion develops HCC. The combined use of CCL4 and DEN often results in HCC development (30,31).

Thioacetamide (TAA) is well-known for creating liver fibrosis models in rodents. Administering 100-200 mg/kg TAA intraperitoneally three days a week for 3-4 weeks can create this model. Also, adding 200 μg/L TAA to drinking water for 6-18 weeks induces liver fibrosis (32,33).

Diet-Induced HCC Carcinogenesis Model

A cancer model can be created by adding methionine to a diet deficient in choline. Diets deficient in choline and L-amino acids can also induce HCC. A diet deficient in choline and L-amino acids can lead to 100% cancer development in rats and mice within 52 weeks (30).

Alcohol-Induced HCC Carcinogenesis Model

HCC can be induced in 16-week-old experimental mice by administering alcohol for seven weeks followed by DEN injection.

Oncogenic, Transgenic Mice in HCC

Downregulation of glycine N-methyl-transferase (GNMT) is common in human HCC. In mice, GNMT knockout (GNMT-/-) transgenic models can create chronic hepatitis, fatty liver, and HCC models, developing multiple HCC lesions up to 5 mm in size within 16 months. These models are often used to identify biomarkers for early diagnosis of hepatocarcinogenesis (34).

Cholangiocellular Carcinoma (CCA) Models

Chemically-Induced CCA Models

Administering the *O. viverrini* parasite intragastrically to mice and adding 0.0025% dimethylnitrosamine (DMN) to the drinking water after four weeks can induce CCA in 100% of the animals. Additionally, administering DMN to Syrian hamsters with biliary duct ligation can induce CCA in 40% of the animals (35).

Male albino rats given 0.03% TAA in their drinking water for eight months develop cystadenomas, and after 12 months of TAA treatment, 100% of the subjects develop CCA. The significant advantage of TAA animal models is the initiation of carcinogenesis without surgical procedures, though these models are primarily limited to rats (36).

In rats, chronic use of high doses (8 mg/kg) of Furan for 15 months can lead to 98% CCA development (37).

Cholestatic CCA Models

This model is achieved by ligating the left medial bile duct (LMBDL), leading to cholestasis. Following ligation, adding DMN to the treatment can induce CCA development in 40% of the subjects after 40 weeks. In another model, chronic cholestasis is induced by LMBDL following bi-weekly intraperitoneal injections of DEN. One week after LMBDL, mice are given DEN again in corn oil via oral gavage. 28 weeks after these applications, 50% of the subjects develop CCA (35).

Xenotransplant CCA Models

Heterotopic CCA models can be created by injecting human tumor cells or tissue subcutaneously into the flank area of immunodeficient mice. These ectopic CCA models allow for drug studies. However, the main disadvantages of these models are the ectopic location of the tumor ignoring microenvironment factors, and the effects of the immune system due to the use of immunodeficient mice (1).

Gastric Cancer Models

Chemically Induced Gastric Cancer Models

Various chemical carcinogens are utilized to explore the mechanisms of gastric cancer development. Researchers have particularly focused on N-nitroso compounds produced by anaerobic bacteria in the stomach after the intake of nitrates and nitrites, considered significant inducers of human cancer. N-methyl-N-nitro-N-nitrosoguanidine (MNNG) is the first nitrosamine shown by researchers to induce stomach tumors in rats. Subjects exposed to 400 ppm MNNG in drinking water for 50 weeks develop stomach adenocarcinoma in 63% of cases (38).

In a model developed with MNU, BALB/c mice receiving weekly intragastric intubation of 0.5 mg MNU develop squamous cell carcinoma in the fore-stomach, with most subjects dying from this squamous cell carcinoma. However, if the fore-stomach is surgically removed before MNU treatment, well-differentiated stomach adenocarcinoma develops in 100% of the subjects within 40 weeks (39).

Gastric Cancer Model Induced by Helicobacter Infection

Due to the key role of *H. pylori* infection in the etiology and pathogenesis of gastric cancer, researchers have developed gastric cancer models with Helicobacter species. Gastric cancer development can be induced in wild ferrets infected with *H. mustelae* and exposed to a single dose of 100 mg/kg MNNG (39).

Gastric Cancer Model with Gastrin Knockout Mice

Many laboratories have reported that gastrin knockout (GASKO or GAS-/-) mice are susceptible to stomach cancer. Hypergastrinemic mice (INS-GAS) develop corpus cancers, while GAS-/- mice develop antral stomach cancers (40).

Gastric Cancer Model with TFF1 Knockout Mice

In humans and mice, TFF1 (pS2) proteins are normally expressed in the epithelial cells of the stomach mucosa. This protein is abnormally expressed in various diseases and cancers of the gastrointestinal tract. To elucidate the function of TFF1, researchers have created TFF1-/- mice by disrupting the TFF1 gene through homologous recombination. These mice, lacking TFF1 expression, display markedly elongated stomach folds and hyperplastic gastric epithelial development, with 30% developing multifocal intraepithelial or intramucosal carcinomas (41). Additionally, there are many other models used for gastric cancer development.

Colorectal Cancer (CRC) Models

Chemically Induced CRC Models

For the induction of sporadic CRC in animal models, direct inducers like azoxymethane (AOM) and indirect carcinogenesis inducer 1,2-dimethylhydrazine (DMH) are used. DMH is a specific pro-carcinogen agent for the colon, activated in the liver and transported to the intestine with bile. It promotes the production of free radicals causing oxidative damage to the DNA of colon and liver cells. In male Wistar albino rats, the subcutaneous injection of 20 mg/kg DMH once a week for 12 weeks leads to the development of colon adenocarcinomas after an average of 8 months (44). AOM is an active metabolite of DMH, primarily affecting organs like the liver, lungs, and colon, with lesion occurrence proportional to exposure time and administered dose (42).

CRC Model with Enema

This model involves the induction of transient colitis in nude mice using a 3% dextran sulfate sodium (DSS) enema, followed by the transanal transplantation of human colon cancer cells (LS174T), leading to CRC development. Two weeks later, a 95% tumor development rate can be observed in the rectums, although significant metastasis may not be observed (43,44).

Acetic Acid-Induced CRC Model

Irritation of the rectum with a 4% acetic acid solution for two minutes, followed by washing of the distal rectum with 6 ml phosphate-buffered saline, disrupts the epithelial cell layer of the distal rectal mucosa. After these procedures, a CRC cell line (CT-26) or human CRC cell line (HCT-116) can be transanally transplanted to induce CRC (43).

Transanal Low-Dose Electrocoagulation Technique CRC Model

In immunodeficient and nude mice, CRC can be developed by transplanting human (LS-174T and HT-29) and murine (CRL-2638 and CRL-2639) colon cancer cell lines transanally after transanal low-dose mucosal electrocoagulation. This technique results in CRC development in 87.5% of mice (43,45).

Genetically Modified Animals in CRC Models

In the development of CRC, tumor suppressor genes such as APC, DCC, p53, and MCC; oncogenes like K-ras, SRC, and C-myc; DNA repair genes including hMSH2, hMSH6, hMLH1, hPMS1, hPMS2, as well as DC44 and COX-2 genes play roles. Numerous genetically modified animal models have been developed from these genes involved in CRC development (43,46). APCmin animals are genetically modified animals with a mutation in the APC gene. "Min" stands for multiple intestinal neoplasia. Similar to familial adenomatous polyposis cases, APCmin animals develop colorectal adenomas but die within 120 days. Although the autosomal dominant mutation is lethal for APCmin homozygote animals, heterozygote animals develop tumors in the large and small intestines within 60 days (47). p53 gene knockout animals rarely develop colorectal tumors. However, the combination of APCmin and p53 knockout mutations leads to an abnormal increase in crypt numbers compared to APCmin animals. Similarly, administering AOM to APCmin and p53 knockout animals can also lead to CRC development (43,48).

Metastasis Models

In experimental metastasis models, tumor cells are directly applied to the systemic circulation of immunodeficient

animals. Depending on the application site of the vessel, metastasis develops. Injections into the mouse tail vein result in lung or spleen metastasis; application into the portal vein leads to liver metastasis; injection into the carotid results in brain metastasis; direct injection into the tibia or femur causes bone metastasis; intracardiac application leads to bone and bone marrow metastasis. The general advantages of these applications are their rapid development and the elucidation of the biology of metastasis. However, they do not provide information about the early stages and initial phases of metastasis.

Experimental metastasis models are crucial tools for understanding the complex processes involved in the spread of cancer from a primary tumor to distant organs, a hallmark feature of cancer progression. These models are designed to mimic the dissemination of tumor cells through the body, allowing researchers to study the mechanisms underlying metastasis, evaluate the metastatic potential of different cancer cell lines, and test the efficacy of anti-metastatic therapies (49-51).

Injection Models for Studying Metastasis

1. ***Tail Vein Injection:*** This method involves injecting tumor cells into the tail vein of immunodeficient mice, leading to the development of lung and potentially spleen metastases. It simulates the hematogenous spread of cancer cells and is commonly used to study lung metastasis mechanisms.
2. ***Portal Vein Injection:*** By injecting tumor cells into the portal vein, researchers can specifically target liver metastasis. This model is particularly relevant for cancers known to metastasize to the liver, such as CRC.
3. ***Carotid Injection:*** Injection into the carotid artery allows for the modeling of brain metastasis. This approach is used to study cancers that have a propensity to spread to the brain, including lung, breast, and melanoma.
4. ***Direct Bone Injection:*** Tumor cells can be directly injected into the tibia or femur to create models of bone metastasis. This method is crucial for studying bone-tropic cancers, such as prostate and breast cancers, and understanding the bone microenvironment's role in cancer metastasis.
5. ***Intracardiac Injection:*** This technique involves injecting tumor cells into the left ventricle of the heart, leading to widespread dissemination of cancer cells and metastasis to bone and bone marrow, among other sites. It is used to study the metastatic spread to various organs simultaneously.

Advantages of Experimental Metastasis Models

- ***Rapid Development:*** These models allow for the quick establishment of metastases, enabling timely evaluation of therapeutic interventions.
- ***Biological Insight:*** They provide valuable insights into the biological processes of metastasis, including tumor cell intravasation, circulation, extravasation, and colonization of new tissues.

Limitations

- ***Lack of Early Stage Insights:*** While invaluable for studying late-stage metastasis, these models do not adequately represent the initial steps of the metastatic process, such as local invasion and the early interactions between tumor cells and the microenvironment of the primary site.
- ***Artificial Circumstances:*** The direct injection of tumor cells into circulation or specific organs may bypass important natural barriers and interactions that occur in spontaneous metastasis.

PATIENT-DERIVED XENOGRAFT (PDX) MODELS

Xenografts, derived from the Greek term "Xenos" meaning foreign, are sourced from one organism and transplanted into another. These grafts, which include organs, tissues, or living cells, are predominantly implanted in immunocompetent mice for research purposes. Within cancer research, xenografts play a crucial role in addressing fundamental questions by employing animal models that closely mimic tumor progression observed in human patients (52).

Models incorporating primary carcinoma tissues sourced directly from a patient's tumor are established with minimal passage numbers, specifically fewer than ten transfers from human patients, to maintain the integrity of the original tumor characteristics. These include cellular heterogeneity, clinical biomolecular markers, malignant genetic and phenotypic expressions, tumor architecture, and vascular structure (52,53). The rationale behind developing PDX models lies in the anticipation that they will enhance preclinical evaluation, offering predictive insights into the molecular biology of cancer-relevant to human conditions and patient responsiveness to therapy (54). PDX models have proven beneficial for examining cancer metastasis, drug resistance, personalized medicine approaches, and the preclinical discovery and testing of novel anticancer drugs (55).

Primary or metastatic tumors are harvested via surgical or biopsy techniques and conserved as intact tissue structures (54). This extraction method enables the gradual growth of tumor specimens in immune-deficient mice, marking a pivotal shift towards utilizing patient-derived tumor tissue xenograft models in the exploration of anticancer drugs and therapeutic strategies (56).

The most common implantation site in mice is subcutaneous (on the dorsal side), although orthotopic implantation -transplanting into the same organ as the original tumor- serves as a viable alternative for organs like the pancreas, brain, oral cavity, ovary, and breast. Efforts to implant tumors at the renal capsular site have been made to increase engraftment rates, offering the advantage of preserving tumor histology relative to the primary sample across successive xenograft generations and retaining original genetic and phenotypic traits (54,57).

Additionally, experimental metastasis models employ controlled quantities of tumor cells for metastasis induction. These models require a comparatively short duration for metastasis development, allowing for subsequent identification of metastatic sites (58).

The advancement of cancer drug development faces challenges due to the lack of preclinical cancer models that accurately replicate the clinical evaluation of significant new compounds in human patients. These challenges are being addressed through the use of patient-derived tumor xenografts in immunocompetent mice (preclinical models) such as nude mice, severe combined immunodeficiency mice (SCID), nonobese diabetic (NOD)-SCID gamma mice, recombination-activating gene (Rag), and NOD rag gamma mice (52).

An essential aspect of extensive preclinical studies using PDX models is their capability to prioritize potential clinical indications and contribute to the identification of potential drug efficacy biomarkers. In CRC, studies indicate that PDX models with Kirsten rat sarcoma (KRAS)-mutant do not respond to cetuximab, making KRAS wild-type status a well-documented biomarker for this therapy in preclinical research. Similar observations apply to non-small-cell lung cancer. PDX models also serve as adaptable tools for modeling resistance in clinical treatment protocols, notably in ovarian cancer where exposure to cisplatin initiates resistance to the drug in platinum-sensitive models, mirroring clinical outcomes. This model is utilized to explore new therapeutic agents for use in platinum-resistant patients (59). Breast cancer PDX models effectively recapitulate various tumor biology aspects, making them ideal for translational research endeavors (60).

However, PDXs also have limitations, including differing tumor microenvironments and the inability to undergo genetic modifications or incorporate the immune system due to their development in immunodeficient mice. Consequently, they do not fully represent the host immune system's role. Additionally, they are not suitable for testing immunomodulatory approaches in cancer prevention, lack feasibility for high-throughput drug screening, and do not support biobanking due to genetic heterogeneity and epigenomic instability (61).

GENETICALLY ENGINEERED MOUSE MODELS (GEMMs) FOR CANCER RESEARCH

The inception of genetically engineered mouse models (GEMMs) arose from the necessity to bridge the genetic gap between xenografts and the human tumor's genetic makeup. Recent technological advancements have enabled researchers to precisely modify the mouse genome to either conditionally or permanently alter the expression of crucial genes responsible for tumor development. GEMMs serve as pivotal tools in oncology for delineating molecular pathways, allowing for the manipulation of the genome to mimic the loss or gain of function in oncogenes or tumor suppressor genes. This manipulation directly correlates with tumor phenotype manifestations, thereby validating significant genes as therapeutic targets (52,62,63).

GEMMs have been employed for over two decades in cancer research, primarily due to the mouse's genome sharing a 99% similarity with humans. This, coupled with the extensive molecular toolbox available and their small size, makes mice an economical choice for large-scale studies. Transgenic mice models provide an effective platform for preclinical safety assessments and screening, offering insights into gene functions linked to human diseases and potential treatments (52,64).

Various types of GEMMs have been utilized in chemoprevention research. The initial models, or oncomice, facilitated direct evaluation of specific gene functions in tumor genesis. Subsequent generations, such as those with targeted deletions of the Rb1 and Trp53 genes, displayed a spectrum of cancer phenotypes. More sophisticated models include Cre-inducible gene targeting and Tet-regulatable systems, allowing for precise control over gene expression and the modeling of human cancer with high fidelity. These models include gain-of-function tumor virus models and RNAi gene silencing for loss of function, providing a dynamic approach to studying gene expression levels (62,65,66).

The suitability of GEMMs for human disease research and their predictive value for cancer prevention is paramount. For instance, GEMMs of colon cancer are instrumental in examining chemopreventive drugs' effects on tumors originating from genetic mutations. Similarly, GEMMs for mammary cancer have demonstrated the potential of specific drugs to halt the progression to invasive carcinoma. Nutritional interventions targeting different molecular pathways have shown promise in prostate cancer models, aiming to create more predictive models for human preventive measures (63).

Despite their advantages, GEMMs are not without limitations. They often focus on a limited number of genes, not fully representing the complex heterogeneity of human tumors. The development of GEMMs is time-consuming and expensive, with variable and slow tumor evolution compared to human cancers. Critics argue that the relevance of GEMMs to human cancer is unproven, while proponents believe that more appropriately designed experimental conditions could enhance translational research from GEMMs to human cancer. Essential evaluation criteria for GEMMs include pathological assessment, disease progression, tumor microenvironment, molecular pathways, and environmental factors (62,67,68).

Specific mutations in Kras and P53 in lung cancer GEMMs have shed light on the NFκB pathway's role in tumor development, offering potential therapeutic targets. In HCC, GEMMs have highlighted the importance of genetic diversity in understanding tumor subtypes, providing a platform for bench-to-bedside research, especially with systems allowing for the controlled overexpression of genes like MYC, relevant to human carcinomas (69,70).

ORGANOID MODELS IN CANCER RESEARCH

Over the past decade, the advent of organoid technology has transformed the landscape of primary and clinical research in cancer. Organoids, essentially miniaturized versions of human organs and tissues, accurately replicate the functional attributes and architecture of specific organs. Developed from cancer patient-derived tumor cells placed in a tailored extracellular matrix and specific culture media, cancer organoids offer a dynamic model system (71). These organoids enable detailed molecular and cellular studies, supporting the investigation into cancer's origins and paving the way for new cancer stratification methods for both conventional and targeted treatments through early genetic, transcriptomic, and biochemical profiling (52).

Organoids, embedded within a matrix, are cultures of primary epithelial cells proliferating under the influence of Wnt signaling and mitogens. Stem cells from tissues, when embedded into a three-dimensional matrix, form self-sustaining organoid structures (72). Patient-derived organoids (PDOs) not only mirror the primary tumor's structure but also preserve the genetic and expression

profiles, including copy number alterations (CNAs), transcriptional patterns, and mutation profiles. Despite their detailed representation of various cancers such as HCC, breast, pancreatic, and prostate cancers, PDOs typically lack vital components like immune cells, blood vessels, and stromal cells, posing a challenge for their application in cancer immunotherapy (73).

The relationship between cancer and infectious agents, like *S. enterica* in gallbladder cancer or *H. pylori* in gastric cancer, can be elucidated through co-culturing organoids with these pathogens. This approach has highlighted the significant role of chronic *H. pylori* infection in gastric cancer development, with microinjection of *H. pylori* triggering robust inflammatory responses in the gastric epithelium (73,74).

Organoids derived from healthy organs, through genome sequencing of their clonal cultures, facilitate the analysis of organ-specific mutation spectrums and intratumor heterogeneity by developing clonal cultures from different tumor areas. This genetic stability allows for the examination of mutagenic processes over an extended period (63). Comparative studies of lesions from the same individual through organoid cultures help in understanding tumor evolution, with sequencing confirming common origins and shared driver mutations among organoids from primary and metastatic lesions, indicating these mutations preceded metastatic spread (73).

Organoids also serve as an invaluable tool for studying the tumor microenvironment, exploring the supportive niche created by malignant cells and their surrounding environment, which opens avenues for therapeutic targeting. Traditional in vivo models fall short in capturing the intricate paracrine interactions within cancer organoid cultures, necessitating the development of models that simulate these interactions between cancer cells and the tumor microenvironment (75).

Engrafting organoids into murine models establishes organoid xenografts that enable in vivo studies of human cancer biology, offering insights into malignancies like breast and bladder cancer. For instance, orthotopically transplanted pancreatic cancer organoids can recreate a microenvironment closely resembling human pancreatic cancer, overcoming limitations seen in GEMMs of colon cancer, which typically develop tumors in the small intestine. Orthotopic transplantation into the murine cecum offers a more accurate model for colon cancer (76).

Despite their transformative impact on cancer research, organoids have limitations, including the lack of a complete microenvironment, restricting them to epithelial layer studies. The development of non-epithelial organoid cultures and overcoming challenges in drug response, gene expression, and signaling pathways impacted by growth factors are areas needing further exploration (77,78).

COMPUTATIONAL MODELING IN CANCER TREATMENT AND RESEARCH

In the face of challenges such as tumor heterogeneity, disease complexity, and inadequate clinical diagnostics, leveraging the unique genetic constitution, pharmacokinetic properties, and individual characteristics of patients promises to refine therapy personalization for tumor management. Personalized medicine, emphasizing treatments tailored to individual genetic profiles, is poised to redefine future healthcare paradigms. The leap in systems biology, alongside the surge in high-throughput methodologies and the detailed analysis of various -omics, has shifted the research landscape from traditional hypothesis-led investigations to data-centric studies, fostering the advent of precision medicine for complex conditions like cancer (79).

Computational cancer modeling encompasses digital simulations related to cancer therapies and tumor biology (80). These models, applied extensively in cancer diagnosis, monitoring, and growth prediction, utilize 3D imaging for visual representations of tumors or tissues. Despite their reliance on algorithms and diverse software tools, these models face challenges in consistency and replicability, unlike in vitro cancer models (81).

Currently, expansive computational models are under development to decode signal transduction within human cells, employing platforms like PyBioS3 for designing, modeling, and simulating cellular systems. These models, integrating around 50 cancer-associated signaling pathways, draw on data reflecting the impacts of genetic variations and drug mechanisms (82,83).

Personalized prognoses are rendered by customizing models with next-generation sequencing (NGS) derived-omics data, where biological data science furnishes essential resources and effective tools for simulating biological processes. This enables the construction of robust cancer models based on experimental data, disease progression, and therapeutic strategies. Computational and mathematical models elucidate cancer evolution, offering insights into potential biomarkers within signaling pathways and promising therapeutic targets. Cancer signaling network models, grounded in time-lapse experimental data on protein expression and activity, support the validation of drug target effectiveness and simulation forecasts (84).

Enhancing the translation of findings from cancer models to patient care necessitates aligning experimental outcomes with computational model predictions. For instance, assessing drug effects in digital cell or animal models, followed by model adjustments, allows for personalized patient adaptation (83). Computational systems further aid in cancer research and treatment by facilitating image analysis and interpretation, with computerized tomography recently proposed for assessing personalized cancer responses (83).

Advanced computational models promise to refine experimental designs, reducing reliance on animal models, cutting costs, and enhancing the translational relevance of research outcomes. These models offer insights into molecular changes in disease pathways and serve as effective screens for selecting promising candidates, enriching our understanding of disease mechanisms and drug responses (83).

However, current computational models do not capture the full complexity of the biological systems they simulate. A significant barrier to their application in research and clinical settings is the accuracy of their predictions. One approach to overcoming this challenge involves simplifying models through reduction techniques to manage the complexity and improve predictive validity (83,85).

CONCLUSION

In summarizing the extensive landscape of experimental cancer models in laboratory animals, it becomes evident that the field has made significant strides toward understanding and combatting this complex disease. From the utilization of xenograft models, which bridge the gap between in vitro studies and human clinical scenarios, to the sophisticated GEMMs that offer a closer approximation of human cancer genetics and behavior, researchers have a broad arsenal at their disposal for the exploration of cancer biology and the development of therapies.

The advent of organoid cancer modeling and computational cancer models further underscores the rapid evolution of cancer research methodologies, offering more personalized and precise insights into tumor dynamics and treatment responses. Organoids provide a three-dimensional, microenvironment-aware platform that more accurately reflects the cellular complexity and heterogeneity of tumors, enabling targeted therapy testing and the study of cancer-stem cell interactions within a controlled setting. Meanwhile, computational models stand at the forefront of precision medicine, offering predictive insights into cancer progression and treatment outcomes based on individual genetic profiles, thereby paving the way for customized patient care.

As we advance, the integration of these models -each with its unique strengths and limitations- into a cohesive research framework will be paramount. The synergy between traditional in vivo models, innovative organoid cultures, and computational simulations promises to enhance our understanding of cancer's molecular underpinnings, improve the efficacy of therapeutic interventions, and ultimately, lead to more effective, personalized cancer treatments.

The future of cancer research and treatment lies in the continued refinement of these models, increased collaboration between disciplines, and the integration of emerging technologies. By leveraging the distinct advantages of each model and addressing their respective challenges, the scientific community can hope to unravel the complexities of cancer, offering new hope for patients around the world. The journey from bench to bedside, while fraught with challenges, is illuminated by the potential of these experimental models to transform cancer diagnosis, treatment, and prevention, moving us closer to the ultimate goal of curing this multifaceted disease.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: MAE; Design: MAE; Data Collection/Processing: MAE; Analysis/Interpretation: MAE; Literature Review: MAE; Drafting/Writing: MAE; Critical Review: MAE.

REFERENCES

1. Loeuillard E, Fischbach SR, Gores GJ, Ilyas SI. Animal models of cholangiocarcinoma. Biochim Biophys Acta Mol Basis Dis. 2019;1865(5):982-92.
2. Kwon MC, Berns A. Mouse models for lung cancer. Mol Oncol. 2013;7(2):165-77.
3. Gremonprez F, Willaert W, Ceelen W. Animal models of colorectal peritoneal metastasis. Pleura Peritoneum. 2016;1(1):23-43.
4. Fichtner I, Rolff J, Soong R, Hoffmann J, Hammer S, Sommer A, et al. Establishment of patient derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. Clin Cancer Res. 2008;14(20):6456-68.
5. Hodgkinson CL, Morrow CJ, Li Y, Metcalf RL, Rothwell DG, Trapani F, et al. Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. Nat Med. 2014;20(8):897-903.
6. Walrath JC, Hawes JJ, Van Dyke T, Reilly KM. Genetically engineered mouse models in cancer research. Adv Cancer Res. 2010;106:113-64.
7. Ittmann M, Huang J, Radaelli E, Martin P, Signoretti S, Sullivan R, et al. Animal models of human prostate cancer: the consensus report of the New York meeting of the Mouse Models of Human Cancers Consortium Prostate Pathology Committee. Cancer Res. 2013;73(9):2718-36.
8. Kemp CJ. Animal models of chemical carcinogenesis: driving breakthroughs in cancer research for 100 years. Cold Spring Harb Protoc. 2015;2015(10):865-74.
9. Almosailleakh M, Schwaller J. Murine models of acute myeloid leukaemia. Int J Mol Sci. 2019;20(2):453.
10. Rein A. Murine leukemia viruses: objects and organisms. Adv Virol. 2011;2011:403419.
11. Kellar A, Egan C, Morris D. Preclinical murine models for lung cancer: clinical trial applications. Biomed Res Int. 2015;2015:621324.
12. Üstüner C, Entok E. Experimental animal models for lung cancer. Nucl Med Semin. 2019;5(1):40-8.
13. Jin Y, Liu M, Sa R, Fu H, Cheng L, Chen L. Mouse models of thyroid cancer: Bridging pathogenesis and novel therapeutics. Cancer Lett. 2020;469:35-53.
14. Zhang L, Gaskins K, Yu Z, Xiong Y, Merino MJ, Kebebew E. An in vivo mouse model of metastatic human thyroid cancer. Thyroid. 2014;24(4):695-704.
15. Hiroshima Y, Maawy A, Zhang Y, Zhang N, Murakami T, Chishima T, et al. Patient-derived mouse models of cancer need to be orthotopic in order to evaluate targeted anti-metastatic therapy. Oncotarget. 2016;7(44):71696-702.
16. Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. JAMA. 2013;309(14):1493-501.
17. Cho JY, Sagartz JE, Capen CC, Mazzaferri EL, Jhiang SM. Early cellular abnormalities induced by RET/PTC1 oncogene in thyroid-targeted transgenic mice. Oncogene. 1999;18(24):3659-65.
18. Powell DJ Jr, Russell JP, Li G, Kuo BA, Fidanza V, Huebner K, et al. Altered gene expression in immunogenic poorly differentiated thyroid carcinomas from RET/PTC3p53-/- mice. Oncogene. 2001;20(25):3235-46.

19. Miller KA, Yeager N, Baker K, Liao XH, Refetoff S, Di Cristofano A. Oncogenic Kras requires simultaneous PI3K signaling to induce ERK activation and transform thyroid epithelial cells in vivo. Cancer Res. 2009;69(8):3689-94.
20. Kirschner LS, Qamri Z, Kari S, Ashtekar A. Mouse models of thyroid cancer: A 2015 update. Mol Cell Endocrinol. 2016;421:18-27.
21. Pozo K, Castro-Rivera E, Tan C, Plattner F, Schwach G, Siegl V, et al. The role of Cdk5 in neuroendocrine thyroid cancer. Cancer Cell. 2013;24(4):499-511.
22. Antico Arciuch VG, Russo MA, Dima M, Kang KS, Dasrath F, Liao XH, et al. Thyrocyte-specific inactivation of p53 and Pten results in anaplastic thyroid carcinomas faithfully recapitulating human tumors. Oncotarget. 2011;2(12):1109-26.
23. Tsubura A, Lai YC, Miki H, Sasaki T, Uehara N, Yuri T, et al. Review: Animal models of N-methyl-N-nitrosourea-induced mammary cancer and retinal degeneration with special emphasis on therapeutic trials. In Vivo. 2011;25(1):11-22.
24. Bazm MA, Naseri L, Khazaei M. Methods of inducing breast cancer in animal models: a systematic review. World Cancer Res J. 2018;5(4):e1182.
25. Sydnor KL, Cockrell B. Influence of estradiol-17-beta, progesterone and hydrocortisone on 3-methylcholanthrene-induced mammary cancer in intact and ovariectomized Sprague-Dawley rats. Endocrinology.1963;73:427-32.
26. Lai H, Singh NP. Oral artemisinin prevents and delays the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer in the rat. Cancer Lett. 2006;231(1):43-8.
27. Gao ZG, Tian L, Hu J, Park IS, Bae YH. Prevention of metastasis in a 4T1 murine breast cancer model by doxorubicin carried by folate conjugated pH sensitive polymeric micelles. J Control Release. 2011;152(1):84-9.
28. Calaf GM, Hei TK. Establishment of a radiation-and estrogen-induced breast cancer model. Carcinogenesis. 2000;21(4):769-76.
29. Vesselinovitch SD, Koka M, Mihailovich N, Rao KV. Carcinogenicity of diethylnitrosamine in newborn, infant, and adult mice. J Cancer Res Clin Oncol. 1984;108(1):60-5.
30. Zhang HE, Henderson JM, Gorrell MD. Animal models for hepatocellular carcinoma. Biochim Biophys Acta Mol Basis Dis. 2019;1865(5):993-1002.
31. Kisseleva T, Cong M, Paik Y, Scholten D, Jiang C, Benner C, et al. Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci U S A. 2012;109(24):9448-53.
32. Zaldivar MM, Pauels K, von Hundelshausen P, Berres ML, Schmitz P, Bornemann J, et al. CXC chemokine ligand 4 (Cxcl4) is a platelet-derived mediator of experimental liver fibrosis. Hepatology. 2010;51(4):1345-53.
33. Salguero Palacios R, Roderfeld M, Hemmann S, Rath T, Atanasova S, Tschuschner A, et al. Activation of hepatic stellate cells is associated with cytokine expression in thioacetamide-induced hepatic fibrosis in mice. Lab Invest. 2008;88(11):1192-203.
34. Yang MH, Chen WJ, Fu YS, Huang B, Tsai WC, Arthur Chen YM, et al. Utilizing glycine N-methyltransferasegene knockout mice as a model for identification of missing proteins in hepatocellular carcinoma. Oncotarget. 2017;9(1):442-52.
35. Thamavit W, Pairojkul C, Tiwawech D, Itoh M, Shirai T, Ito N. Promotion of cholangiocarcinogenesis in the hamster liver by bile duct ligation after dimethylnitrosamine initiation. Carcinogenesis. 1993;14(11):2415-7.
36. Praet MM, Roels HJ. Histogenesis of cholangiomas and cholangiocarcinomas in thioacetamide fed rats. Exp Pathol. 1984;26(1):3-14.
37. Maronpot RR, Giles HD, Dykes DJ, Irwin RD. Furan-induced hepatic cholangiocarcinomas in Fischer 344 rats. Toxicol Pathol. 1991;19(4 Pt 2):561-70.
38. Tatematsu M, Yamamoto M, Shimizu N, Yoshikawa A, Fukami H, Kaminishi M, et al. Induction of glandular stomach cancers in Helicobacter pylori-sensitive Mongolian gerbils treated with N-methyl-N-nitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine in drinking water. Jpn J Cancer Res. 1998;89(2):97-104.
39. Hayakawa Y, Fox JG, Gonda T, Worthley DL, Muthupalani S, Wang TC. Mouse models of gastric cancer. Cancers (Basel). 2013;5(1):92-130.
40. Poh AR, O'Donoghue RJ, Ernst M, Putoczki TL. Mouse models for gastric cancer: Matching models to biological questions. J Gastroenterol Hepatol. 2016;31(7):1257-72.
41. Lefebvre O, Chenard MP, Masson R, Linares J, Dierich A, LeMeur M, et al. Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. Science. 1996;274(5285):259-62.
42. Bobek P, Galbavy S, Ozdin L. Effect of oyster mushroom (Pleurotus ostreatus) on pathological changes in dimethylhydrazine-induced rat colon cancer. Oncol Rep. 1998;5(3):727-30.
43. Mittal VK, Bhullar JS, Jayant K. Animal models of human colorectal cancer: Current status, uses and limitations. World J Gastroenterol. 2015;21(41):11854-61.
44. Kishimoto H, Momiyama M, Aki R, Kimura H, Suetsugu A, Bouvet M, et al. Development of a clinically-precise mouse model of rectal cancer. PLoS One. 2013;8(11):e79453.
45. Zigmond E, Halpern Z, Elinav E, Brazowski E, Jung S, Varol C. Utilization of murine colonoscopy for orthotopic implantation of colorectal cancer. PLoS One. 2011;6(12):e28858.
46. DE-Souza ASC, Costa-Casagrande TA. Animal models for colorectal cancer. Arq Bras Cir Dig. 2018;31(2):e1369.
47. Yamada Y, Mori H. Multistep carcinogenesis of the colon in Apc(Min/+) mouse. Cancer Sci. 2007;98(1):6-10.
48. Sakai H, Tsukamoto T, Yamamoto M, Shirai N, Iidaka T, Hirata A, et al. High susceptibility of nullizygous p53 knockout mice to colorectal tumor induction by 1,2-dimethylhydrazine. J Cancer Res Clin Oncol. 2003;129(6):335-40.
49. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer. 2003;3(6):453-8.
50. Nguyen DX, Bos PD, Massagué J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009;9(4):274-84.
51. Weiss L. Metastatic inefficiency. Adv Cancer Res. 1990;54:159-211.
52. Vandamme TF. Use of rodents as models of human diseases. J Pharm Bioallied Sci. 2014;6(1):2-9.

53. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9(6):338-50.
54. Kopetz S, Lemos R, Powis G. The promise of patient-derived xenografts: the best laid plans of mice and men. Clin Cancer Res. 2012;18(19):5160-2.
55. Jin K, Teng L, Shen Y, He K, Xu Z, Li G. Patient-derived human tumour tissue xenografts in immunodeficient mice: a systematic review. Clin Transl Oncol. 2010;12(7):473-80.
56. Shimosato Y, Kameya T, Nagai K, Hirohashi S, Koide T, Hayashi H, et al. Transplantation of human tumors in nude mice. J Natl Cancer Inst. 1976;56(6):1251-60.
57. Cutz JC, Guan J, Bayani J, Yoshimoto M, Xue H, Sutcliffe M, et al. Establishment in severe combined immunodeficiency mice of subrenal capsule xenografts and transplantable tumor lines from a variety of primary human lung cancers: potential models for studying tumor progression-related changes. Clin Cancer Res. 2006;12(13):4043-54.
58. Wettersten HI, Ganti S, Weiss RH. Metabolomic profiling of tumor-bearing mice. Methods Enzymol. 2014;543:275-96.
59. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998-1013.
60. Dobrolecki LE, Airhart SD, Alferez DG, Aparicio S, Behbod F, Bentires-Alj M, et al. Patient-derived xenograft (PDX) models in basic and translational breast cancer research. Cancer Metastasis Rev. 2016;35(4):547-73.
61. Pillai SPS, Uthamanthil RK. PDX models: history and development. In: Uthamanthil R, Tinkey P, editors. Patient derived tumor xenograft models: promise, potential and practice. London: Elsevier; 2017. p.1-12.
62. Abate-Shen C, Brown PH, Colburn NH, Gerner EW, Green JE, Lipkin M, et al. The untapped potential of genetically engineered mouse models in chemoprevention research: opportunities and challenges. Cancer Prev Res (Phila). 2008;1(3):161-6.
63. Green JE, Hudson T. The promise of genetically engineered mice for cancer prevention studies. Nat Rev Cancer. 2005;5(3):184-98.
64. Brandon-Warner E, Schrum LW, Schmidt CM, McKillop IH. Rodent models of alcoholic liver disease: of mice and men. Alcohol. 2012;46(8):715-25.
65. Hanahan D, Wagner EF, Palmiter RD. The origins of oncomice: a history of the first transgenic mice genetically engineered to develop cancer. Genes Dev. 2007;21(18):2258-70.
66. Van Dyke T, Jacks T. Cancer modeling in the modern era: progress and challenges. Cell. 2002;108(2):135-44.
67. Frese KK, Tuveson DA. Maximizing mouse cancer models. Nat Rev Cancer. 2007;7(9):654-58.
68. Olive KP, Tuveson DA. The use of targeted mouse models for preclinical testing of novel cancer therapeutics. Clin Cancer Res. 2006;12(18):5277-87.
69. Porru M, Leonetti C. The role of mouse models in translational cancer research: present and future directions. Transl Med Rep. 2020;4(1):64-9.
70. Brown ZJ, Heinrich B, Greten TF. Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research. Nat Rev Gastroenterol Hepatol. 2018;15(9):536-54.
71. Voskoglou-Nomikos T, Pater JL, Seymour L. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clin Cancer Res. 2003;9(11):4227-39.
72. Sachs N, Clevers H. Organoid cultures for the analysis of cancer phenotypes. Curr Opin Genet Dev. 2014;24:68-73.
73. Huch M, Knoblich JA, Lutolf MP, Martinez-Arias A. The hope and the hype of organoid research. Development. 2017;144(6):938-41.
74. Yang H, Sun L, Liu M, Mao Y. Patient-derived organoids: a promising model for personalized cancer treatment. Gastroenterol Rep (Oxf). 2018;6(4):243-5.
75. Tuveson D, Clevers H. Cancer modeling meets human organoid technology. Science. 2019;364(6444):952-5.
76. Kim J, Koo BK, Knoblich JA. Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol.2020;21(10):571-84.
77. Lo YH, Karlsson K, Kuo CJ. Applications of organoids for cancer biology and precision medicine. Nat Cancer. 2020;1(8):761-73.
78. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, et al. Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov. 2017;7(5):462-77.
79. Savoia C, Volpe M, Grassi G, Borghi C, Agabiti Rosei E, Touyz RM. Personalized medicine-a modern approach for the diagnosis and management of hypertension. Clin Sci (Lond). 2017;131(22):2671-85.
80. Barbolosi D, Ciccolini J, Lacarelle B, Barlési F, André N. Computational oncology--mathematical modelling of drug regimens for precision medicine. Nat Rev Clin Oncol. 2016;13(4):242-54.
81. Morgan MM, Johnson BP, Livingston MK, Schuler LA, Alarid ET, Sung KE, et al. Personalized in vitro cancer models to predict therapeutic response: challenges and a framework for improvement. Pharmacol Ther. 2016;165:79-92.
82. Wierling C, Kessler T, Ogilvie LA, Lange BM, Yaspo ML, Lehrach H. Network and systems biology: essential steps in virtualising drug discovery and development. Drug Discov Today Technol. 2015;15:33-40.
83. Ogilvie LA, Kovachev A, Wierling C, Lange BMH, Lehrach H. Models of models: a translational route for cancer treatment and drug development. Front Oncol. 2017;7:219.
84. Jean-Quartier C, Jeanquartier F, Jurisica I, Holzinger A. In silico cancer research towards 3R. BMC Cancer. 2018;18(1):408.
85. Jones W, Alasoo K, Fishman D, Parts L. Computational biology: deep learning. Emerging Top Life Sci. 2017;1(3):257-74.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1503737

Duzce Med J, 2024;26(S1):47-54
Düzce Tıp Fak Derg, 2024;26(S1):47-54

Experimental Animal Models in Respiratory Diseases

Solunum Hastalıklarında Deneysel Hayvan Modelleri

Pınar YILDIZ GÜLHAN[1,2]
0000-0002-5347-2365

[1]Department of Chest Diseases, Düzce University Faculty of Medicine, Düzce, Türkiye
[2]Experimental Animals Application and Research Center, Düzce University, Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Pınar YILDIZ GÜLHAN
pinaryildiz691@hotmail.com

Received / Geliş Tarihi : 23.04.2024
Accepted / Kabul Tarihi : 01.06.2024
Available Online / Çevrimiçi Yayın Tarihi : 23.06.2024

ABSTRACT

Respiratory diseases are among the leading causes of morbidity and mortality worldwide. Various animal models are used to understand the pathogenesis of these diseases and develop novel therapeutic strategies. Each model offers the opportunity to examine the multifaceted nature of pulmonary health, from common afflictions such as asthma and chronic obstructive pulmonary disease (COPD) to interstitial lung diseases. While these models provide a unique opportunity to understand normal physiology and disease pathophysiology and to test potential treatments for diseases, all animal models have inherent limitations. This review focuses on experimental models of common respiratory diseases such as asthma, COPD, and pulmonary fibrosis. The advantages, disadvantages, and translational potential to human disease of each model are discussed. Asthma models include mice, guinea pigs, and Drosophila, while elastase-induced emphysema, cigarette smoke exposure, and genetically modified mice are used for COPD. For pulmonary fibrosis, bleomycin, adenoviral TGF-β1 vector, silica, and genetically modified mice models are available. These models have provided valuable insights into disease mechanisms and aided in identifying new therapeutic targets. However, it is important to note that no single model fully recapitulates human disease, and each has its own unique advantages and limitations. Therefore, careful consideration of the translatability of findings from preclinical studies to humans is crucial.
Keywords: Experimental animal; lung diseases; pathogenesis.

ÖZ

Akciğer hastalıkları, dünya genelinde morbidite ve mortalitenin önde gelen nedenlerindendir. Bu hastalıkların patogenezini anlamak ve yeni tedavi stratejileri geliştirmek için çeşitli hayvan modelleri kullanılmaktadır. Her model, astım ve kronik obstrüktif akciğer hastalığı (KOAH) gibi yaygın rahatsızlıklardan interstisyel akciğer hastalıklarına kadar akciğer sağlığının çok yönlü doğasını inceleme fırsatı sunar. Bu modeller normal fizyolojiyi ve hastalık patofizyolojisini anlamak ve hastalıklara yönelik potansiyel tedavileri test etmek için eşsiz bir fırsat sağlarken, tüm hayvan modellerinin doğası gereği sınırlamaları vardır. Bu derlemede, astım, KOAH ve pulmoner fibroz gibi yaygın akciğer hastalıklarının deneysel modellerine odaklanılmıştır. Her modelin avantajları, dezavantajları ve insan hastalığına translasyonel potansiyeli tartışılmaktadır. Astım modelleri arasında fareler, kobaylar ve Drosophila bulunurken, KOAH için elastazla indüklenen amfizem, sigara dumanına maruziyet ve genetik olarak değiştirilmiş fareler kullanılmaktadır. Pulmoner fibroz için ise bleomisin, adenoviral TGF-β1 vektörü, silika ve genetik olarak değiştirilmiş fare modelleri mevcuttur. Bu modeller, hastalık mekanizmalarına dair değerli bilgiler sağlamış ve yeni terapötik hedeflerin belirlenmesine yardımcı olmuştur. Bununla birlikte, her modelin insan hastalığını tam olarak yansıtmadığı ve her birinin kendine özgü avantajları ve sınırlamaları olduğu unutulmamalıdır. Bu nedenle, klinik öncesi çalışmalarda elde edilen bulguların insanlara uygulanabilirliğini dikkatlice değerlendirmek önemlidir.
Anahtar kelimeler: Akciğer hastalıkları; deney hayvanı; patogenez.

INTRODUCTION

Animal models have been instrumental in investigating normal physiology, the pathophysiology of diseases, and testing the safety and efficacy of novel therapeutics in preclinical studies (1). From commonly encountered afflictions such as asthma and chronic obstructive pulmonary disease (COPD) to the more nuanced realms of interstitial lung diseases, each model offers a unique lens through which we can examine the multifaceted nature of pulmonary health. While these models provide a unique opportunity to understand normal physiology, and disease pathophysiology, and test potential therapies for diseases, all animal models inherently possess limitations (2). The first limitation is that the genetic and physiological differences between experimental animals and humans can lead to an incomplete replication of human diseases. For instance, the lung structure and immune system of mice differ from those of humans. Therefore, lung disease models created in mice may not reflect all the characteristics of the disease in humans. The second limitation is that lung disease models created in experimental animals may not fully reflect the progression and severity of the disease. In humans, lung diseases often develop over a long period, whereas in experimental animals, this process can be accelerated. This can affect the natural course of the disease and the response to treatment. In addition to these limitations, ethical concerns and the welfare of experimental animals should also be considered. The use of experimental animals should be reduced as much as possible and alternative methods should be investigated (3).

In this review, it was aimed to write about different experimental models of respiratory diseases aiming to unravel the complex mechanisms underlying their pathophysiology.

ASTHMA MODEL

Asthma, a global health issue affecting an estimated 300 million people across all ages, is characterized by fluctuating respiratory symptoms like wheezing, shortness of breath, chest tightness, and cough, alongside varying limitations in exhaling air. The condition's prevalence is on the rise. This chronic inflammatory disease causes structural changes in the airways, including increased mucus production, tissue scarring, abnormal cell growth, and enlarged airway muscles (4,5). Animal models play a crucial role in deciphering the underlying disease mechanisms and assessing the safety and effectiveness of potential new therapies before they are tested in humans (6).

Various animal species have been utilized in experimental asthma models, including Drosophila, rats, guinea pigs, cats, dogs, pigs, primates, and horses. However, in the last two decades, the most commonly studied species have been mice, particularly BALB/c mice (7).

Drosophila Melanogaster

The respiratory organ of the fruit fly *D. melanogaster* is the tracheal system, a network of epithelial tubes that branch throughout the body, carrying gases to tissues and organs. This network takes in gases from respiratory openings called spiracles, which provide an external connection to the system, and transports them to tissues and organs through tracheal branches that branch out in the body (8).

In particular, the fruit fly *Drosophila* has been successfully positioned as a genetically tractable model for studying the molecular architecture underlying various chronic lung diseases, such as asthma, COPD, and lung cancer. Although insects share surprising commonalities with human lungs in terms of structure, physiology, organogenesis, and innate immune system, they have a very simple, entirely epithelial airway system (9).

Guinea Pig

Guinea pigs do not naturally have asthma; however, they are known to exhibit immediate hypersensitivity reactions in their lungs (10). Ovalbumin (OVA) is commonly used as a sensitizer in inducible animal models (such as mice and rats) of T2-heavy asthma. Guinea pigs can also be sensitized to OVA or other stimuli, triggering IgE-mediated mechanisms similar to the human asthma phenotype, with eosinophilia and increased airway responsiveness (11).

OVA can be administered through various routes, including peritoneal, subcutaneous, and aerosol forms. Low-dose OVA (10 μg) can induce early asthmatic responses (EARs) with IgE and IgG1 production (12-13). Higher doses (100 μg) can trigger both early and late asthmatic responses (LARs) (11).

In asthmatic humans, acute allergic hypersensitivity reactions in the airways (airway smooth muscle contraction, eosinophil infiltration, airway hyperresponsiveness, and mucus production) are partially mediated by activation of histamine H1 and cysteinyl leukotriene (cysLT)-1 receptors. These receptor activations have also been observed in guinea pigs (14). Due to these similarities, guinea pigs have played a significant role in the development of asthma treatments; for example, leukotriene receptor antagonists (such as Montelukast) and phosphodiesterase (PDE3/4) inhibitors (such as ensifentrine) have been developed in these models (3,11).

Despite their benefits, guinea pigs have some limitations as an asthma model. They have obligate nasal breathing due to their long soft palate, possess seven lung lobes with a specific branching pattern, and their lung parenchyma is more fragile than that of humans. Chronic asthma is generally not inducible in guinea pigs, as they develop tolerance to allergens and do not exhibit non-specific hyperresponsiveness. Additionally, bronchoconstriction in guinea pigs is mostly mediated by histamine, which limits the translational value for human asthma since antihistamines have limited efficacy in humans. Other challenges include a longer gestation period and limited availability of reactive and transgenic lines. Efforts are ongoing to develop more assays, identify translatable markers, and increase the availability of monoclonal antibodies to make research in guinea pigs more feasible (11,15).

Mice Models

Mice, not prone to developing asthma naturally, require artificial induction of asthma-like reactions for research purposes. Acute allergic responses to inhaled allergens in mice are commonly studied to understand the immunological and inflammatory mechanisms underlying asthma and identify novel targets for managing allergic inflammation. Various allergens, such as OVA, house dust mites, fungal extracts, and cockroach extracts, are utilized depending on the specific condition being replicated (16).

Allergens used in animal models are OVA, house dust mites such as *Dermatophagoides pteronyssinus* (*Der p*) or *D. farinae* (*Der f*), mite allergens (Der p 1, Der f 1, Der p 23, etc.), fungi (*Aspergillus fumigatus*, *Alternaria alternata*), cockroach extracts, Ascaris antigens, cotton dust, ragweed and latex (*Hevea brasiliensis*). The allergen selected depends on the condition to be replicated and can be used individually or in combination (17).

BALB/c mice, known for their tendency to develop a strong T helper cell 2 (Th2)-biased immune response, are the most frequently used strain in antigen-challenge models (18). However, other strains like C57BL/6 and A/J have also been employed successfully (19). OVA, derived from chicken egg, is a widely used allergen that effectively induces allergic lung inflammation in laboratory animals.

Mouse models for allergic asthma involve sensitizing the animal to a foreign protein, typically OVA, through intraperitoneal injections with an adjuvant to enhance immunogenicity (20). Following sensitization, the animal is challenged with further antigen exposure through aerosol inhalation or nasal drip, triggering an inflammatory response in the lungs. This response is characterized by eosinophil influx, epithelial thickening, and airway hyperresponsiveness. The specific methods used for sensitization and challenge can vary among investigators, but a typical approach involves two-spaced intraperitoneal injections followed by a week of rest and then daily exposure to 1% OVA aerosol for three days (21). Allergic inflammation typically peaks one or two days after the final challenge, although the precise time course of this process remains incompletely understood.

Chronic allergen challenge models expose the airways to low allergen levels repeatedly for extended periods, up to 12 weeks. These models often utilize various allergens, including OVA, house dust mite extract, or grass pollen, without always requiring adjuvant co-administration. They simulate long-term allergen exposure and its effects on airway inflammation and remodeling (22-24).

While mouse allergen challenge models are invaluable in asthma research, the complex and diverse nature of asthma makes it unlikely that any single model can fully recapitulate the clinical disease. Therefore, studies focus on modeling specific asthma phenotypes rather than attempting to replicate all features of asthma in a single model. This approach allows for targeted investigation of particular disease mechanisms and potential therapeutic interventions.

- Acute Allergen Challenge Models: These are commonly used to investigate lung inflammation and airway hyperresponsiveness. However, they have limitations, particularly in relating findings to chronic asthma.
- Chronic Allergen Challenge Models: These appear to be able to reproduce some features of chronic asthma. This allows them to address fundamental questions regarding both the pathogenesis of asthma and new therapeutic approaches.

Both acute and chronic allergen challenge models have limitations that need to be considered when integrating findings from the animal model into human disease. These limitations include:

- The lung structure of mice differs from that of humans.
- The mouse immune system responds differently from the human immune system.
- Mouse models may not fully reflect environmental factors.

Therefore, while mouse allergen challenge models are an important tool in asthma research, careful consideration must be given to how the results translate to human disease (16).

CHRONIC OBSTRUCTIVE PULMONARY DISEASE MODEL

COPD is a debilitating, progressive lung disease characterized by breathing difficulties, chronic inflammation, and tissue breakdown. Due to ethical considerations, animal models are indispensable in COPD research involving human subjects. These models enhance our understanding of COPD's underlying mechanisms, including its physiology, pathophysiology, and treatment responses. While not perfectly replicating all aspects of the human disease, animal models provide valuable insights into the processes involved in COPD (25).

Various approaches exist to simulate COPD in animal models. These include exposing animals to cigarette smoke (the primary cause of COPD), inflammatory stimuli like lipopolysaccharide, proteolytic enzymes such as elastase, and genetic modifications. The choice of model depends on the specific research goals (26-28).

The subsequent discussion will delve into the diverse models employed in COPD research.

Elastase-Induced Animal Model of Emphysema

Since the 1970s, the role of elevated protein breakdown in COPD's development has garnered significant research interest. In COPD patients, an imbalance exists between protein-degrading elastases (MMP family) and anti-elastases in lung tissues. Excessive elastase release from inflammatory cells can damage the lung parenchyma, leading to emphysema. Notably, genetic alpha-1-antitrypsin deficiency, which disrupts the elastase-antielastase balance, has been directly linked to emphysema-like changes (29,30).

To model emphysema, a simple and cost-effective method involves instilling elastase drops into the trachea. This approach disrupts the protease-antiprotease balance in lung tissue, both removing protective factors and generating numerous inflammatory factors, thereby accelerating alveolar wall rupture and fusion, ultimately leading to emphysema. Commonly used elastases in this model include:

Papain

A plant-derived proteolytic enzyme and the first elastase used to create an emphysema model. In the 1960s, a successful rat emphysema model was first created using papain. Although the use of different doses of papain was tried in subsequent studies, no significant difference was found between doses. Therefore, the instillation of 2 mg/kg papain into the trachea in a single dose has been accepted as a relatively suitable method (31).

Porcine Pancreatic Elastase (PPE)

Commonly used to create emphysema models in animals, PPE is obtained from porcine pancreas. PPE not only acts as a protease, disrupting the protease-antiprotease balance but also acts as an oxidant, causing oxidative stress.

Thanks to this dual effect, the alveoli expand significantly in the experimental animal model. Therefore, PPE is often used to create emphysema. Usage doses vary between 6 and 24 U. Methods for creating emphysema with PPE generally include intratracheal instillation, tracheotomy injection, and atomizer inhalation. It usually takes 4 to 6 weeks to create emphysema-like changes with these methods (30,32).

Human Neutrophil Elastase (HNE)

A serine protease that plays an important role in the inflammatory process of COPD. Protease/anti-protease imbalance causes excessive hydrolysis of elastin and structural proteins that give elasticity to lung tissue by extracellular HNE. Since HNE's ability to enter the alveolar septum and break down elastic fibers is weak, it is rarely used to create emphysema today (33).

While papain is the earliest method used, PPE is more commonly preferred due to its ease of use and effectiveness. HNE is not preferred due to its weak efficacy (30)

Emphysema Model Creation with Cigarette Smoke Exposure

In the realm of COPD research, cigarette smoke exposure holds significant importance, as approximately 90% of COPD patients are smokers. Cigarette smoke exposure is a major risk factor for emphysema, and animal models employing cigarette smoke exposure have been instrumental in understanding its development.

In 1990, Wright et al. (34) first succeeded in creating a guinea pig emphysema model through cigarette smoke exposure. They found that long-term smoking caused changes in the center of the lobules, creating emphysema, as in humans. Long-term cigarette smoke exposure in animals can cause an inflammatory response in the lungs, mostly consisting of macrophages (35). As a result, the bronchial lumen narrows and the bronchial cartilage tissue is damaged. This leads to alveolar rupture, fusion, and emphysema formation, just as in humans exposed to cigarette smoke. Passive smoking-induced emphysema can mimic the pathogenesis of human emphysema as closely as possible and provide a basis for basic and clinical research on human emphysema.

The airway and lung structures of experimental animals differ between species and from humans. Guinea pigs are among the most sensitive animals to cigarette smoke. Rats, on the other hand, show some resistance to cigarette smoke, but there are also differences in sensitivity between different rat species. The experimental duration for a passive smoking-induced COPD animal model is relatively long and its stability is also relatively low (36).

Cigarette smoke exposure can be broadly classified in two ways; the first method is the partial exposure (nose or head only) method. van der Strate et al. (37) studied C57BL/6J mice that inhaled cigarette smoke through their noses twice a day, 2 cigarettes each time, 10 puffs per cigarette. The results showed that pulmonary alveoli expanded with increasing exposure time. At the same time, B lymphocytes in the lung tissues of smoking mice increased, similar to those seen in human emphysema.

The other method is the whole-body exposure method (38). In this method, the experimental animal is placed in a box completely filled with smoke. Valenca et al. (39) exposed C57BL/6 mice to cigarette smoke 3 times a day, 3 cigarettes each time. After 60 days, emphysema-like changes were observed in the lungs, with increased alveolar macrophages, extracellular matrix changes, and increased MMP-12 expression.

The passive smoking method is quite popular due to its low cost, simple application, high success rate, and elimination of experimental differences in a more objective environment. The duration of cigarette smoke exposure in the experimental emphysema model may vary depending on various factors such as the type of cigarette used, the method of exposure, duration and frequency, smoke density, and the species and age of the animals.

Animal Models of Chemical-Induced Emphysema

Many chemicals common in air pollution, such as nitric oxide (NO_2), can cause emphysema and inflammation in animals. These chemicals include:

- Nitric Oxide (NO_2)
- Lipopolysaccharide (LPS)
- Ozone (O_3)
- Cadmium chloride ($CdCl_2$)
- Hyaluronidase enzyme (intravenous injection)
- Ovalbumin dust (inhalation)

Nitric Oxide (NO_2)

In an experimental setting, emphysema can be induced in animals by controlling the NO_2 concentration and inhalation duration. Wegmann et al. (40) observed that long-term exposure of mice to 20 x 106 volume ratio NO_2 for 14 hours a day for 25 days resulted in emphysema due to oxidative stress.

Lipopolysaccharide (LPS)

Lipopolysaccharide primarily causes inflammation in airway and lung tissue by stimulating neutrophils, monocytes, and endothelial cells. These cells secrete a series of inflammatory mediators such as TNF-α, IL-1, and trigger protease-antiprotease imbalance, leading to emphysema (41).

Cadmium Chloride ($CdCl_2$)

Snider et al. (42) created an animal model of emphysema by instilling 0.5 ml of 0.025% $CdCl_2$ solution into the trachea of golden-mantled ground squirrels in a single dose.

Gene-Knockout Animal Models of Emphysema

Advancements in molecular biology and the Human Genome Project have enabled scientists to explore the intricate relationship between genes and diseases, leading to the development of emphysema animal models through genetic manipulation. By editing genes related to emphysema, researchers hope to uncover new insights into this complex condition.

Spontaneous emphysema, initially discovered in spotted mice in the 1970s, has been linked to abnormal connective tissue mechanisms and cross-linking of collagen and elastin. Long-term animal experiments have identified spontaneous emphysema in various mouse strains, including Tit-skin, Beige, Blotchy, and Palliad mice (43).

With the advancement of molecular biology, emphysema animal models created using gene knockout methods are widely utilized in emphysema research. In recent years, an increasing number of studies have employed gene knockout techniques to replicate animal models (30).

Liang et al. (44) found that mice with a knockout of the Abhd2 gene exhibited emphysema-like changes in the

lungs due to excessive inflammation cytokines and protease gene expression, increased macrophage numbers, abnormal apoptosis, and resistance to the deficiency or loss of protease inhibitors. The models showed a similar progressive emphysema development in formation, development process, and clinical pathology. Therefore, examining the genetic susceptibility and environmental factors of emphysema is of great importance.

ANIMAL MODELS FOR IDIOPATHIC PULMONARY FIBROSIS (IPF)

Idiopathic pulmonary fibrosis (IPF) is a chronic, incurable lung disease characterized by fibrosis (stiffness), inflammation, and tissue damage in the lungs (45). Damage to the alveolar epithelium and abnormal wound healing are key factors in the disease's progression. IPF typically affects individuals around 65 years old, with a 3-5 year survival rate post-diagnosis and an estimated annual incidence of 5.6 per 100,000 people (46).

While the exact causes of IPF remain elusive, a combination of genetic and environmental factors is suspected. Continuous micro-injuries to the aging alveolar epithelium disrupt epithelial-fibroblast communication, triggering myofibroblasts to produce and activate collagen-rich extracellular matrix. This excessive matrix accumulation leads to irreversible alveolar collapse, impairing gas exchange and making breathing difficult. In the absence of a cure, treatment focuses on slowing fibrosis progression, maintaining comfort, and providing palliative care in advanced stages (47,48).

Given the lack of curative options, further research using animal models that closely resemble human IPF is crucial for developing potential therapies. While spontaneous pulmonary fibrosis in domestic animals like cats and dogs offers some insights, rodent models remain essential for investigating disease pathogenesis and conducting preclinical therapeutic evaluations. A variety of established and emerging experimental models provide valuable information about disease mechanisms, aiding in the identification of novel therapeutic targets for clinical trials.

Bleomycin Model

Bleomycin, a chemotherapeutic antibiotic derived from the bacterium Streptomyces verticillatus, is utilized in cancer treatment but carries the risk of acute lung damage and fibrosis as adverse effects in humans (49). While effective against cancer, it can also induce scar tissue formation in the lungs.

The bleomycin model, an animal model employed to study lung fibrosis, presents a combination of strengths and weaknesses. Recognized by the ATS workshop as the most well-established model for preclinical testing, it offers researchers a reliable tool for obtaining consistent results (50). Bleomycin inflicts damage upon lung cells, swiftly triggering inflammation and fibrosis through an initial surge in proinflammatory cytokines, followed by an increase in profibrotic factors. The effects of fibrosis become evident within a week and peak in 3-4 weeks. However, a notable limitation is that bleomycin-induced fibrosis is transient and histopathologically distinct from the persistent fibrosis observed in human IPF (51).

Mouse responses to bleomycin exhibit genetic variations, mirroring the diversity seen in humans. For instance, C57Bl/6 mice demonstrate greater susceptibility compared to Balb/c mice, likely due to differences in cytokine and protease expression patterns (52). Most research utilizes young mice aged 8-12 weeks, corresponding to human puberty. Notably, male mice are more susceptible to bleomycin-induced lung injury than females, similar to the pattern observed in humans, although the underlying mechanisms remain incompletely understood (53).

Inhalation (intratracheal) is the most prevalent and well-established method of bleomycin administration, favored for its ease of use and ability to confine lung damage to the lungs, mimicking the human disease. However, alternative routes such as intraperitoneal, subcutaneous, and intravenous administration exist (50).

In the bleomycin model, interventions during the inflammatory phase predominantly exert anti-inflammatory effects and are considered "preventive treatment." To investigate the "therapeutic" effects of antifibrotic agents, intervention after the inflammatory period (usually after day 7) is recommended, highlighting the importance of optimizing treatment timing in this model. This recommendation aligns with those put forth by the European Respiratory Society Task Force for optimizing experimental models of lung diseases (Figure 1, 54).

Interestingly, Peng et al. (55) reported that repeated low-dose intratracheal bleomycin administration could potentially induce irreversible lung injury lasting 24 weeks. However, this approach is impractical for most studies due to high mortality rates, cost, and time constraints. Therefore, factors like administration route, intervention timing, and fibrosis persistence warrant careful consideration to maximize the effectiveness of the bleomycin model in developing treatments for pulmonary fibrosis.

Adenoviral TGF-β1 Vector Model

In this model, an adenovirus acts as a carrier to deliver the TGF-β1 gene to lung cells. TGF-β1, a protein pivotal in lung fibrosis, is produced by lung cells due to the introduced gene, ultimately leading to fibrosis development. Importantly, TGF-β1 plays a crucial role in IPF.

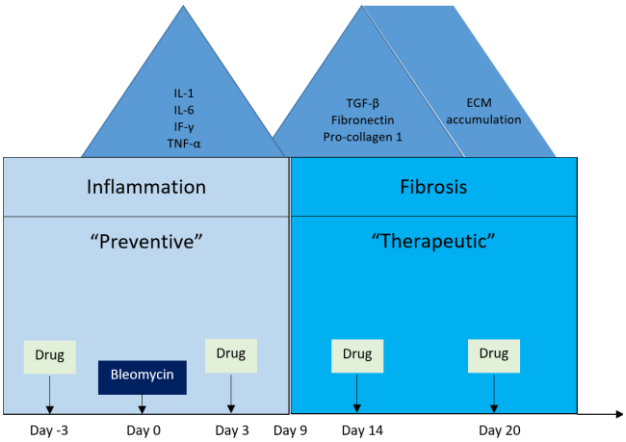

Figure 1. Bleomycin administration triggers an acute inflammatory response for up to 8 days, followed by fibrogenic changes and matrix deposition leading to lung structure distortion out to 28 or 35 days. Treatments within the initial 7 days are considered "preventive," while interventions after days 7-10 are deemed "therapeutic" (54).

The adenoviral TGF-β1 model offers a more accurate representation of the fibrosis environment compared to the bleomycin model. Unlike the bleomycin model, where fibrosis resolves over time, fibrosis in this model persists for an extended period, up to day 64, mirroring the persistent nature of fibrosis in IPF (56).

Intratracheal administration of AdTGF-β1 triggers high expression levels of activated TGF-β1, resulting in mild initial inflammation and rapid lung fibrosis. Notably, this model also induces pulmonary hypertension in rats, a common and severe complication in IPF patients with a poorer prognosis. Consequently, this model proves valuable for investigating the pathogenesis of pulmonary hypertension secondary to pulmonary fibrosis (57).

Genetically Modified Mouse Models of Lung Fibrosis

Genetic predisposition plays a significant role in the development of lung fibrosis. Mutations in genes such as surfactant protein-C (58), surfactant protein-A (59), telomerase reverse transcriptase (TERT), and telomerase RNA component (TERC) have been associated with familial interstitial pneumonia (FIP), a hereditary form of lung fibrosis (60). Genetically modified mouse models, based on known FIP mutations or common alleles found in IPF, offer valuable insights into the pathogenesis of this disease.

Silica Model

Inhalation of silica (quartz) dust is a known cause of lung fibrosis in humans, making silica administration a widely used method in animal models of the disease (61). Silica particles are believed to induce fibrosis through their uptake by macrophages, which then produce pro-fibrotic cytokines like TNF-α, platelet-derived growth factor (PDGF), and TGF-β. A major advantage of the silica model is the continuous stimulation provided by the slow clearance of silica particles from the lungs. However, the model also has limitations. The development of fibrotic nodules can take up to 16 weeks, and the resulting fibrosis lacks the characteristic histological features of usual interstitial pneumonia (UIP) seen in IPF. Moreover, the delivery of aerosolized silica requires specialized and expensive equipment. Similar to bleomycin-induced damage, the development of fibrosis in this model is species-dependent, with Balb/c mice exhibiting resistance to fibrosis development (62).

CONCLUSION

The animal experimental models have provided valuable insights into disease mechanisms and aided in identifying new therapeutic targets. However, it is important to note that no single model fully recapitulates human disease, and each has unique advantages and disadvantages. The use of experimental animals in modeling lung diseases presents certain limitations. While lung diseases in humans usually develop over a long period of time, this process can be accelerated in experimental animals. This may affect the natural course of the disease and response to treatment. In addition, ethical concerns and the welfare of experimental animals must also be taken into account. Awareness of these limitations is crucial for the accurate interpretation of research findings and their applicability to humans.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: PYG; Design: PYG; Data Collection/Processing: PYG; Analysis/Interpretation: PYG; Literature Review: PYG; Drafting/Writing: PYG; Critical Review: PYG.

REFERENCES

1. Ware LB. Modeling human lung disease in animals. Am J Physiol Lung Cell Mol Physiol. 2008;294(2):L149-50.
2. Shapiro SD. Animal models of asthma: Pro: Allergic avoidance of animal (model[s]) is not an option. Am J Respir Crit Care Med. 2006;174(11):1171-3.
3. Fröhlich E. Animals in respiratory research. Int J Mol Sci. 2024;25(5):2903.
4. Global Initiative for Asthma. Global strategy for asthma management and prevention. USA: Global Initiative for Asthma; 2016.
5. Shinagawa K, Kojima M. Mouse model of airway remodeling: strain differences. Am J Respir Crit Care Med. 2003;168(8):959-67.
6. Kumar RK, Herbert C, Yang M, Koskinen AM, McKenzie AN, Foster PS. Role of interleukin-13 in eosinophil accumulation and airway remodelling in a mouse model of chronic asthma. Clin Exp Allergy. 2002;32(7):1104-11.
7. Aun MV, Bonamichi-Santos R, Arantes-Costa FM, Kalil J, Giavina-Bianchi P. Animal models of asthma: utility and limitations. J Asthma Allergy. 2017;10:293-301.
8. Ehrhardt B, El-Merhie N, Kovacevic D, Schramm J, Bossen J, Roeder T, et al. Airway remodeling: The Drosophila model permits a purely epithelial perspective. Front Allergy. 2022;3:876673.
9. Wagner C, Uliczka K, Bossen J, Niu X, Fink C, Thiedmann M, et al. Constitutive immune activity promotes JNK- and FoxO-dependent remodeling of Drosophila airways. Cell Rep. 2021;35(1):108956.
10. Ressmeyer AR, Larsson AK, Vollmer E, Dahlèn SE, Uhlig S, Martin C. Characterisation of guinea pig precision-cut lung slices: comparison with human tissues. Eur Respir J. 2006;28(3):603-11.
11. Woodrow JS, Sheats MK, Cooper B, Bayless R. Asthma: the use of animal models and their translational utility. Cells. 2023;12(7):1091.
12. Yamaguchi T, Kohrogi H, Honda I, Kawano O, Sugimoto M, Araki S, et al. A novel leukotriene antagonist, ONO-1078, inhibits and reverses human bronchial contraction induced by leukotrienes C4 and D4 and antigen in vitro. Am Rev Respir Dis. 1992;146(4):923-9.

13. Malo PE, Bell RL, Shaughnessy TK, Summers JB, Brooks DW, Carter GW. The 5-lipoxygenase inhibitory activity of zileuton in in vitro and in vivo models of antigen-induced airway anaphylaxis. Pulm Pharmacol. 1994;7(2):73-9.
14. Adner M, Canning BJ, Meurs H, Ford W, Ramos Ramírez P, van den Berg MPM, et al. Back to the future: Re-establishing guinea pig in vivo asthma models. Clin Sci (Lond). 2020;134(11):1219-42.
15. Skappak C, Ilarraza R, Wu YQ, Drake MG, Adamko DJ. Virus-induced asthma attack: The importance of allergic inflammation in response to viral antigen in an animal model of asthma. PLoS ONE. 2017;12(7):e0181425.
16. Nials AT, Uddin S. Mouse models of allergic asthma: acute and chronic allergen challenge. Dis Model Mech. 2008;1(4-5):213-20.
17. Zosky GR, Sly PD. Animal models of asthma. Clin Exp Allergy. 2007;37(7):973-88.
18. Boyce JA, Austen KF. No audible wheezing: nuggets and conundrums from mouse asthma models. J Exp Med. 2005;201(12):1869-73.
19. Kumar RK, Herbert C, Foster PS. The "classical" ovalbumin challenge model of asthma in mice. Curr Drug Targets. 2008;9(6):485-94.
20. Kool M, Soullie T, van Nimwegen M, Willart MA, Muskens F, Jung S, et al. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. J Exp Med. 2008;205(4):869-82.
21. Wagers S, Lundblad LK, Ekman M, Irvin CG, Bates JH. The allergic mouse model of asthma: normal smooth muscle in an abnormal lung? J Appl Physiol (1985). 2004;96(6):2019-27.
22. Temelkovski J, Hogan SP, Shepherd DP, Foster PS, Kumar RK. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. Thorax. 1998;53(10):849-56.
23. Fernandez-Rodriguez S, Ford WR, Broadley KJ, Kidd EJ. Establishing the phenotype in novel acute and chronic murine models of allergic asthma. Int Immunopharmacol. 2008;8(5):756-63.
24. Jungsuwadee P, Benkovszky M, Dekan G, Stingl G, Epstein MM. Repeated aerosol allergen exposure suppresses inflammation in B-cell deficient mice with established allergic asthma. Int Arch Allergy Immunol. 2004;133(1):40-8.
25. Ghorani V, Boskabady MH, Khazdair MR, Kianmeher M. Experimental animal models for COPD: a methodological review. Tob Induc Dis. 2017;15:25.
26. Groneberg DA, Chung KF. Models of chronic obstructive pulmonary disease. Respir Res. 2004;5(1):18.
27. Mortaz E, Adcock IA. Limitation of COPD studies in animal modeling. Tanaffos. 2012;11(3):7-8.
28. Eltom S, Stevenson C, Birrell MA. Cigarette smoke exposure as a model of inflammation associated with COPD. Curr Protoc Pharmacol. 2013;5(5)64.
29. Costa CH, Rufino R, Lapa E Silva JR. Inflammatory cells and their mediators in COPD pathogenesis. Rev Assoc Med Bras (1992). 2009;55(3):347-54. Portuguese.
30. Liang GB, He ZH. Animal models of emphysema. Chin Med J (Engl). 2019;132(20):2465-75.
31. Herget J, Palecek F, Cermáková M, Vízek M. Pulmonary hypertension in rats with papain emphysema. Respiration. 1979;38(4):204-12.
32. Gülhan PY, Ekici MS, Niyaz M, Gülhan M, Erçin ME, Ekici A, et al. Therapeutic treatment with abdominal adipose mesenchymal cells does not prevent elastase-induced emphysema in rats. Turk Thorac J. 2020;21(1):14-20.
33. Lucas SD, Gonçalves LM, Cardote TAF, Correia HF, Rui M, Guedes RC. Structure based virtual screening for discovery of novel human neutrophil elastase inhibitors. Med Chem Comm. 2012;3(10):1299-304.
34. Wright JL, Churg A. Cigarette smoke causes physiologic and morphologic changes of emphysema in the guinea pig. Am Rev Respir Dis. 1990;142(6 Pt 1):1422-8.
35. John G, Kohse K, Orasche J, Reda A, Schnelle-Kreis J, Zimmermann R, et al. The composition of cigarette smoke determines inflammatory cell recruitment to the lung in COPD mouse models. Clin Sci (Lond). 2014;126(3):207-21.
36. Shore S, Kobzik L, Long NC, Skornik W, Van Staden CJ, Boulet L, et al. Increased airway responsiveness to inhaled methacholine in a rat model of chronic bronchitis. Am J Respir Crit Care Med. 1995;151(6):1931-8.
37. van der Strate BW, Postma DS, Brandsma CA, Melgert BN, Luinge MA, Geerlings M, et al. Cigarette smoke-induced emphysema: a role for the B cell? Am J Respir Crit Care Med. 2006;173(7):751-8.
38. Churg A, Cosio M, Wright JL. Mechanisms of cigarette smoke-induced COPD: insights from animal models. Am J Physiol Lung Cell Mol Physiol. 2008;294(4):L612-31.
39. Valenca SS, da Hora K, Castro P, Moraes VG, Carvalho L, Porto LC. Emphysema and metalloelastase expression in mouse lung induced by cigarette smoke. Toxicol Pathol. 2004;32(3):351-6.
40. Wegmann M, Fehrenbach A, Heimann S, Fehrenbach H, Renz H, Garn H, et al. NO2-induced airway inflammation is associated with progressive airflow limitation and development of emphysema-like lesions in C57bl/6 mice. Exp Toxicol Pathol. 2005;56(6):341-50.
41. Gupta V, Banyard A, Mullan A, Sriskantharajah S, Southworth T, Singh D. Characterization of the inflammatory response to inhaled lipopolysaccharide in mild to moderate chronic obstructive pulmonary disease. Br J Clin Pharmacol. 2015;79(5):767-76.
42. Snider GL, Lucey EC, Faris B, Jung-Legg Y, Stone PJ, Franzblau C. Cadmium-chloride-induced air-space enlargement with interstitial pulmonary fibrosis is not associated with destruction of lung elastin. Implications for the pathogenesis of human emphysema. Am Rev Respir Dis. 1988;137(4):918-23.
43. Keil M, Lungarella G, Cavarra E, van Even P, Martorana PA. A scanning electron microscopic investigation of genetic emphysema in tight-skin, pallid, and beige mice, three different C57 BL/6J mutants. Lab Invest. 1996;74(2):353-62.
44. Liang R, Jin SD, Zhang X, Liu LJ, Rong HF. Abhd2 genes and emphysema pathogenesis research. Progr Modern Biomedicine. 2011;11:4430-3. Chinese.
45. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet. 2011;378(9807):1949-61.

46. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. Lancet. 2017;389(10082):1941-52.
47. Glass DS, Grossfeld D, Renna HA, Agarwala P, Spiegler P, DeLeon J, et al. Idiopathic pulmonary fibrosis: Current and future treatment. Clin Respir J. 2022;16(2):84-96.
48. Senanayake S, Harrison K, Lewis M, McNarry M, Hudson J. Patients' experiences of coping with Idiopathic Pulmonary Fibrosis and their recommendations for its clinical management. PLoS One. 2018;13(5):e0197660.
49. Reinert T, Baldotto CSR, Nunes FAP, Scheliga AAS. Bleomycin-induced lung injury. J Cancer Res. 2013;2013:480608.
50. Jenkins RG, Moore BB, Chambers RC, Eickelberg O, Königshoff M, Kolb M, et al. An official American Thoracic Society Workshop report: use of animal models for the preclinical assessment of potential therapies for pulmonary fibrosis. Am J Respir Cell Mol Biol. 2017;56(5):667-79.
51. Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2008;294(2):L152-60.
52. Stefanov AN, Fox J, Depault F, Haston CK. Positional cloning reveals strain-dependent expression of Trim16 to alter susceptibility to bleomycin-induced pulmonary fibrosis in mice. PLoS Genet. 2013;9(1):e1003203.
53. Redente EF, Jacobsen KM, Solomon JJ, Lara AR, Faubel S, Keith RC, et al. Age and sex dimorphisms contribute to the severity of bleomycin-induced lung injury and fibrosis. Am J Physiol Lung Cell Mol Physiol. 2011;301(4):L510-8.
54. Moeller A, Ask K, Warburton D, Gauldie J, Kolb M. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? Int J Biochem Cell Biol. 2008;40(3):362-82.
55. Peng R, Sridhar S, Tyagi G, Phillips JE, Garrido R, Harris P, et al. Bleomycin induces molecular changes directly relevant to idiopathic pulmonary fibrosis: a model for "active" disease. PLoS One. 2013;8(4):e59348.
56. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J Clin Invest. 1997;100(4):768-76.
57. Bellaye PS, Yanagihara T, Granton E, Sato S, Shimbori C, Upagupta C, et al. Macitentan reduces progression of TGF-β1-induced pulmonary fibrosis and pulmonary hypertension. Eur Respir J. 2018;52(2):1701857.
58. Thomas AQ, Lane K, Phillips J 3rd, Prince M, Markin C, Speer M, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am J Respir Crit Care Med. 2002;165(9):1322-8.
59. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. Am J Hum Genet. 2009;84(1):52-9.
60. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. N Engl J Med. 2007;356(13):1317-26.
61. Davis GS, Leslie KO, Hemenway DR. Silicosis in mice: effects of dose, time, and genetic strain. J Environ Pathol Toxicol Oncol. 1998;17(2):81-97.
62. Degryse AL, Lawson WE. Progress toward improving animal models for idiopathic pulmonary fibrosis. Am J Med Sci. 2011;341(6):444-9.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1481630

Duzce Med J, 2024;26(S1):55-64
Düzce Tıp Fak Derg, 2024;26(S1):55-64

Experimental Animal Models in Neurological Diseases

Nörolojik Hastalıklarda Deneysel Hayvan Modelleri

Neslihan ŞİRİN[1]
0000-0002-3470-0043
Şerif DEMİR[2]
0000-0002-2548-1969

[1]Experimental Animals Application and Research Center, Düzce University, Düzce, Türkiye

[2]Department of Physiology, Düzce University Faculty of Medicine, Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Şerif DEMİR
serifdemir@duzce.edu.tr

Received / Geliş Tarihi : 28.02.2024
Accepted / Kabul Tarihi : 03.04.2024
Available Online / Çevrimiçi Yayın Tarihi : 10.05.2024

ABSTRACT

The human brain is a structure that controls billions of neurons and trillions of connections. Having a unique anatomy with countless neurons and connections makes its understanding even more complex. The brain, divided into different regions for specialized functions such as memory, movement, sensation, and emotions, holds great significance in human cognition and behavior. Centuries of research, coupled with advancements in technology, have propelled neuroscience forward, facilitating the understanding of the neurological, behavioral, and structural characteristics of the brain. Developing treatments for neurological disorders such as Alzheimer's, Parkinson's, multiple sclerosis, amyotrophic lateral sclerosis, migraine, epilepsy, and schizophrenia as well as understanding the complex mechanisms of these diseases, require the exploration of new treatment methods, drugs, and products through direct experimentation on humans, which raises ethical concerns. Therefore, experimental animal models are needed in the treatment of neurodegenerative diseases. There are currently many experimental animal models developed to elucidate the pathophysiological characteristics of neurological disorders. The aim of this review was to summarize the experimental models of neurodegenerative diseases developed today in sections. While recognizing that an experimental animal model may not fully replicate the disease process in humans, it can at least provide guidance in understanding the disease.
Keywords: Neurological diseases; experimental animal models.

ÖZ

İnsan beyni, milyarlarca nöronu ve trilyonlarca bağlantıyı kontrol eden bir yapıdır. Eşsiz bir anatomiye sahip olan bu yapının sayısız nöron ve bağlantıya sahip olması, onun anlaşılmasını daha da karmaşık hale getirmektedir. Hafıza, hareket, duyu ve duygular gibi özelleşmiş fonksiyonlar için farklı bölgelere ayrılmış olan beyin, insanın biliş ve davranışında büyük öneme sahiptir. Yüzyıllardır süren araştırmalar, teknolojinin de gelişmesiyle sinirbilimini ileriye taşımış, beynin nörolojik, davranışsal ve yapısal özelliklerinin anlaşılmasını sağlamıştır. Alzheimer, Parkinson, multiple skleroz, amyotrofik lateral skleroz, migren, epilepsi ve şizofreni gibi nörolojik bozukluklara yönelik tedavilerin geliştirilebilmesi ve hastalıkların karmaşık mekanizmalarının anlaşılması için yeni tedavi yöntemlerinin, ilaç ve ürünlerinin doğrudan insanlarla çalışılması etik sorunlar doğuracağından nörodejeneratif hastalıkların tedavisinde, deneysel hayvan modellerine ihtiyaç duyulmaktadır. Nörolojik bozuklukların fizyopatolojik özelliklerini aydınlatmak için hali hazırda geliştirilmiş birçok deneysel hayvan modeli mevcuttur. Bu derlemenin amacı, günümüzde geliştirilen nörodejeneratif hastalıklara yönelik deneysel modellerin bölümler halinde özetlenmesidir. Bir deneysel hayvan modeli insandaki hastalık sürecini tamamen karşılayamasa bile en azından hastalığın anlaşılmasında yol gösterici olabilir.
Anahtar kelimeler: Nörolojik hastalıklar; deneysel hayvan modelleri.

INTRODUCTION

Neurological diseases encompass a wide range of disorders characterized by various abnormalities in the nervous system. These diseases affect the functions of the brain, brainstem, spinal cord, and other nerves, deeply impacting individuals' quality of life (1). Experimental animal models are frequently utilized to understand the complex mechanisms of neurological diseases and to develop treatment methods (2). In this review, basic information and disease pathophysiology of Alzheimer's, Parkinson's, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), migraine, epilepsy, and schizophrenia were mentioned under the main heading, with experimental animal models developed in recent times summarized under a subheading.

ALZHEIMER'S DISEASES AND MODELS

Alzheimer's disease (AD) is a prevalent neurodegenerative disease worldwide that leads to progressive dementia. In the clinical presentation of AD, a decrease in memory, language, judgment, and behavior is observed, while in its pathophysiology, mitochondrial dysfunction, hormonal imbalance, calcium dysregulation, increased oxidative stress, and neuroinflammation are seen (3). The disease exhibits variability in its onset, progression, and pathology, and it is classified as early-onset and late-onset (4). The specific markers of AD are neurofibrillary tangles composed of amyloid-beta (Aβ) plaques and hyperphosphorylated tau proteins. Aβ triggers mitochondrial oxidative stress, leading to disruptions in the electron transport chain and abnormalities in calcium regulation, resulting in increased harmful radicals and decreased ATP production. The decreases observed in the levels of enzymes related to energy metabolism in the brain cells of AD patients have been reported to lead to protein/DNA alterations and neuronal death (5,6). The most commonly used animal species in modeling AD are mice, however, the use of rats is also mentioned (7). In modeling, both transgenic and non-transgenic species are used. Transgenic models include those featuring amyloid precursor protein (APP), tau protein, and transgenic models capable of expressing both. Among non-transgenic models, there are induced models using substances such as streptozotocin, amyloid, colchicine, aluminum, zinc, and lipopolysaccharide (LPS) (6,7).

Transgenic AD Models

To model certain features of AD, transgenic animal models have been generated by incorporating mutant genes into the existing genetic structure or by modifying genes. Mice are the most commonly used species for transgenic modeling due to their ease of manipulation and accessibility.

APP transgenic AD model

The overexpression of APP leads to the release of harmful Aβ peptides, causing damage to neurons. Although dense plaques, gliosis, and early spatial memory impairment are exhibited in the cortex and hippocampus regions of this model, it does not sufficiently mimic the symptoms of AD (7,8).

Tau protein transgenic AD model

In this model, which can mimic some aspects of early-onset AD, excessive phosphorylation of the tau protein is induced. Excessive phosphorylated tau inhibits axonal transport, leading to the formation of tangled structures in the soma and dendrites of neurons. These alterations in AD significantly impair cognitive functions by affecting vital brain regions for learning and memory (7).

APP and Tau double transgenic AD model

This model involves genetically modifying animals to express both mutant forms of the APP and tau protein, thus enabling to explore the interactions between APP and tau and their roles in the development and progression of AD. However, while this model replicates some aspects of the disease, it may not fully capture the entire spectrum of AD pathology seen in humans (9).

Non-transgenic AD Models

Streptozotocin-induced AD model

Intracerebroventricular (ICV) injection of streptozotocin triggers free radical formation and significantly affects rat cognitive function. Developing Aβ and tau neuropathology with the ICV infusion model takes time (10).

Aβ-induced AD model

Aβ oligomers are derived from the breakdown of APP and are associated with neuronal damage, cognitive impairment, and memory loss. Aβ administered via the ICV route triggers inflammatory processes, disrupts calcium balance, increases the release of reactive oxygen species (ROS), triggers DNA/protein alterations, mitochondrial dysfunction, and various neurotoxic mechanisms such as apoptosis (11). The model not only exhibits AD-like behavioral abnormalities but also displays the Aβ pathology shared by both familial and sporadic AD, which is a common feature (12).

Colchicine-induced AD model

When colchicine derived from certain lily plants is applied to the brain, it triggers excessive free radical production and causes DNA damage, particularly affecting hippocampal cells and pathways severely. This condition leads to cholinergic neuron loss, decreased learning ability, and memory loss. However, the neurotoxic mechanism of colchicine is not fully understood (13,14).

Aluminum-induced AD model

When aluminum salts are administered intracerebrally or peripherally, they trigger the formation of neurofibrillary tangles. The neurodegenerative effect of aluminum varies depending on the route of administration, type of salt, animal species, dosage, and duration of exposure, but aluminum levels in the brain increase with age. Accumulated aluminum in neurons interacts with tau proteins, contributing to the neurofibrillary pathology of AD (15,16).

Zinc-induced AD model

Zinc, which is important for growth, cognitive functions, and neurotransmission regulation, is abundantly present in the hippocampus and cortex brain regions. Positioned in synaptic vesicles, zinc is released excessively into the synaptic cleft when cellular function is disrupted, leading to the generation of ROS and affecting enzymes and cellular respiration. This ultimately leads to the activation of apoptosis and consequently neuronal loss. Zinc has a role in Aβ accumulation, hence its effect in AD (17).

Lipopolysaccharide-induced AD model

The LPS present in the structure of gram-negative bacteria strongly stimulates microglia and astrocytes, triggering the production of endogenous IL-1 and beta-APP. This inflammation, responsible for neuronal degeneration in brain regions and affecting spatial memory, is used in AD research (18).

PARKINSON'S DISEASES AND MODELS

Parkinson's disease (PD) is a late-onset, progressive neurodegenerative disorder characterized by four primary motor symptoms (resting tremor, rigidity, bradykinesia, and postural instability). The disease is associated with degeneration of nigrostriatal dopaminergic neurons and the presence of Lewy bodies (containing alpha-synuclein and ubiquitin) (19). In PD, treatment aims to manage symptoms, and for a long time, levodopa, a precursor to dopamine, has been used. In animal modeling using rodents to test new treatment strategies for PD, neurotoxic models stand out more prominently compared to genetic models. Neurotoxic agents such as 6-OHDA (6-hydroxydopamine), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), rotenone, paraquat, and LPS are frequently used in a disease that typically manifests with late-onset symptoms in about 90% of cases (20). Additionally, models induced by medications such as haloperidol, fluphenazine, reserpine, and methamphetamine are observed (21).

Toxin-induced PD Models

6-OHDA-induced PD model

6-OHDA is similar to endogenous catecholamines and accumulates pathologically in catecholaminergic neurons when exposed. Neurotoxic effects emerge due to oxidative damage. Damage to the dopaminergic pathway induces asymmetric rotation in subjects, allowing the study of PD (22).

MPTP-induced PD model

The lipophilic MPTP is converted into the toxic metabolite MPP (1-methyl-4-phenylpyridinium) in the body. It enters the mitochondria of dopaminergic neurons and interferes with complex I of the electron transport chain, inhibiting the oxidation reaction. Irreversible damage leads to dopamine deficiency. The MPTP model exhibits many characteristics of PD, including dopaminergic neurodegeneration, motor deficits, the formation of alpha-synuclein aggregates, and neuroinflammation (23,24).

Rotenone-induced PD model

Rotenone, both an insecticide and herbicide, is a lipophilic neurotoxin. In models predominantly using rodents, the toxin inhibits mitochondrial complex 1, increases ROS, reduces dopamine-glutathione levels, induces oxidative damage, and blocks mitosis, thereby preventing cell proliferation. Lewy bodies resulting from damage to the nigrostriatal dopaminergic pathway can be examined (25,26).

Paraquat-induced PD model

Paraquat, a herbicide with neurotoxic properties that cannot cross the blood-brain barrier, causes degeneration in dopaminergic neurons by generating ROS when applied systemically (27).

Lipopolysaccharide-induced PD model

LPS, which triggers microglial activation and sets the stage for the degeneration of dopaminergic neurons, can be administered stereotactically, systemically, and intranasally (28).

Drug-induced PD model

Haloperidol and fluphenazine cause functional dopamine deficiency in postsynaptic receptor regions, while reserpine and methamphetamine affect vesicles. These drugs represent the reduced dopamine levels in PD (21).

Genetic-induced PD Models

Six genes; alpha-synuclein, LRRK2, VPS35, Parkin, PINK1, and DJ-1, are associated with PD. However, genetic PD models created in animals do not completely align with the behaviors observed in humans (29).

MULTIPLE SCLEROSIS AND MODELS

MS is a chronic autoimmune disorder where the immune system attacks the myelin sheath of the central nervous system, mistaking it as foreign. In the unit of oligodendrocyte-myelin-axon, myelin protects and nourishes the axon while increasing its cross-sectional diameter. This structure becomes disrupted due to MS, leading to lesion formations in the white matter of the brain. In MS, decreased axonal density and volume in affected areas and seemingly normal central nervous system tissue contribute to brain and spinal cord atrophy, resulting in permanent disability (30). Among the MS animal models; experimental autoimmune encephalomyelitis (EAE), a viral-induced chronic demyelinating disease known as Theiler's murine encephalomyelitis virus (TMEV) infection, and toxin-induced demyelination are included (31).

EAE MS Model

Initially, monkeys and guinea pigs were used to establish the model, but nowadays, mice and rats are predominantly used. Here, autoimmunity against components of the central nervous system is induced through immunization with antigens derived from the basic myelin protein in susceptible mice. Paralysis starting in the tail after induction is followed by manifestations in the hind and forelimbs. Among the shortcomings of the most commonly studied EAE model are limited information about the progression of MS, challenges in studying remyelination, and inadequate treatment procedures targeting neuronal growth (31).

Virus-induced MS Model

Epidemiological studies have suggested that a viral infection early in life, in the presence of a specific genetic background, may lead to an immune-mediated attack against the central nervous system, but so far, no specific virus has been identified as a potential cause or contributor to MS. The most commonly studied viral animal model of MS is the TMEV model. In this method, which can be applied in susceptible mice, the chronic demyelination phase of MS can be examined. However, a disadvantage of the model is that demyelination and remyelination occur simultaneously (31).

Toxin-induced MS Model

Among the toxins used to model MS by inducing demyelination, ethidium bromide, and cuprizone are prominent. An important aspect of these toxins is their assistance in studying the process of remyelination. However, toxins that induce demyelination due to myelin loss and oligodendrocyte death in white matter areas are insufficient in representing all aspects of MS expression. They focus only on myelin loss and cannot simulate all inflammatory processes and immune system dysfunction (32).

AMYOTROPHIC LATERAL SCLEROSIS AND MODELS

ALS is a late-onset, progressive, etiology completely unknown neurodegenerative disease that affects cortical and spinal motor neurons. The onset of ALS symptoms in patients is actually indicative of the loss of approximately 50-70% of motor neurons. While the majority of cases arise sporadically, about 5-10% are familial. Prominent symptoms include weakness in the extremities and difficulty swallowing. As the disease progresses, daily activities decrease, and cognitive losses increase (33).

According to current data, the incidence of ALS is between 6 and 38 cases per million, and an effective treatment for the disease has not yet been discovered (34). Although ALS is generally known as a motor neuron disorder, nowadays, there is a significant amount of evidence suggesting that ALS is a non-cell autonomous disease involving astrocytes, oligodendrocytes, microglia, and immune cells. For the onset and progression of the disease, various factors have been proposed, such as excessive calcium and glutamate excitotoxicity, oxidative stress, axonal dysfunction, neuroinflammation, errors in proteins and RNA, mitochondrial stress, and damage. Animal models, often using rodents, are commonly employed to investigate early symptoms, motor neuron loss, muscle weakness, and other ALS symptoms. However, limitations exist as these models may not fully reflect ALS, and there are constraints on how applicable therapeutic approaches tested on these models will be in humans. Thus, human clinical studies still hold a crucial position (33).

Genetic ALS Models

Many of the animal models used to investigate the pathogenesis and biochemical mechanisms of ALS are studies involving genetic interventions. So far, close to 50 genes associated with ALS have been discovered. A mutation in the human superoxide dismutase 1 (SOD1) gene is reported to be a significant factor in ALS. Among other important mutated genes are TARDBP (TAR DNA binding protein), C9orf72 (chromosome 9 open reading frame 72), and FUS (Fused in Sarcoma). Transgenic animals carrying mutated genes such as VABP (VAMP-associated protein B), OPTN (Optineurin), VCP (Valosin Containing Protein), UBQLN2 (Ubiquilin-2), MATR3 (Matrin 3), TBK1 (TANK-Binding Kinase-1) have been used as models to understand the pathogenesis of ALS and test new therapies by reflecting similar symptoms observed in ALS patients (motor neuron loss, muscle denervation, tremors, paralysis, death) (35).

Environmentally-induced ALS Models

Dietary model of ascorbic acid deficiency (36), rodent model infected with motor neuron antigens from different species (37,38), L-BMAA (beta-N-methylamino-L-alanine) induced model (39,40), neurotoxin-induced model (41-43) have been created to mimic environmentally induced ALS. Since these exposures can lead to negative changes and mutations in genes, categorizing environmentally induced models separately from genetic models may not be an entirely accurate classification.

MIGRAINE AND MODELS

Migraine is a neurovascular disorder characterized by a one-sided, throbbing headache, the intensity of which can increase in response to factors such as movement, sound, and light. Migraine attacks can often be accompanied by symptoms like nausea, vomiting, and sensitivity to light. Migraine, affecting 18% of women and 6% of men, arises through the separate or combined effects of environmental and genetic factors. Although all mechanisms in migraines have not yet been fully elucidated, evidence suggests that the triggering of sensitization in the trigeminovascular system, which activates inflammatory and vasodilatory processes, is the cause of the headache (44). When sensory nerve fibers in the trigeminovascular system are activated, the release of vasoactive agents is induced, leading to vasodilation and dural plasma extravasation, resulting in neurogenic inflammation, and ultimately, an increase in the severity of pain is observed (45). Migraine is classified as with aura and without aura. In migraines with aura, visual, sensory, or motor symptoms are observed before or at the onset of the headache, and these symptoms are referred to as "aura." For example, flickering lights, dots or lines, needles, or numbness are signs of an aura. Migraine without aura starts suddenly, there are no aura symptoms, and it is generally severe (46,47). The majority of information regarding the pathophysiology of migraine has been obtained from animal models developed to investigate the nociceptive pathways of the trigeminovascular system and their transmissions reaching the brainstem and diencephalic nuclei (45). Among the experimental animal models currently used to understand the underlying mechanisms of migraine, evaluate treatment options, and develop new therapeutic strategies, there are models such as the cortical spreading depression model (48), electrical stimulation model (49), inflammatory mediator-induced migraine model (50), chemical-induced migraine model (51), and genetic migraine model (52).

Cortical Spreading Depression Model

Cortical spreading depression is induced by microinjection of potassium chloride into the brain through craniotomy, and test data is observed through an electrical activity recorder (53). Cortical spreading depression stimulates ipsilateral trigeminal axons surrounding cortical blood vessels, leading to extravasation of plasma proteins in the dura mater, activating mechanisms that induce c-Fos expression in the caudal trigeminal nucleus, causing disruption of the blood-brain barrier, enabling the activation of the trigeminovascular system, and resulting in the release of chemicals such as H+, K+, nitric oxide, and neurotransmitters into the extracellular space (48). These observed effects contribute to the understanding of migraine pathophysiology.

Electrical Stimulation Migraine Model

The model is designed to achieve trigeminovascular activation by placing a bipolar stimulating electrode near the meningeal arteries and superior sagittal sinus dural vessels. Upon activation of trigeminovascular regions stimulable by nociceptive stimulation with this electrical stimulus, Fos immunoreactivity is triggered, and efforts are made to examine neuronal populations in the trigeminovascular system (49).

Inflammatory Mediator-Induced Migraine Model

A model is created by intracranial injection of an inflammatory mixture containing prostaglandin, histamine, serotonin, and bradykinin in rodents. By interpreting the data through myographic and histological analyses, various aspects of migraine pain can be evaluated, and pain mechanisms can be scrutinized (50). The disadvantage of the model is that it alters blood-brain barrier activities and directly activates central brain regions (49).

Chemically Induced Migraine Model

The nitroglycerin model, a frequently employed method for peripheral application among chemical-induced migraine models, is conducted by administering a single dose of intraperitoneal nitroglycerin injection to rodents to trigger hyperalgesia. In the model where trigeminal and cortical structures associated with migraine pain are

sensitized, phenotypically, attacks similar to spontaneous migraine attacks are observed (51). In a recent study, repeated nitroglycerin injections are reported to hold promise for investigating the chronic course of migraine (54).

Genetic Migraine Model

Transgenic mouse models are being created to gain insights into the phenotypic consequences of mutations playing a role in migraine pathophysiology. These models primarily mimic familial hemiplegic migraine, a rare subtype of migraine with aura. So far, genes associated with migraine, namely FHM1 CACNA1A, FHM2 ATP1A2, and FHM3 SCN1A, have been identified. Mutations in these genes lead to disruptions in neuronal voltage-gated calcium channels, voltage-gated sodium channels, and Na+/K+ ATPase activities, resulting in migraine attacks. Detailed research on transgenic models enables the identification of genes associated with migraine (52).

EPILEPSY AND MODELS

Epilepsy is a neurological disorder characterized by abnormal electrical activities of brain cells. Sudden and uncontrolled increases in brain electrical activity can lead to recurrent seizures (55). Among the conditions that disrupt normal functioning and cause epilepsy seizures are genetic factors (56), brain injury (57), hormonal changes (58), infections (59), neurological disorders (60), etc. In individuals with epilepsy, irregularities in the regulation of the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the central nervous system have been reported. This irregularity can lead to increased sensitivity to seizure activity and a decrease in the ability to regulate and terminate seizures (61). Since not all epileptic seizures have the same pathophysiology due to the large number of them, it is not possible to make definitive statements about all components of the disease (62). The classification of epilepsy models is primarily based on focal onset, generalized onset, and unknown onset seizures. Subcategories can be grouped as motor and non-motor onset seizures (63). In epilepsy research, animal experiments are widely used to examine specific pathophysiology, develop new treatments, and produce therapeutic drugs. Studies often utilizing rodent species aim to investigate focal and generalized epileptic seizure models. Additionally, rats and mice are also utilized in genetic studies. These models can be created through induction with chemical agents, electrical stimulation, or genetic interventions (64).

Focal Epileptic Seizures

Focal seizures occur due to disruptions in the stability of neuron membranes leading to hyperexcitability. Seizures are characterized by motor, sensory, or autonomic symptoms that remain confined to a specific region. These symptoms may involve sensory perceptions such as vision, hearing, taste, smell, and touch, or be associated with motor manifestations such as muscle spasms, tremors, and shaking (65). In focal seizures, if information about awareness can be obtained, grouping can also be done as 'aware or impaired awareness' (63).

Chemically-Induced Focal Epileptic Seizure Models

Among the most commonly used agents for creating focal epilepsy models by chemical induction are penicillin (66), pilocarpine (67), and kainic acid (68). These agents are typically administered intracerebrally. Penicillin acts on GABA receptors (69), pilocarpine on muscarinic receptors involved in the onset of seizures (70), and kainic acid on glutamate receptors (70) to induce epileptic seizures.

Electrically Induced Focal Epileptic Seizure Models

Electrical stimulation is applied to brain regions containing limbic structures such as the amygdala, hippocampus, perirhinal cortex, and piriform cortex. The kindling model is a process where low-intensity electrical stimulation is repeatedly delivered to a brain region until it triggers an epileptic seizure (This model can also be created with a chemical agent). Electrodes are implanted into the limbic region via stereotaxy for electrical stimulation, typically using 60 Hz sinusoidal or biphasic square wave pulses lasting for 1 second. Stimulation is continued until a 5-stage seizure progression (mouth and facial clonus, head nodding, forelimb clonus, rearing, and rearing and falling) is observed (70).

Generalized Epileptic Seizures

Seizures in which epileptic activity starts simultaneously in both hemispheres of the brain are called generalized seizures. These seizures typically originate from deep regions of the brain and are characterized by prominent motor or behavioral symptoms often accompanied by loss of consciousness. There are many different subtypes of these seizures, which often begin at a young age, including tonic-clonic seizures, clonic seizures, myoclonic seizures, and atonic seizures (63).

Chemically-Induced Generalized Epileptic Seizure Models

Pentylenetetrazol is a GABA receptor antagonist convulsant commonly used in experimental models to induce tonic-clonic seizures. Behavioral changes (leg dystonia, generalized clonic, and clonic-tonic convulsions) in seizures initiated by intraperitoneal administration can be observed to examine various parameters such as onset time, duration, intensity, and frequency of seizures. In addition, the effectiveness of antiepileptic drugs in preventing or reducing seizure activity can also be evaluated (71,72). Furthermore, pentylenetetrazol, used in the kindling model as a chemical inducer, not only clarifies the convulsive effect but also allows the examination of behavioral and cognitive changes, thanks to long-term injections (73).

Flurothyl is a volatile liquid with convulsant vapor. Flurothyl, an antagonist of GABA receptors, is commonly used to induce neonatal generalized tonic-clonic seizures. During the experimental phase, animals are exposed to flurothyl in an airtight environment until tonic extension develops in their front and hind limbs, thus inducing seizures. When exposure to the agent is halted, the seizures also cease. This model provides information about epilepsy in the neonatal period (70,74).

Electrically-Induced Generalized Epileptic Seizure Models

The maximal electroshock seizures serve as a model where generalized tonic-clonic seizures are induced by delivering electroshocks through electrodes. Stimulation triggers seizures characterized by tonic extension of the forelimbs and hind limbs. The efficacy of drugs is often evaluated by measuring the duration of tonic maximum extension of the hind limb. Due to its ease of use, it is frequently employed in antiepileptic drug research, and phenytoin, discovered through this model, is used as an anticonvulsant medication (70).

SCHIZOPHRENIA AND MODELS

Schizophrenia is a complex psychiatric disorder characterized by symptoms such as detachment from reality, cognitive distortions, emotional abnormalities, and perceptual disturbances. These symptoms can significantly impair an individual's functionality and lead to difficulties in daily life. Although the pathophysiology of schizophrenia is not fully understood, abnormalities in brain regions like the frontal lobe, temporal lobe, and limbic system have been detected in brain imaging studies, and these have been associated with imbalances in neurotransmitters such as dopamine, glutamate, and serotonin systems (75,76). Schizophrenia symptoms are categorized as positive, negative, and cognitive symptoms. Positive symptoms include hallucinations and delusions, as well as poor insight, while negative symptoms consist of anergia, apathy, and social withdrawal, and cognitive symptoms include a decrease in working memory and executive function ability (76,77). The lack of specific biomarkers for schizophrenia hinders successful diagnosis and treatment. Despite the incomplete parallelism between animal models of schizophrenia and the human condition, attention is directed toward animal studies aimed at unraveling the pathophysiology of schizophrenia and devising treatment modalities. Within the spectrum of schizophrenia models lie neurodevelopmental models, pharmacological/physiological models, and genetic models (76).

Neurodevelopmental Schizophrenia Models

During the perinatal and neonatal processes, factors kind of stress (78) and viral infections (79), which affect brain development, increase the risk of schizophrenia, thus enabling neurodevelopmental investigations. Animal models are used to induce viral infections such as influenza virus (80), herpes simplex virus (81), and cytomegalovirus (82) during the embryonic period, thereby activating neuroinflammatory processes. Thus, schizophrenia symptoms and developmental processes are investigated. The stress induced by social isolation after weaning in animals leads to hyperlocomotor activity associated with schizophrenia and a decrease in cognitive functions in the adult stages. It is used in the research of new antipsychotics (83).

Pharmacological / Physiological Schizophrenia Models

The association of schizophrenia with the dopamine, glutamate, and serotonin systems has led to in-depth research on these pathways. In pharmacological/physiological models, substances that are generally effective on psychotropic drugs or neurotransmitters are often used. The dopaminergic hyperfunction model, dopaminergic hypofunction model, serotonergic model, and glutamatergic hypofunction model are examined in this category (84).

Dopaminergic Hyperfunction Model

The amphetamine used in the dopaminergic hyperfunction model reflects conditions associated with the positive symptoms of schizophrenia when administered intermittently at increasing doses (85).

Dopaminergic Hypofunction Model

The agent used in the rarely used dopaminergic hypofunction model is 6-OHDA. It is reported to be useful for modeling specific aspects of the negative symptoms of schizophrenia (86).

Serotonergic Model

The serotonergic model is a sustained substitute model following amphetamine administration, and it utilizes 2,5-dimethoxy-4-iodoamphetamine, an agonist of serotonergic receptors. Its use is not as prominent as other models due to the potential limitation of the therapeutic properties of neuropsychiatric drugs (87).

Glutamatergic Hypofunction Model

The agents used in the glutamatergic hypofunction model are phencyclidine, ketamine, and MK-801 (dizocilpine). Phencyclidine has a simple, non-time-consuming, and inexpensive administration process, and after administration, psychomotor hyperactivity and psychosis-like behaviors are immediately observed (88). Intraperitoneal administration of ketamine, an NMDA receptor antagonist, allows observation of negative and cognitive schizophrenia symptoms (89). Dizocilpine, representing hyperlocomotion and behavioral symptoms well, is mentioned in the literature as a suitable agent for mimicking acute psychotic symptoms of schizophrenia (90).

Genetic Schizophrenia Models

Genetic manipulations are used in animals to study the effects of specific genes associated with schizophrenia in humans. To understand the underlying biological mechanisms of schizophrenia and develop new treatment methods, genetic models such as DISC1 (disrupted in schizophrenia 1) models (91,92), NRG1 (neuregulin 1) models (93,94), COMT (catechol-o-methyltransferase) models (95,96), and 22q11.2 deletion syndrome models (97-99) are utilized. These genes are linked to an increased risk of schizophrenia and are utilized to model behaviors and neurodevelopmental disorders associated with the condition.

CONCLUSION

In conclusion, experimental animal models play a crucial role in elucidating the mechanisms underlying neurological diseases such as Alzheimer's, Parkinson's, MS, ALS, migraine, epilepsy, and schizophrenia. These models, mimicking important aspects of neurodegenerative diseases, hold a significant place in investigating disease mechanisms and developing potential treatment methods. Through collaboration among scientists, clinicians, and animal welfare advocates, we hope to harness the power of experimental models in the treatment of neurological diseases that affect a large portion of individuals and society.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: NŞ, ŞD; Design: NŞ, ŞD; Data Collection/Processing: NŞ; Analysis/Interpretation: NŞ; Literature Review: NŞ; Drafting/Writing: NŞ; Critical Review: NŞ, ŞD.

REFERENCES

1. Gowers WR. A manual of diseases of the nervous system. Philadelphia: P. Blakiston's Son & Co.; 1899.
2. Bansal PK, Deshmukh R. Animal models of neurological disorders: principle and working procedure for animal models of neurological disorders. Singapore: Springer; 2018.
3. Mucke L. Neuroscience: Alzheimer's disease. Nature. 2009;461(7266):895-7.
4. Piaceri I, Nacmias B, Sorbi S. Genetics of familial and sporadic Alzheimer's disease. Front Bosci (Elite Ed). 2013;5(1):167-77.
5. Rajmohan R, Reddy PH. Amyloid-Beta and phosphorylated tau accumulations cause abnormalities at synapses of Alzheimer's disease neurons. J Alzheimers Dis. 2017;57(4):975-99.
6. Shree S, Bhardwaj R, Kashish, Deshmukh R. Non-transgenic animal models of Alzheimer's disease. In: Bansal PK, Deshmukh R, editors. Animal models of neurological disorders: principle and working procedure for animal models of neurological disorders. Singapore: Springer Singapore; 2017. p.3-22.
7. Li X, Bao X, Wang R. Experimental models of Alzheimer's disease for deciphering the pathogenesis and therapeutic screening (Review). Int J Mol Med. 2016;37(2):271-83.
8. Sasaguri H, Nilsson P, Hashimoto S, Nagata K, Saito T, De Strooper B, et al. APP mouse models for Alzheimer's disease preclinical studies. EMBO J. 2017;36(17):2473-87.
9. Sahara N, Lewis J. Amyloid precursor protein and tau transgenic models of Alzheimer's disease: insights from the past and directions for the future. Future Neurol. 2010;5(3):411-20.
10. Kamat PK. Streptozotocin induced Alzheimer's disease like changes and the underlying neural degeneration and regeneration mechanism. Neural Regen Res. 2015;10(7):1050-2.
11. Pal A, Rani I, Pawar A, Picozza M, Rongioletti M, Squitti R. Microglia and astrocytes in Alzheimer's disease in the context of the aberrant copper homeostasis hypothesis. Biomolecules. 2021;11(11):1598.
12. Kim HY, Lee DK, Chung BR, Kim HV, Kim Y. Intracerebroventricular injection of amyloid-β peptides in normal mice to acutely induce Alzheimer-like cognitive deficits. J Vis Exp. 2016;(109):53308.
13. Kumar A, Dogra S, Prakash A. Neuroprotective effects of Centella asiatica against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress. Int J Alzheimers Dis. 2009;2009:972178.
14. Kumar A, Seghal N, Naidu PS, Padi SS, Goyal R. Colchicines-induced neurotoxicity as an animal model of sporadic dementia of Alzheimer's type. Pharmacol Rep. 2007;59(3):274-83.
15. Savory J, Herman MM, Ghribi O. Mechanisms of aluminum-induced neurodegeneration in animals: Implications for Alzheimer's disease. J Alzheimers Dis. 2006;10(2-3):135-44.
16. Walton JR. An aluminum-based rat model for Alzheimer's disease exhibits oxidative damage, inhibition of PP2A activity, hyperphosphorylated tau, and granulovacuolar degeneration. J Inorg Biochem. 2007;101(9):1275-84.
17. Watt NT, Whitehouse IJ, Hooper NM. The role of zinc in Alzheimer's disease. Int J Alzheimers Dis. 2011;2011:971021.
18. Zhan X, Stamova B, Sharp FR. Lipopolysaccharide associates with amyloid plaques, neurons and oligodendrocytes in Alzheimer's disease brain: a review. Front Aging Neurosci. 2018;10:42.
19. Dickson DW. Neuropathology of Parkinson disease. Parkinsonism Relat Disord. 2018;46(Suppl 1):S30-S3.
20. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron. 2003;39(6):889-909.
21. Sharma N, Jamwal S, Singh S, Gill HK, Bansal PK. Animal models of Parkinson's disease. In: Bansal PK, Deshmukh R, editors. Animal models of neurological disorders: principle and working procedure for animal models of neurological disorders. Singapore: Springer Singapore; 2017. p.23-42.
22. Blandini F, Armentero MT, Martignoni E. The 6-hydroxydopamine model: News from the past. Parkinsonism Relat Disord. 2008;14(Suppl 2):S124-9.
23. Masilamoni GJ, Smith Y. Chronic MPTP administration regimen in monkeys: a model of dopaminergic and non-dopaminergic cell loss in Parkinson's disease. J Neural Transm (Vienna). 2018;125(3):337-63.
24. Smeyne RJ, Jackson-Lewis V. The MPTP model of Parkinson's disease. Brain Res Mol Brain Res. 2005;134(1):57-66.
25. Jagmag SA, Tripathi N, Shukla SD, Maiti S, Khurana S. Evaluation of models of Parkinson's disease. Front Neurosci. 2016;9:503.
26. Perier C, Bové J, Vila M, Przedborski S. The rotenone model of Parkinson's disease. Trends Neurosci. 2003;26(7):345-6.
27. Berry C, La Vecchia C, Nicotera P. Paraquat and Parkinson's disease. Cell Death Differ. 2010;17(7):1115-25.
28. Deng I, Corrigan F, Zhai G, Zhou XF, Bobrovskaya L. Lipopolysaccharide animal models of Parkinson's disease: Recent progress and relevance to clinical disease. Brain Behav Immun Health. 2020;4:100060.
29. Kalinderi K, Bostantjopoulou S, Fidani L. The genetic background of Parkinson's disease: current progress and future prospects. Acta Neurol Scand. 2016;134(5):314-26.
30. Gupta S, Kour S, Deshmukh R. Animal models of multiple sclerosis (MS). In: Bansal PK, Deshmukh R, editors. Animal models of neurological disorders: principle and working procedure for animal models of neurological disorders. Singapore: Springer Singapore; 2017. p.263-76.
31. Procaccini C, De Rosa V, Pucino V, Formisano L, Matarese G. Animal models of Multiple sclerosis. Eur J Pharmacol. 2015;759:182-91.
32. Bjelobaba I, Begovic-Kupresanin V, Pekovic S, Lavrnja I. Animal models of multiple sclerosis: Focus on experimental autoimmune encephalomyelitis. J Neurosci Res. 2018;96(6):1021-42.
33. Zarei S, Carr K, Reiley L, Diaz K, Guerra O, Altamirano PF, et al. A comprehensive review of amyotrophic lateral sclerosis. Surg Neurol Int. 2015;6:171.

34. Aktekin MR, Uysal H. Epidemiology of amyotrophic lateral sclerosis. Turk J Neurol. 2020;26(3):187-96.
35. Bonifacino T, Zerbo RA, Balbi M, Torazza C, Frumento G, Fedele E, et al. Nearly 30 years of animal models to study amyotrophic lateral sclerosis: a historical overview and future perspectives. Int J Mol Sci. 2021;22(22):12236.
36. den Hartog Jager WA. Experimental amyotrophic lateral sclerosis in the guinea-pig. J Neurol Sci. 1985;67(2):133-42.
37. Engelhardt JI, Appel SH, Killian JM. Motor neuron destruction in guinea pigs immunized with bovine spinal cord ventral horn homogenate: experimental autoimmune gray matter disease. J Neuroimmunol. 1990;27(1):21-31.
38. Engelhardt JI, Appel SH, Killian JM. Experimental autoimmune motoneuron disease. Ann Neurol. 1989;26(3):368-76.
39. de Munck E, Muñoz-Sáez E, Miguel BG, Solas MT, Ojeda I, Martínez A, et al. β-N-methylamino-l-alanine causes neurological and pathological phenotypes mimicking amyotrophic lateral sclerosis (ALS): The first step towards an experimental model for sporadic ALS. Environ Toxicol Pharmacol. 2013;36(2):243-55.
40. de Munck E, Muñoz-Sáez E, Miguel BG, Solas MT, Martínez A, Arahuetes RM. Morphometric and neurochemical alterations found in l-BMAA treated rats. Environ Toxicol Pharmacol. 2015;39(3):1232-45.
41. Wilson JM, Petrik MS, Moghadasian MH, Shaw CA. Examining the interaction of apo E and neurotoxicity on a murine model of ALS-PDC. Can J Physiol Pharmacol. 2005;83(2):131-41.
42. Tabata RC, Wilson JM, Ly P, Zwiegers P, Kwok D, Van Kampen JM, et al. Chronic exposure to dietary sterol glucosides is neurotoxic to motor neurons and induces an ALS-PDC phenotype. Neuromolecular Med. 2008;10(1):24-39.
43. Smitt PAES, de Jong JM. Animal models of amyotrophic lateral sclerosis and the spinal muscular atrophies. J Neurol Sci. 1989;91(3):231-58.
44. Harriott AM, Strother LC, Vila-Pueyo M, Holland PR. Animal models of migraine and experimental techniques used to examine trigeminal sensory processing. J Headache Pain. 2019;20(1):91.
45. Noseda R, Burstein R. Migraine pathophysiology: Anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain. Pain. 2013;154(Supll 1):S44-53.
46. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. Annu Rev Physiol. 2013;75:365-91.
47. Lipton RB, Bigal ME, Steiner TJ, Silberstein SD, Olesen J. Classification of primary headaches. Neurology. 2004;63(3):427-35.
48. Eikermann-Haerter K, Moskowitz MA. Animal models of migraine headache and aura. Curr Opin Neurol. 2008;21(3):294-300.
49. Andreou AP, Summ O, Charbit AR, Romero-Reyes M, Goadsby PJ. Animal models of headache: from bedside to bench and back to bedside. Expert Rev Neurother. 2010;10(3):389-411.
50. Lukács M, Haanes KA, Majláth Z, Tajti J, Vécsei L, Warfvinge K, et al. Dural administration of inflammatory soup or Complete Freund's Adjuvant induces activation and inflammatory response in the rat trigeminal ganglion. J Headache Pain. 2015;16:564.
51. Sufka KJ, Staszko SM, Johnson AP, Davis ME, Davis RE, Smitherman TA. Clinically relevant behavioral endpoints in a recurrent nitroglycerin migraine model in rats. J Headache Pain. 2016;17:40.
52. Barrett CF, van den Maagdenberg AMJM, Frants RR, Ferrari MD. Familial hemiplegic migraine. Adv Genet. 2008;63:57-83.
53. Moskowitz MA, Nozaki K, Kraig RP. Neocortical spreading depression provokes the expression of c-fos protein-like immunoreactivity within trigeminal nucleus caudalis via trigeminovascular mechanisms. J Neurosci. 1993;13(3):1167-77.
54. Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A. Characterization of a novel model of chronic migraine. Pain. 2014;155(2):269-74.
55. Cano A, Fonseca E, Ettcheto M, Sánchez-López E, de Rojas I, Alonso-Lana S, et al. Epilepsy in neurodegenerative diseases: related drugs and molecular pathways. Pharmaceuticals (Basel). 2021;14(10):1057.
56. Poduri A, Lowenstein D. Epilepsy genetics--past, present, and future. Curr Opin Genet Dev. 2011;21(3):325-32.
57. Olney JW. Excitatory transmitters and epilepsy-related brain damage. Int Rev Neurobiol. 1985:27:337-62.
58. Taubøll E, Sveberg L, Svalheim S. Interactions between hormones and epilepsy. Seizure. 2015;28:3-11.
59. Vezzani A, Fujinami RS, White HS, Preux PM, Blümcke I, Sander JW, et al. Infections, inflammation and epilepsy. Acta Neuropathol. 2016;131(2):211-34.
60. Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. J Clin Invest. 2005;115(8):2010-7.
61. Sarlo GL, Holton KF. Brain concentrations of glutamate and GABA in human epilepsy: A review. Seizure. 2021;91:213-27.
62. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia. 2010;51(4):676-85.
63. Fisher RS. The New Classification of Seizures by the International League Against Epilepsy 2017. Curr Neurol Neurosci Rep. 2017;17(6):48.
64. Löscher W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure. 2011;20(5):359-68.
65. Ighodaro ET, Maini K, Arya K, Sharma S. Focal Onset Seizure. [Updated 2023 Sep 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
66. Prabhu S, Chabardès S, Sherdil A, Devergnas A, Michallat S, Bhattacharjee M, et al. Effect of subthalamic nucleus stimulation on penicillin induced focal motor seizures in primate. Brain Stimul. 2015;8(2):177-84.
67. Curia G, Longo D, Biagini G, Jones RSG, Avoli M. The pilocarpine model of temporal lobe epilepsy. J Neurosci Methods. 2008;172(2):143-57.

68. Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. Neurosci Biobehav Rev. 2013;37(10 Pt 2):2887-99.
69. Sutter R, Rüegg S, Tschudin-Sutter S. Seizures as adverse events of antibiotic drugs. Neurology. 2015;85(15):1332-41.
70. Raol YH, Brooks-Kayal AR. Experimental models of seizures and epilepsies. Prog Mol Biol Transl Sci. 2012;105:57-82.
71. Gholami M, Saboory E, Zare S, Roshan-Milani S, Hajizadeh-Moghaddam A. The effect of dorsal hippocampal administration of nicotinic and muscarinic cholinergic ligands on pentylenetetrazol-induced generalized seizures in rats. Epilepsy Behav. 2012;25(2):244-9.
72. JelenkoviĆ A, JovanoviĆ M, NinkoviĆ M, MaksimoviĆ M, BokonjiĆ D, BoŠKoviĆ B. Nitric oxide (NO) and convulsions induced by pentylenetetrazol. Ann N Y Acad Sci. 2002;962:296-305.
73. Becker A, Grecksch G, Thiemann W, Höllt V. Pentylenetetrazol-kindling modulates stimulated dopamine release in the nucleus accumbens of rats. Pharmacol Biochem Behav. 2000;66(2):425-8.
74. Isaeva E, Isaev D, Khazipov R, Holmes GL. Selective impairment of GABAergic synaptic transmission in the flurothyl model of neonatal seizures. Eur J Neurosci. 2006;23(6):1559-66.
75. Hany M, Rehman B, Azhar Y, Chapman J. Schizophrenia. [Updated 2023 Mar 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
76. Winship IR, Dursun SM, Baker GB, Balista PA, Kandratavicius L, Maia-de-Oliveira JP, et al. An overview of animal models related to schizophrenia. Can J Psychiatry. 2019;64(1):5-17.
77. McCutcheon RA, Reis Marques T, Howes OD. Schizophrenia-An Overview. JAMA Psychiatry. 2020;77(2):201-10.
78. Ratajczak P, Kus K, Murawiecka P, Słodzińska I, Giermaziak W, Nowakowska E. Biochemical and cognitive impairments observed in animal models of schizophrenia induced by prenatal stress paradigm or methylazoxymethanol acetate administration. Acta Neurobiol Exp (Wars). 2015;75(3):314-25.
79. Carter CJ. Schizophrenia susceptibility genes directly implicated in the life cycles of pathogens: cytomegalovirus, influenza, herpes simplex, rubella, and Toxoplasma gondii. Schizophr Bull. 2008;35(6):1163-82.
80. Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. Brain Behav Immun. 2008;22(4):469-86.
81. Engel JA, Zhang J, Bergström T, Conradi N, Forkstam C, Liljeroth A, et al. Neonatal herpes simplex virus type 1 brain infection affects the development of sensorimotor gating in rats. Brain Res. 2000;863(1):233-40.
82. Rothschild DM, O'Grady M, Wecker L. Neonatal cytomegalovirus exposure decreases prepulse inhibition in adult rats: Implications for schizophrenia. J Neurosci Res. 1999;57(4):429-34.
83. Gaskin PL, Alexander SP, Fone KC. Neonatal phencyclidine administration and post-weaning social isolation as a dual-hit model of 'schizophrenia-like' behaviour in the rat. Psychopharmacology (Berl). 2014;231(12):2533-45.
84. Białoń M, Wąsik A. Advantages and limitations of animal schizophrenia models. Int J Mol Sci. 2022;23(11):5968.
85. Featherstone RE, Rizos Z, Kapur S, Fletcher PJ. A sensitizing regimen of amphetamine that disrupts attentional set-shifting does not disrupt working or long-term memory. Behav Brain Res. 2008;189(1):170-9.
86. Rezvani AH, Eddins D, Slade S, Hampton DS, Christopher NC, Petro A, et al. Neonatal 6-hydroxydopamine lesions of the frontal cortex in rats: Persisting effects on locomotor activity, learning and nicotine self-administration. Neuroscience. 2008;154(3):885-97.
87. Canal CE, Morgan D. Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. Drug Test Anal. 2012;4(7-8):556-76.
88. Kalinichev M, Robbins MJ, Hartfield EM, Maycox PR, Moore SH, Savage KM, et al. Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague-Dawley rats: a locomotor activity and gene induction study. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32(2):414-22.
89. Jeevakumar V, Driskill C, Paine A, Sobhanian M, Vakil H, Morris B, et al. Ketamine administration during the second postnatal week induces enduring schizophrenia-like behavioral symptoms and reduces parvalbumin expression in the medial prefrontal cortex of adult mice. Behav Brain Res. 2015;282:165-75.
90. Shahzad S, Ahmad S, Madiha S, Khaliq S, Liaquat L, Sadir S, et al. Dizocilpine induced psychosis-like behavior in rats: A possible animal model with full spectrum of schizophrenia. Pak J Pharm Sci. 2017;30(Suppl 6):2423-7.
91. Pletnikov MV, Ayhan Y, Xu Y, Nikolskaia O, Ovanesov M, Huang H, et al. Enlargement of the lateral ventricles in mutant DISC1 transgenic mice. Mol Psychiatry. 2008;13(2):115.
92. Shen S, Lang B, Nakamoto C, Zhang F, Pu J, Kuan SL, et al. Schizophrenia-related neural and behavioral phenotypes in transgenic mice expressing truncated Disc1. J Neurosci. 2008;28(43):10893-904.
93. Karl T, Duffy L, Scimone A, Harvey RP, Schofield PR. Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes Brain Behav. 2007;6(7):677-87.
94. Chesworth R, Downey L, Logge W, Killcross S, Karl T. Cognition in female transmembrane domain neuregulin 1 mutant mice. Behav Brain Res. 2012;226(1):218-23.
95. Janetsian-Fritz SS, Timme NM, Timm MM, McCane AM, Baucum Ii AJ, O'Donnell BF, et al. Maternal deprivation induces alterations in cognitive and cortical function in adulthood. Transl Psychiatry. 2018;8(1):71.

96. Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, et al. Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. Proc Natl Acad Sci U S A. 1998;95(17):9991-6.
97. Bassett AS, Chow EW. Schizophrenia and 22q11.2 deletion syndrome. Curr Psychiatry Rep. 2008;10(2):148-57.
98. Saito R, Koebis M, Nagai T, Shimizu K, Liao J, Wulaer B, et al. Comprehensive analysis of a novel mouse model of the 22q11.2 deletion syndrome. Transl Psychiatry. 2020;10(1):35.
99. Sun Z, Williams DJ, Xu B, Gogos JA. Altered function and maturation of primary cortical neurons from a 22q11.2 deletion mouse model of schizophrenia. Transl Psychiatry. 2018;8(1):85.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1493348

Duzce Med J, 2024;26(S1):65-71
Düzce Tıp Fak Derg, 2024;26(S1):65-71

Animal Experiments Used in Experimental Neuroscience Research: Learning, Memory, Anxiety, Depression and Motor Function Behavioural Experiments

Deneysel Sinirbilim Araştırmalarında Kullanılan Hayvan Deneyleri: Öğrenme, Hafıza, Anksiyete, Depresyon ve Motor Fonksiyon Davranış Deneyleri

Güven AKÇAY
0000-0003-3418-8825

Department of Biophysics, Bolu Abant İzzet Baysal University Faculty of Medicine, Bolu, Türkiye

ABSTRACT

Behavioral experiments have been conducted since the classical conditioning research of Ivan Pavlov in 1904. Experimental research plays an important role in understanding the mechanisms of diseases, preventing these diseases, and developing effective treatment methods. Research using animal models is very important to understand the mechanisms of these diseases and to develop effective treatment strategies. Animal models are widely used in the research of a treatment method, the development of novel treatment protocols, and the discovery of new drug molecules. The efficacy of the drug to be developed is very important both for testing whether the animal model is formed before starting the research and for the effectiveness of the drug in treatment and for the elucidation of the mechanisms to be investigated. Therefore, evaluations are usually made with behavioral experiments. Each behavioral experiment has its own advantages and disadvantages. Therefore, the researcher should be aware of these advantages and limitations before choosing the most appropriate behavioral experiment. This review aimed to describe the most commonly used learning, memory, anxiety, depression, and motor function behavioral experimental protocols in experimental models such as Alzheimer's, epilepsy, migraine, neuropathic pain, schizophrenia, Parkinson's, cerebral ischemia, and traumatic brain injury.
Keywords: Anxiety; depression; learning; memory; motor function.

ÖZ

Davranış deneyleri, 1904'te Ivan Pavlov'un klasik koşullanma araştırmalarından beri yapılmaktadır. Hastalıkların mekanizmalarının anlaşılması, bu hastalıkların önlenmesi ve etkin tedavi yöntemlerinin geliştirilmesinde, deneysel çalışmalar önemli rol oynar. Hayvan modelleri kullanılarak yapılan araştırmalar, bu hastalıkların mekanizmalarını aydınlatabilmek ve etkin tedavi stratejileri geliştirebilmek için oldukça önemlidir. Bir tedavi yönteminin araştırılmasında, yeni tedavi protokollerinin geliştirilmesinde ve yeni ilaç moleküllerinin keşfedilmesinde, hayvan modelleri yaygın olarak kullanılır. Geliştirilecek olan ilacın etkinliği hem araştırılmaya başlanmadan önce hayvan modelinin oluşup oluşmadığının test edilmesi için hem de ilacın tedavideki etkinliği açısından ve hem de araştırılacak mekanizmaların aydınlatılması için oldukça önemlidir. Bundan dolayı genellikle davranış deneyleri ile değerlendirmeler yapılır. Her davranış deneyinin kendine özgü avantajları ve dezavantajları vardır. Bu nedenle araştırmacı, davranış deneyini seçmeden önce bu avantajların ve kısıtlamaların farkında olarak en uygun davranış deneyini tercih etmelidir. Bu derlemede Alzheimer, epilepsi, migren, nöropatik ağrı, şizofreni, Parkinson, serebral iskemi ve travmatik beyin hasarı gibi deneysel modellerde en sık kullanılan öğrenme, hafıza, anksiyete, depresyon ve motor fonksiyon davranışsal deney protokollerinin detaylı olarak tanımlanması amaçlandı.
Anahtar kelimeler: Anksiyete; depresyon; hafıza; öğrenme; motor fonksiyon.

Corresponding Author
Sorumlu Yazar
Güven AKÇAY
guvenakcayibu@gmail.com

Received / Geliş Tarihi : 07.03.2024
Accepted / Kabul Tarihi : 07.05.2024
Available Online / Çevrimiçi Yayın Tarihi : 31.05.2024

INTRODUCTION

Experimental animals; human health protection, improvement, and complementary therapy prioritized in the discovery, and development of methods is a group of living organisms. A medicine or treatment method can be used on human beings through many trials and researches in order to become must pass. Animal models are the most preferred research method for the prevention and treatment of diseases in humans (1).

Behavioral experiments have been conducted since the classical conditioning research of Ivan Pavlov in 1904, and human behavior has been investigated through experiments on animals. The effectiveness of these models is tested by behavioral experiments such as cognitive, emotional, and motor functions such as learning, memory, locomotor activity, anxiety, and depression. The complex structure and signaling pathways of nervous systems make it difficult to understand the neurobiology and pathophysiology of diseases and also prevent the development of radical treatments for these diseases. Experimental studies play an important role in understanding the mechanisms of neurological diseases, preventing these diseases, and applying effective treatment methods. Research using animal models plays a vital role in elucidating the mechanisms of these diseases and developing effective treatment strategies (2). Rodents (e.g. mice, rats, gerbils) and non-rodent mammals (e.g. dogs, rabbits, cats, pigs, chimpanzees) are frequently used in the research of a treatment modality, the development of new treatment protocols, and the discovery of new drug molecules. In animal models, basic neuronal mechanisms of both normal and abnormal brain function are investigated. Each model has its own advantages and disadvantages. Therefore, the researcher should be aware of these advantages and limitations before choosing a particular model and should prefer the most appropriate model for his/her study (2).

In order to demonstrate the efficacy of a drug or treatment in animal experiments, the accuracy of the animal model must first be tested. In this review, the most commonly used learning, memory, anxiety, depression, and motor function behavioral experimental protocols in experimental models such as Alzheimer's, epilepsy, migraine, neuropathic pain, schizophrenia, Parkinson's, cerebral ischemia, and traumatic brain injury, etc. (3-8) were described in detail.

MOTOR FUNCTION BEHAVIOR EXPERIMENTS

Open Field Test

Open field test (OF) is a test generally used to assess locomotor activity (9). OF experiments are measured in a square experimental setup with a height of 40 cm and 80x80 cm in rat experiments, and in a square experimental setup with a height of 40 cm and 40x40 cm in mouse experiments (Figure 1). The areas are usually divided into 16 small squares equal to each other. At the beginning of the experiment, the animals are placed one by one in the center of this area and their movements are recorded by the camera for 5 minutes. Before each test, the OF setup must be thoroughly cleaned against the odor stimulus. The total distance (cm), velocity (cm/s), and the number of squares entered are measured as locomotor activity parameters (9).

Rotarod Test

The rotarod test is used to measure motor performance and coordination of animals (Figure 2). Animals are acclimatized to the rotarod for 3 consecutive days before the experiment. During this time, animals (for rats) are trained to walk on a rotating rod at a constant speed of 2 rpm for a maximum of 12 min. On the test day, after the animals are placed in their individual compartments in the rotarod device in the opposite direction of the rod (7.3 cm diameter) rotating at a uniform speed between 4 and 40 rpm for a period of 5 min, the time spent on the rod is automatically recorded (10). The recording time is set to 300 seconds and 3 consecutive measurements are made with rest periods of 5 minutes.

LEARNING AND MEMORY BEHAVIORAL EXPERIMENTS

Morris Water Maze

The Morris water maze test is highly preferred in learning and memory experiments. Especially working and reference memory are assessed. A circular water tank with a diameter of 150 cm and a height of 45 cm is used in the Morris Water Maze test setup. In the experimental room, visual clues are placed around the experimental setup. The height of the water should be 30 cm and the temperature should be 22±2 C^{o}. The circular tank is divided into 4 equal quadrants and a 10 cm diameter platform is placed 2 cm below the water surface in the escape quadrant. In the learning phase of the experiment, rats/mice are randomly released into the tank and allowed to swim for 90 seconds. Animals that find the platform within this time are allowed to stay on the platform for 30 seconds. Within 120 seconds, rats/mice that could not find the platform were slowly guided to the platform and allowed to stay on the platform for 30 seconds. For 5 days, 4 trials are performed daily at 30-minute intervals and the platform is removed at the end of the trials on the 6th day. During the experiment, whether learning occurred or not was evaluated with a 30-second probe trial in a water tank without a platform. Meanwhile, the arrival time of the mice to the platform, swimming paths, and swimming speeds of the mice were recorded by a video camera system (11).

Radial Arm Maze

The radial arm maze (RAM) is used to measure spatial learning and memory. The RAM consists of eight arms with a food zone at the end of the arm. In the room where the experimental setup is located, the RAM is surrounded by a large number of visual objects. Animals are habituated by exploring the maze for 5 minutes a day for 3 days. On the first day of habituation, animals are gradually allowed to access food from all arms. Following habituation, each trial is administered twice a day for 4 days. The 2nd, 3rd, 5th, and 7th arms have chocolate, while the other arms have no chocolate. The animal is placed in the center of the apparatus on each trial and working and reference memory are assessed (12). The maze was thoroughly cleaned with 70% ethanol and dried before each trial. With a video monitoring system in RAM, it is usually measured by three parameters; (i) the number of reference memory errors (RME, visits to unbaited arms), (ii) the number of working memory errors (WME, visits to previously visited arms in the same trial) and (iii) the accuracy index (number of first entries to baited arms/total

entries to all arms). Reference memory is associated with long-term memory for information that remains consistent across repeated trials (memory for the positions of unbaited arms), whereas working memory is associated with short-term memory, where the information to be recalled changes on each trial (memory for the positions of arms that have already been visited on each trial).

Novel Object Recognition Test

The novel object recognition test is especially used in attention and short-term memory studies and consists of three stages: habituation, training, and retention (Figure 3). In the habituation phase, animals are placed in the center of a 40 cm high, 80x80 cm setup and allowed to walk around for 5 minutes without any objects in the environment. In the training phase, animals are left in the center and allowed to examine two objects placed in the environment for 5 minutes. Between the phases, the apparatus should be cleaned with 70% ethanol to prevent the animals from moving according to the sense of smell. In the retention phase, one of the objects is replaced with a novel object, and the animals' behavior is recorded for 5 minutes. In this process, animals are expected to spend more time examining the novel object (13). In the novel object recognition test, the discrimination index and time spent on the novel object (s) values are analyzed. Discrimination Index = ((Time spent on the new object - Time spent on the old object) / Total time)*100.

Object Location Test

The object location test is especially used in short-term and spatial memory researches and consists of three stages: habituation, training, and retention (Figure 4). In the habituation phase, animals are placed in the center of a

Figure 1. Locomotor activity experimental phases

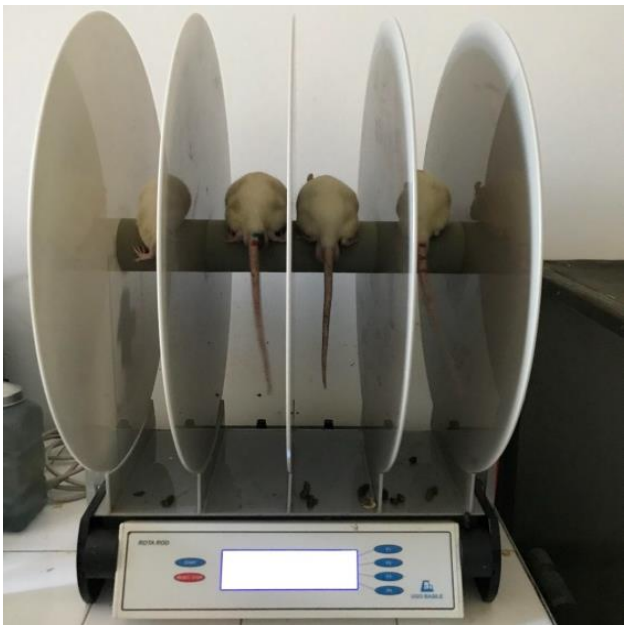

Figure 2. Rotarod system

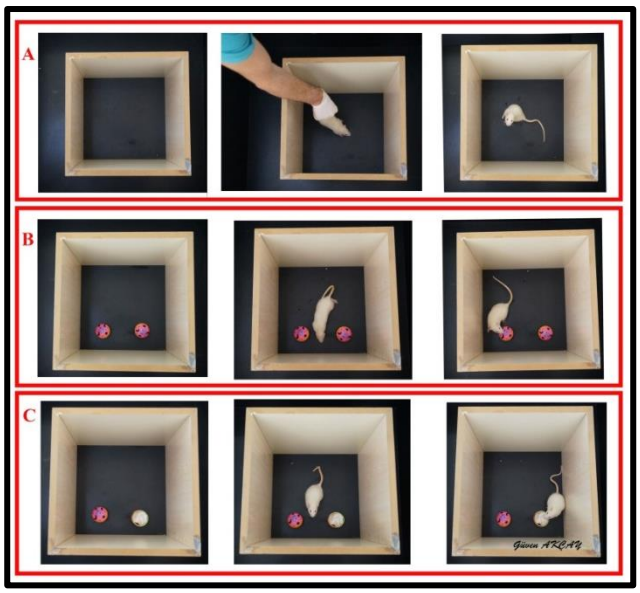

Figure 3. Experimental phases of novel object recognition test; a) habituation, b) training, and c) retention phases

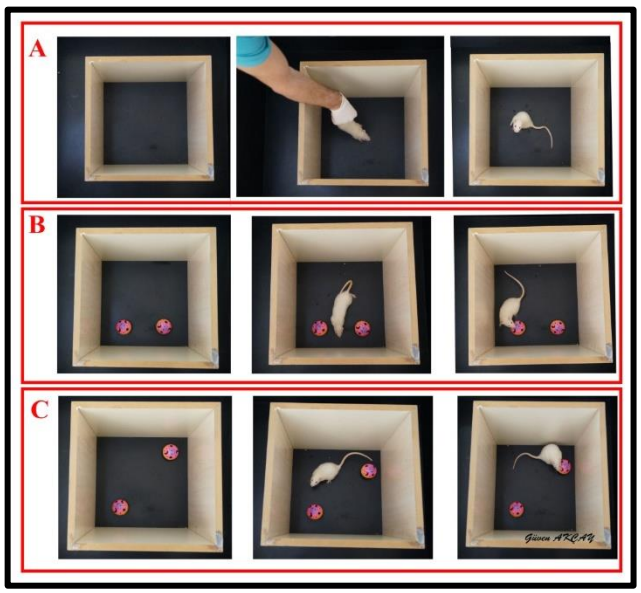

Figure 4. Experimental phases of the object localization test; a) habituation, b) training, and c) retention phases

40 cm high, 80x80 cm environment, and allowed to move around for 5 minutes without any objects in the environment. In the training phase, the animals are released from the center and allowed to examine two objects placed in the environment for 5 minutes. Between the stages, the maze setup is cleaned with 70% ethanol to prevent the animals from moving according to the sense of smell. In the recall phase, one of the objects is replaced and the behavior of the animals is recorded for 5 minutes. Animals are expected to spend more time examining the relocated object (14). In the object localization test, the discrimination index and the time spent on the displaced object (s) values are analyzed. Discrimination Index = ((Time spent on the displaced object - Time spent on the non-displaced object) / Total time)*100.

Y-maze Test

The Y-maze test is widely used to assess both the short-term memory and spatial memory of rats (9). The Y-maze test is a three-arm apparatus for animals, each arm is 50 cm long, 20 cm wall height, and 10 cm width, and the angle between the arms is 120° (Figure 5). The walls of the experiment room are equipped with visual clues. The arms of the maze are named as 'initial', 'other', and 'new' arms. In the first part of the experiment, rats are left at the end of the initial arm, and each animal is given 15 minutes to freely examine the other arms while the new arm is completely closed. At the end of 15 minutes, the animals are returned to the cages and the Y-labyrinth apparatus is cleaned with 70% ethanol to prevent them from moving according to the sense of smell during the experiment. One hour later to test short-term memory and 24 hours later to test long-term memory, a new arm is opened and the animals are returned to the initial arm of the maze and allowed to move freely in all three arms for 5 minutes and their behavior is recorded by a camera. To evaluate memory, the rate of entry into the new arm and the rate of time spent in the new arm are analyzed (9).

ANXIETY BEHAVIORAL EXPERIMENTS

Open Field Test

The open-field test is a test used to assess anxiety and some depression-like behaviors (Figure 6). The number of crossovers and the percentage of time spent in the outer/inner quadrant are used as depression parameters (3).

Elevated Plus Maze Test

It is a preferred test, especially in the determination of emotional behaviors and in the interpretation of long-term anxiety responses. Animals are housed in a plus-shaped enclosure with an arm width of 10 cm, an arm length of 45 cm, a wall height of 9 cm, and a floor height of 68 cm. Two arms are surrounded by walls and the other two arms are completely open. Each animal is released at the center where the arms overlap and its behavior is recorded for 5 minutes. The time spent in the open and closed arms and the number of entries into the open and closed arms recorded by the video camera are evaluated (15).

DEPRESSION BEHAVIORAL EXPERIMENTS

Forced Swim Test

The forced swim test is a test used in depression research, especially in testing short-term depression. In this model, animals are placed in a cylinder filled with water. After a certain period of time, the animals assume a posture of "immobility" in the water, a posture that reflects the depressed state of mind of animals whose body shape has lost hope (Figure 7). Symptoms of depression: Hopelessness, immobility, reduced escape behavior. Parameters measured: time spent in escape behavior, immobility, swimming, climbing, etc. Antidepressant effect: significant reduction in immobility (16).

Light-Dark Model

Animals are placed in a 60×60×60×45 cm 2-compartment set-up consisting of a light and a dark compartment. The chamber wall has an opening of 7×7 cm between the two compartments. The bright and novel environment causes

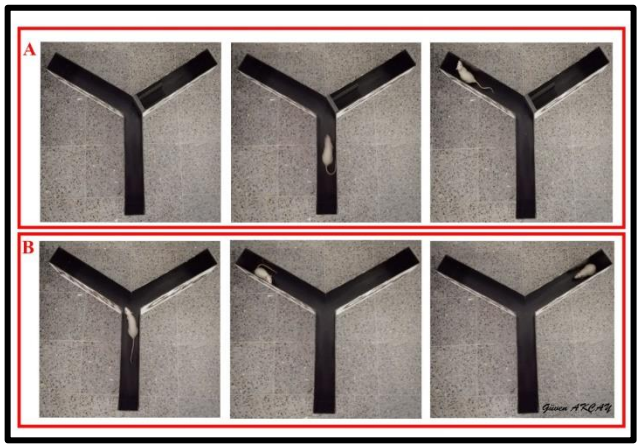

Figure 5. Y maze test experiment phases; a) training, and b) retention phases

Figure 6. Presentation of the path traveled by the experimental groups in the open field setup with lines and inner/outer quadrant transitions

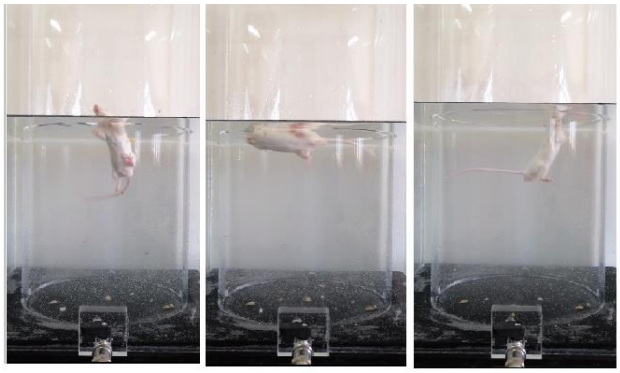

Figure 7. Forced swim test setup

photophobia for the animals and therefore the time spent in the dark area (movement) and the number of transitions is a sign of photophobic behavior. For 5 minutes, the time spent in both areas, the time spent moving in both areas and the number of transitions between the compartments are measured (17).

Catatonia Test

Catatonia is a psychomotor syndrome in which motor excitement, stereotypy, and stupor can be observed. Rats with genetic catatonia and pendulum-like movements in the anterior half of the body have physiological and behavioral changes similar to those observed in schizophrenia and depression in humans and can be considered as incomplete experimental models of these pathologies (18). Catatonia, a postural disorder, is defined as "freezing" in animals and is a simple and easily applicable method (10). Animals are slowly placed on a 25x35 cm vertical wire grid (5 mm spacing). The time the animals are completely immobilized on the grid is measured and recorded with a stopwatch (Figure 8). The severity of cataleptic behavior is assessed by measuring the longest period of immobility within a 2-minute observation period (10).

NEUROPATHIC PAIN BEHAVIORAL EXPERIMENTS

Hot Plate Test

The hot plate test assesses thermal hyperalgesia and its effects on the thermal nociceptive threshold (4,19). The surface of the hot plate apparatus was preheated and maintained at a constant temperature of 55±0.1 °C (Figure 9). Animals are placed into glass funnels on the heated surface and the time between the rat's placement and the first response (foot licking, jumping, or rapid raising of the paws) is recorded as the paw withdrawal latency. The cut-off time is set at 20 seconds to avoid tissue damage. The hind paw retraction time (sec) is measured for each animal (20).

Tail Flick Test

Thermal hyperalgesia is assessed by tail-flick test (21). The animal automatically raises its tail when it feels uncomfortable. Briefly, 2 cm of the distal tail is immersed in a water bath at 52.5±0.2 °C. The time for the animals to shake the tail is recorded as the tail shake latency; to avoid damage to the tail tissues, the cutting latency is set at 15 seconds (4,22).

Randall-Selitto Test

Mechanical hyperalgesia is measured with the Randall-Selitto test. The Randall-Selitto test involves applying evenly increasing mechanical pressure to the animal's paw (23). This pressure causes pain leading to an escape response. Animals are immobilized and grasped with one hand. Their hind paws are subjected to a linear increasing pressure until the paw retracts or vocalizations occur. The force (grams) with which the paw is withdrawn is recorded. 3-4 consecutive measurements are made at 5 min intervals and the retraction threshold for each animal is calculated by averaging the force at which the animal retracts the paw (Figure 10). The cutting force is determined as 250 g. The average of three consecutive tests with 1-minute inter-stimulus intervals is considered as the muscle pressure threshold (4,24).

Acetic Acid-Induced Writhing Test

Subjects are injected intraperitoneally with 0.3% acetic acid (10 ml/kg) to induce hyperalgesia. 5 minutes after the administration of acetic acid, the writhing (abdominal stretching/contraction) of the subjects is monitored for 5 minutes and the number of writhing is evaluated (25).

Figure 8. Catatonia test setup

Figure 9. Hot plate test setup

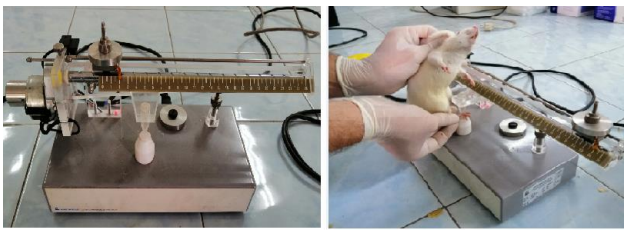

Figure 10. Randall-Selitto test setup

CONCLUSION

Behavioral animal experiments play an important role in understanding the biological basis of human behavior and disease. Studying with animals ensures high scientific quality, reliable and reproducible results. There are many different types of behavioral experiments. They all aim to test the accuracy of behavior in subjects, such as locomotor activity, learning, memory, depression, anxiety, and pain, as well as the effectiveness of the treatment.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: The author thanks to Dr. Dilara NEMUTLU SAMUR for her support in the experimental studies and the editing of the experimental photographs.

Author Contributions: Idea/Concept: GA; Design: GA; Data Collection/Processing: GA; Analysis/Interpretation: GA; Literature Review: GA; Drafting/Writing: GA; Critical Review: GA.

REFERENCES

1. Bovenkerk B, Kaldewaij F. The use of animal models in behavioural neuroscience research. Curr Top Behav Neurosci. 2015;19:17-46.
2. Agar A, Akcay G. Nörolojik hastaliklarin deneysel hayvan modelleri, vol 1. Ankara: Akademisyen Kitabevi; 2022. Turkish.
3. Akcay G, Derin N. The effects of tDCS on depression and anxiety disorders induced by sub-chronic stress. Turk Hij Den Biyol Derg. 2022;79(2):267-78.
4. Akcay G, Nemutlu Samur D, Derin N. Transcranial direct current stimulation alleviates nociceptive behavior in male rats with neuropathic pain by regulating oxidative stress and reducing neuroinflammation. J Neurosci Res. 2023;101(9):1457-70.
5. Akçay, G. Weight drop models in traumatic brain injury. Mid Blac Sea J Health Sci. 2023;9(2):375-84.
6. Akcay G. Therapeutic effects of transcranial direct current stimulation on ketamine-induced schizophrenia-like behaviors and oxidative stress. Med Science. 2023;12(1):63-9.
7. Akcay G, Tamerer S. Transcranial direct current stimulation ameliorates motor and cognitive functions by regulating neuronal excitotoxicity in experimental Parkinson's disease model. Cukurova Med J. 2023;48(3):919-28.
8. Akcay G, Baydemir R. Therapeutic effects of transcranial direct current stimulation on loss of motor function caused by experimental mild traumatic brain injury. Cukurova Med J. 2023;48(3):972-8.
9. Akçay G, Aslan M, Kipmen Korgun D, Çeker T, Akan E, Derin N. Effects of transcranial direct current stimulation on the glutamatergic pathway in the male rat hippocampus after experimental focal cerebral ischemia. J Neurosci Res. 2024;102(1):e25247.
10. Nemutlu Samur D, Akçay G, Yıldırım S, Özkan A, Çeker T, Derin N, et al., Vortioxetine ameliorates motor and cognitive impairments in the rotenone-induced Parkinson's disease via targeting TLR-2 mediated neuroinflammation. Neuropharmacology, 2022;208:108977.
11. Danışman B, Akçay G, Gökçek-Saraç Ç, Kantar D, Aslan M, Derin N. The role of acetylcholine on the effects of different doses of sulfite in learning and memory. Neurochem Res. 2022;47(11):3331-43.
12. Özkan A, Bülbül M, Derin N, Sinen O, Akçay G, Parlak H, et al. Neuropeptide-S affects cognitive impairment and depression-like behavior on MPTP induced experimental mouse model of Parkinson's disease. Turk J Med Sci. 2021;51(6):3126-35.
13. Johnston IN, Tan M, Cao J, Matsos A, Forrest DRL, Si E, et al. Ibudilast reduces oxaliplatin-induced tactile allodynia and cognitive impairments in rats. Behav Brain Res. 2017;334:109-18.
14. Wagner BA, Braddick VC, Batson CG, Cullen BH, Miller LE, Spritzer MD. Effects of testosterone dose on spatial memory among castrated adult male rats. Psychoneuroendocrinology. 2018;89:120-30.
15. Akcay G, Bahadir A, Tatar Y, Nuri Atalar M, Babur C, Taylan Ozkan A. Investigation of the effects of Toxoplasma gondii on behavioral and molecular mechanism in bradyzoite stage. Brain Res. 2024;1828:148762.
16. Zhao Y, Li H, Fang F, Qin T, Xiao W, Wang Z, Ma S. Geniposide improves repeated restraint stress-induced depression-like behavior in mice by ameliorating neuronal apoptosis via regulating GLP-1R/AKT signaling pathway. Neurosci Lett. 2018;676:19-26.
17. Kawashita E, Kanno Y, Ikeda K, Kuretake H, Matsuo O, Matsuno H. Altered behavior in mice with deletion of the alpha2-antiplasmin gene. PLoS One. 2014;9(5):e97947.
18. Plekanchuk VS, Prokudina OI, Ryazanova MA. Social behavior and spatial orientation in rat strains with genetic predisposition to catatonia (GC) and stereotypes (PM). Vavilovskii Zhurnal Genet Selektsii. 2022;26(3):281-9.
19. Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. Brain Stimul. 2016;9(2):209-17.
20. Santos DS, Lopes BC, Medeiros LF, Assumpção JAF, de Souza A, Salvi AA, et al. Transcranial direct current stimulation (tDCS) induces analgesia in rats with neuropathic pain and alcohol abstinence. Neurochem Res. 2020;45(11):2653-63.
21. Hacısüleyman L, Saraç B, Joha Z. Analgesic effects of vilazodone, indatraline, and talsupram in a rat model of neuropathic pain. Turk J Pharm Sci. 2022;19(3):336-42.
22. Arslan R, Aydin S, Nemutlu Samur D, Bektas N. The possible mechanisms of protocatechuic acid-induced central analgesia. Saudi Pharm J. 2018;26(4):541-5.
23. Zhang Y, Ma S, Ke X, Yi Y, Yu H, Yu D, Li Q, Shang Y, Lu Y, Pei L. The mechanism of Annexin A1 to modulate TRPV1 and nociception in dorsal root ganglion neurons. Cell Biosci. 2021;11(1):167.

24. De la Luz-Cuellar YE, Rodríguez-Palma EJ, Franco-Enzástiga Ú, Déciga-Campos M, Mercado F, Granados-Soto V. Spinal dopaminergic D1 and D5 receptors contribute to reserpine-induced fibromyalgia-like pain in rats. Brain Res. 2023;1799:148167.
25. Girotra P, Singh SK. A comparative study of orally delivered PBCA and ApoE coupled BSA nanoparticles for brain targeting of sumatriptan succinate in therapeutic management of migraine. Pharm Res. 2016;33(7):1682-95.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1503734

Duzce Med J, 2024;26(S1):72-78
Düzce Tıp Fak Derg, 2024;26(S1):72-78

Experimental Animal Models in Obstetrics and Gynecology

Jinekolojik Rahatsızlıklarda Kullanılan Deneysel Hayvan Modelleri

Mert İLHAN
0000-0001-5042-3915

Department of Pharmacognosy, Düzce University Faculty of Pharmacy, Düzce, Türkiye

ABSTRACT

This study focuses on two major diseases affecting women's reproductive health: endometriosis and polycystic ovary syndrome (PCOS). Endometriosis is characterized as an estrogen-dependent condition, highlighting estrogen's role in understanding the disease's development and treatment strategies. Rat and mouse models are crucial for comprehending the pathophysiology of endometriosis and testing new therapeutic approaches. These models are particularly valuable in evaluating the effects of hormones and immune system modulators on endometriosis. Conversely, experimental models of PCOS emphasize the central role of hyperandrogenism in the development of this condition. Models induced by substances like dehydroepiandrosterone, testosterone propionate, and letrozole provide insights into the metabolic and endocrinological disruptions associated with PCOS. The letrozole-induced model, in particular, helps in understanding the relationship between hormonal imbalances and the onset of PCOS. Experimental models of both diseases offer critical knowledge for both basic science research and clinical applications. They provide essential data for understanding the pathophysiology of these conditions and developing new treatment strategies. This study demonstrates how findings from experimental models can improve women's reproductive health and lead to more effective treatments for these diseases. An enhanced understanding of hormonal and immune system mechanisms will guide future research and offer innovative solutions for treating these conditions.
Keywords: Gynecological disorders; endometriosis; polycystic ovary syndrome.

ÖZ

Bu çalışma kadınların üreme sağlığını etkileyen iki önemli hastalık olan endometriyoz ve polikistik over sendromu (PKOS) için oluşturulan hayvan modellerine odaklanmaktadır. Endometriyozun östrojene bağımlı bir durum olarak karakterize edilmesi, östrojenin hastalığın gelişimi ve tedavi stratejilerinin anlaşılmasındaki rolünü vurgulamaktadır. Sıçan ve fare modelleri, endometriyozun patofizyolojisini anlamak ve yeni tedavi yaklaşımlarını test etmek için çok önemlidir. Bu modeller özellikle hormonların ve bağışıklık sistemi modülatörlerinin endometriyoz üzerindeki etkilerinin değerlendirilmesinde önem arz etmektedir. PKOS'un deneysel modelleri bu durumun gelişiminde hiperandrojenizmin merkezi rolünü vurgulamaktadır. Dehidroepiandrosteron, testosteron propiyonat ve letrozol gibi maddelerin neden olduğu modeller, PKOS ile ilişkili metabolik ve endokrinolojik bozulmalara ilişkin öngörü sağlamaktadır. Özellikle letrozolün neden olduğu model, hormonal dengesizlikler ile PKOS'un başlangıcı arasındaki ilişkinin anlaşılmasına yardımcı olmaktadır. Her iki hastalığın deneysel modelleri, hem bilimsel araştırmalar hem de klinik araştırmalar için kritik bilgiler sunmaktadır. Bu hastalıkların patofizyolojisini anlamak ve yeni tedavi stratejileri geliştirmek için gerekli verileri sağlamaktadır. Bu çalışma, deneysel modellerden elde edilen bulguların kadınların üreme sağlığını nasıl iyileştirebileceğini ve bu hastalıklara yönelik daha etkili tedavilere nasıl yol açabileceğini göstermektedir. Hormonal ve bağışıklık sistemi mekanizmalarının daha iyi anlaşılması gelecekteki araştırmalara yol gösterecek ve bu durumların tedavisi için yenilikçi çözümler sunacaktır.
Anahtar kelimeler: Jinekolojik rahatsızlıklar; endometriyoz; polikistik over sendromu.

Corresponding Author
Sorumlu Yazar
Mert İLHAN
mertilhan@duzce.edu.tr

Received / Geliş Tarihi : 19.04.2024
Accepted / Kabul Tarihi : 01.06.2024
Available Online /
Çevrimiçi Yayın Tarihi : 23.06.2024

INTRODUCTION

Endometriosis is a chronic illness that is influenced by estrogen and affects 5-10% of women who are in their reproductive years (1). While some cases may be asymptomatic, the primary symptoms of this condition include dysmenorrhea, persistent pelvic discomfort, pain during sexual activity, infertility, and uterine bleeding. These symptoms can significantly affect the patient's psychological condition and quality of life (2). The condition is defined by the presence of endometrial-like tissue in abnormal locations, leading to a persistent inflammatory reaction, adhesions, and scar formation that changes the structure of the pelvic region. From a clinical standpoint, endometriosis is classified into three categories: peritoneal surface lesions, ovarian cysts (endometriomas), and deep infiltrating endometriosis. The classification of this condition is commonly based on the updated American Fertility Society classification (1), which divides it into four stages: minimum, mild, moderate, and severe. Retrograde menstruation has been suggested as a possible explanation for the creation of endometriosis in the peritoneal cavity during the menstrual cycle. However, given that retrograde menstruation has been detected in almost 90% of women without health issues, it is plausible that additional elements, such as hormonal, immunological, genetic, and epigenetic pathways, may also play a role in the development and advancement of the condition (3,4).

Polycystic ovarian syndrome (PCOS) is a common metabolic and hormonal condition that affects women during their reproductive years. The syndrome is characterized by metabolic symptoms including insulin resistance, obesity, and an increase in risk factors for cardiovascular disease. It also involves endocrine symptoms such as high levels of male hormones (hyperandrogenemia), irregular or infrequent menstrual periods (oligomenorrhea), absence of menstrual periods (amenorrhea), and hirsutism (5). PCOS patients have disruptions in the mechanisms that regulate follicular growth due to alterations in the endocrine system balance, leading to observable morphological abnormalities in the ovaries. Increased levels of luteinizing hormone (LH) disrupt the communication between granulosa cells and oocytes, as well as the growth of follicles and oocytes. It also results in the antral follicles staying tiny (6). Global standards for the evaluation and treatment of PCOS have advanced the detection of PCOS during the past thirty years. In 1990, the National Institutes of Health (NIH) established criteria for PCOS, including hyperandrogenism, oligo-ovulation, and the elimination of other potential causes such as Cushing’s syndrome and hyperprolactinemia (7). In 2004, the Rotterdam criteria were revised to diagnose PCOS. The current Rotterdam criteria encompass oligo- or anovulation, clinical and/or biochemical indications of hyperandrogenism, and the presence of polycystic ovaries. The International Guidelines for the Evaluation and Treatment of PCOS endorse a clinical diagnosis that requires the presence of at least two out of the three Rotterdam criteria (8). The prevalence of the disease differs among populations depending on whether the NIH or Rotterdam criteria are employed. Treatments for PCOS include blocking excess androgen production, correcting menstrual irregularities, maintaining the endometrium, enhancing fertility, and addressing metabolic issues. Each of these factors is crucial for the well-being of individuals with PCOS. Treatments primarily alleviate symptoms of PCOS (9).

Experimental animal models provide necessary data to understand the pathophysiology of diseases such as endometriosis and PCOS and to develop new treatment strategies. This review aims to demonstrate how findings from experimental models can improve women's reproductive health and shed light on more effective treatments for these diseases.

EXPERIMENTAL MODELS FOR ENDOMETRIOSIS

Uterine tissue has been effectively transplanted to aberrant places in small laboratory animals such as rats, mice, hamsters, and rabbits. This procedure has been documented in various studies conducted by Grümmer (10).

The rat and mouse models have been the main subjects of recent developments among non-primate models. In these experimental models of endometriosis, the uteri are surgically removed and dissected into tiny fragments. These fragments are subsequently reinserted into the peritoneal cavity, often by using sutures. The majority of these investigations did not distinguish the endometrium from other tissues. Both compartments, the endometrium and myometrium, were implanted (11,12). In rats, the uterine tissue undergoes development and forms fluid-filled, oval-shaped, cystic formations consisting of endometrial and myometrial tissue. The cysts experience growth but reach a stable size after approximately 2 months and maintain their viability for a minimum of 10 months (12). The ectopic uterine fragments in mice exhibit histological features resembling those of human disease. These characteristics encompass the formation of several, well-supplied lesions that consist of stroma, cysts, and endometrial glands. Importantly, the localization of these lesions within the abdomen is not dependent on their peritoneal location (11). Only a few experiments have been conducted in rats and mice to separate the endometrium from the myometrium and inoculate the endometrium to ectopic places. These investigations were performed by Katsuki et al. (13) in rats, and by Somigliana et al. (14), Hirata et al. (15), and Yao et al. (16) in mice. In Somigliana et al.'s (14) investigation, they took extracted endometrial tissue and carefully divided it into small fragments before delicately placing them back into the peritoneal cavity of recipient mice that shared the same genetic makeup. Both the donor and recipient mice underwent ovariectomy and were administered estrogen therapy. All animals that received the treatment showed signs of endometriosis in the peritoneum after 3 weeks. Additionally, new blood vessel formation was identified on the surface of the lesions. Nevertheless, the 'take-rate', which refers to the proportion of lesions obtained from a certain number of randomly injected endometrial pieces, averaged 30% of the inoculated tissue. Hirata et al. (15) established a homologous mouse model utilizing 'green mice' to improve the diagnosis of size and location of ectopic endometriotic lesions following transplantation. They were able to demonstrate a substantial correlation between the weight of endometriotic lesions and the assessed fluorescence intensity. The fluorescence

exhibited a notable increase in the mice that received estrogen supplementation, in comparison to the control animals. This finding provides evidence for the reliance of these abnormal endometrial lesions on estrogen.

UTILIZATION OF THE HOMOLOGOUS MODEL

Endometriosis is a condition in women that is influenced by estrogen and the reduction of estrogen in the blood helps to shrink the abnormal growths in other areas of the body (17). The mouse model, in which uterine tissue is transplanted and displays dependence on steroid hormones, has been extensively utilized to assess the responsiveness of lesions to steroid hormones and medications that disrupt steroid activity. The formation of ectopic endometrial tissue in both rodent species was found to be reliant on estrogen, similar to the situation in humans (12,18). In a study conducted by Schor et al. (19) in 1999, it was shown that rats that had their ovaries removed and were implanted with uterine tissue showed superior recovery of ectopic fragments when given with estrogen alone following ovariectomy, compared to those treated with a combination of estrogen and progesterone. In a study conducted by Fang et al. (20), it was found that estrogen has a significant impact on the size of implants in mice. They also showed that progesterone's ability to inhibit the growth of endometriotic tissue that depends on estrogen is due to the progesterone receptor remaining intact. Progesterone was observed to inhibit this growth in the uterine tissues of normal mice, while mice without the progesterone receptor did not show the same suppression.

Creating a hypoestrogenic condition can help promote the regression of uterine ectopic implants in rats. This can be achieved using methods such as ovariectomy or the injection of GnRH agonists, as demonstrated in studies by Kudoh et al. (21) and Sakata et al. (22).

The suppression of ovulation can be achieved by several methods, including the use of natural progestational compounds such as Kudoh et al. (21), synthetic progestational compounds like levonorgestrel or dienogest as mentioned by Jones (23) and Katsuki et al. (13), or through danazol medication as demonstrated by Sakata et al. (22). In this model, the desired effects can be obtained by decreasing the concentration of estrogen by the use of antiestrogens, or by utilizing selective estrogen (24).

The receptor modulator raloxifen or aromatase inhibitors, which disrupt estrogen production can be used for therapy (21,25). Furthermore, the autologous rat model has been widely employed for research on immune-modulating medicines and anti-inflammatory medications in endometriosis. Uchiide et al. (26) showed that when uterine tissue is transplanted in rats, it causes the accumulation of cells associated with allergic inflammation in the peritoneal stroma that is connected to the ectopic uterine tissue. Administering interferon-α-2b through intraperitoneal or subcutaneous treatment in rats resulted in a decrease in the size of induced lesions. This was observed through consecutive laparotomies conducted over a period of up to 4 months (27). Similarly, the use of the immunomodulator loxoribine in rats (28) and the intraperitoneal injection of interleukin-12 in a syngeneic mouse model also led to a reduction in lesion size (14). In addition, the development of artificially induced endometriosis in rats was inhibited by recombinant human tumor necrosis factor (TNF)-binding protein-1 (r-hTBP-1), which is the soluble form of TNF receptor type I (29). The same outcome was observed when rats were treated with pentoxifylline, a substance known for its anti-inflammatory properties and ability to decrease the production of inflammatory cytokines without causing a decrease in estrogen levels (30). Similar results were also observed with ciglitazone, a compound that binds to proliferator-activated receptor-γ (PPAR-γ) (31). In addition, the use of cyclooxygenase-2 (COX-2) inhibitors has been found to decrease the initial development of ectopic implants in rats (32). Similarly, the application of different non-steroidal anti-inflammatory drugs, including celecoxib, indomethacin, sulinac, and ibuprofen (but not aspirin), has also been shown to reduce the development of ectopic implants in a mouse model for endometriosis (33). Moreover, this mouse model offers the chance to study the impact of environmental pollutants on the formation of abnormal uterine implants. Previous studies have shown that prior exposure to dioxin before the surgical production of endometriosis leads to a proportional increase in the size of endometriotic sites in rats and mice. This effect was particularly pronounced in mice, as shown by Cummings et al. (11). In a novel study, Dinulescu et al. (34) introduced the initial mouse model of de-novo endometriosis. When the oncogene K-ras was activated in ovarian surface epithelial cells, it resulted in the development of benign epithelial lesions that showed histomorphological features resembling human endometriosis. However, this activation did not occur in cells of the peritoneal lining. Furthermore, almost 50% of the animals exhibited the formation of peritoneal endometriosis 8 months following the activation of ovarian surface epithelial cells by K-ras. When Pten is conditionally deleted, it can contribute to the development of endometrioid ovarian carcinoma in humans. In addition, the expression of K-ras can lead to the formation of metastatic endometrioid ovarian adenocarcinomas. Thus far, no genetic alterations of K-ras have been detected in cases of human endometriosis (35). Endometriosis in women is characterized by intense pelvic discomfort and a notable decrease in fertility (36). Researchers have examined the impact of abnormal lesions on reproductive ability in laboratory animals. While Cummings et al. (11) did not witness a decline in fertility among mice, they did observe a decrease in reproductive capacity among rats with artificially induced endometriosis. This decline could be attributed, at least in part, to an elevated count of luteinized unruptured ovarian follicles (37). Furthermore, the possibility of pelvic adhesions has not been ruled out. Additionally, the heightened activation of inflammatory cells in the peritoneum might potentially impact fertility in individuals with endometriosis. Steinleitner et al. (38) provided evidence that pentoxifylline, a substance, can counteract macrophage-mediated subfertility in mice. A medication that counteracts the impact of excessive activation of macrophages, and the drug-induced suppression of macrophage activation improved fertility in a hamster model of endometriosis (39). Recently, published research has examined the impact of ectopic endometrial lesions on pain responses in rats with endometriosis. The autotransplanted ectopic endometrial cystic pieces establish their own innervation, consisting of

both sympathetic efferent and sensory fibers (40). This innervation may have a broad impact on the nervous system. This is corroborated by the discovery that vaginal pain sensitivity was heightened in rats with endometrial cysts, mirroring the condition observed in humans with endometriosis. Furthermore, the rats exhibited vaginal hyperalgesia, as shown by Berkley et al. (41), which is a symptom commonly associated with heightened pelvic discomfort in people. There is speculation that neuroactive substances found in the endometrial cysts could stimulate nociceptive responses.

The afferents have an impact on the central brain mechanisms related to vaginal pain perception, as well as on reproductive processes through interactions between internal organs (40). It is yet unclear if the rats in this endometriosis model show any persistent pelvic pain symptoms other than vaginal hyperalgesia.

ANDROGEN-INDUCED PCOS RODENT MODELS

PCOS is primarily characterized by hyperandrogenism. An etiologic theory of PCOS suggests that being exposed to an excessive amount of androgens throughout the early stages of life can result in the development of PCOS during maturity. Over 30 years ago, it was documented that increased levels of circulating androgens in rodents had an impact on the development of ovarian follicles and the production of cysts (42). Various androgens, such as dehydroepiandrosterone (DHEA), testosterone propionate (TP), and 5α-dihydrotestosterone (DHT), have been administered to rats either by daily injections or subcutaneous implants to create an acute form of PCOS. It is crucial to acknowledge that there are variations in the way endocrine hormones and ovarian histology are reported in various models, leading to some discrepancy among research. Furthermore, several studies have failed to evaluate cardiometabolic parameters, and the impact of daily androgen injection and/or therapy on physiological indicators such as body weight, stress markers, or food consumption is typically not documented (43). In these rodent models, the development of PCOS is temporary and relies on the administration of androgen hormones. Therefore, the return to the regular reproductive/ovarian cycle happens after the injection of androgens is stopped.

PCOS INDUCED BY DHEA

DHEA is the initial androgen hormone that increases during the peripubertal period in females (44). Research has shown that approximately 50% of the T hormone produced in the follicles can come from DHEA in the bloodstream (45). Additionally, 25% of individuals with PCOS have higher than usual levels of DHEA in their bloodstream (46). Roy et al. (47) initially employed DHEA to produce PCOS in rats. Normally, prepubertal rats that have not yet reached sexual maturity and are around 22 days old, receive a daily injection of DHEA (6 mg/100 g body weight, diluted in 0.2 mL of sesame oil) for a period of 20-27 days. Following therapy, rats experience a cessation of menstrual cycles and a lack of ovulation (48).

PCOS INDUCED BY TP

Testosterone is administered to young female rats in order to stimulate the development of polycystic ovaries (49). This procedure involves the daily injection of TP (1 mg/100 g body weight dissolved in propylene glycol) into 21-day-old animals for a maximum of 35 days (49).

PCOS INDUCED BY ESTROGEN

Estradiol valerate (EV) is a type of estrogen that has a long-lasting effect. When it is given, it disrupts the normal functioning of the hypothalamus and pituitary gland, which leads to irregular release and storage of LH. LH is recognized as a crucial causative element in the progression of PCOS. Administering a 2 mg dosage of EV to young adult cyclic rats results in anovulation and the development of polycystic ovaries after 8 weeks (50).

PCOS INDUCED BY LETROZOLE

Aromatase is the primary enzyme responsible for converting testosterone and androstenedione into estradiol (E2) and estrone, respectively. The expression of this gene is prevalent in various human organs, including the placenta, ovary, and testis (51). PCOS development may be attributed to reduced ovarian aromatase activity, according to one of the pathophysiologic hypotheses (52). Letrozole is a type of medication known as a nonsteroidal aromatase inhibitor. It works by reducing the conversion of androgens (male hormones) to estrogens (female hormones) in the ovary. This leads to a rise in testosterone levels and a decrease in E2 production (51). Elevated levels of T in the ovaries are very probable to directly induce polycystic ovaries in rats treated with letrozole (53). The decrease in estrogen diminishes the inhibitory effect on LH synthesis in the pituitary, leading to elevated levels of LH (54), which in turn enhances the secretion of T by theca cells. Normally, female rats that are 6 weeks old (at the age of puberty) are given letrozole orally at dosages of 0.1, 0.5, and 1.0 mg/kg every day for a period of 21 days. As a result, they have a lack of menstrual cycles and exhibit histological and biochemical characteristics similar to those seen in human PCOS.

CONCLUSION

This study examines experimental models of two significant diseases affecting women's reproductive health: endometriosis and PCOS. Endometriosis is particularly characterized as an estrogen-dependent disease, highlighting the role of estrogen in understanding the disease's development and treatment strategies through experimental models. Rat and mouse models are crucial in comprehending the pathophysiology of endometriosis and testing new therapeutic approaches. These models are especially valuable in assessing the effects of hormones and immune system modulators on endometriosis. On the other hand, experimental models of PCOS emphasize the central role of hyperandrogenism in the development of this condition. Models induced by substances like DHEA, TP, and letrozole provide valuable insights into the metabolic and endocrinological disruptions associated with PCOS. Particularly, the letrozole-induced model helps in understanding the relationship between hormonal imbalances and the onset of PCOS. The experimental models of both diseases offer critical knowledge for both basic science research and clinical applications. The models of endometriosis and PCOS provide essential data for better understanding the pathophysiology of these conditions and developing new treatment strategies. This

study demonstrates how findings from the use of experimental models can be utilized to improve women's reproductive health and develop more effective treatments for these diseases. Furthermore, a better understanding of hormonal and immune system-related mechanisms will guide future research and offer innovative solutions in the treatment of these diseases. In conclusion, the experimental models of endometriosis and PCOS are indispensable tools for better understanding and treating these diseases. These models reveal the underlying mechanisms of these complex conditions affecting women's reproductive health, contributing significantly to future research and clinical applications.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: Mİ; Design: Mİ; Data Collection/Processing: Mİ; Analysis/Interpretation: Mİ; Literature Review: Mİ; Drafting/Writing: Mİ; Critical Review: Mİ.

REFERENCES

1. Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al. ESHRE guideline: Management of women with endometriosis. Hum Reprod. 2014;29(3):400-12.
2. Vitale SG, La Rosa VL, Rapisarda AMC, Lagana AS. Impact of endometriosis on quality of life and psychological well-being. J Psychosom Obstet Gynaecol. 2017;38(4):317-19.
3. Lagana AS, Garzon S, Franchi M, Casarin J, Gullo G, Ghezzi F. Translational animal models for endometriosis research: A long and windy road. Ann Transl Med. 2018;6(22):431.
4. Lagana AS, Vitale SG, Salmeri FM, Triolo O, Ban Frangez H, Vrtacnik-Bokal E, et al. Unus pro omnibus, omnes pro uno: A novel, evidence-based, unifying theory for the pathogenesis of endometriosis. Med Hypotheses. 2017;103:10-20.
5. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. Nat Rev Dis Primers. 2016;2:16057.
6. Sander VA, Hapon MB, Sicaro L, Lombardi EP, Jahn GA, Motta AB. Alterations of folliculogenesis in women with polycystic ovary syndrome. J Steroid Biochem Mol Biol. 2011;124(1-2):58-64.
7. Carmina E. Diagnosis of polycystic ovary syndrome: from NIH criteria to ESHRE-ASRM guidelines. Minerva Ginecol. 2004;56(1):1-6.
8. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25.
9. Koçak S. PCOS animal models: An approach induced by dehydroepiandrosterone. Exp Appl Med Sci. 2021;2(1):136-45.
10. Grümmer R. Animal models in endometriosis research. Hum Reprod Update. 2006;12(5):641-9.
11. Cummings AM, Metcalf JL, Birnbaum L. Promotion of endometriosis by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats and mice: time-dose dependence and species comparison. Toxicol Appl Pharmacol. 1996;138(1):131-9.
12. Vernon MW, Wilson EA. Studies on the surgical induction of endometriosis in the rat. Fertil Steril. 1985;44(5):684-94.
13. Katsuki Y, Takano Y, Futamura Y, Shibutani Y, Aoki D, Udagawa Y, et al. Effects of dienogest, a synthetic steroid, on experimental endometriosis in rats. Eur J Endocrinol. 1998;138(2):216-26.
14. Somigliana E, Vigano P, Rossi G, Carinelli S, Vignali M, Panina-Bordignon P. Endometrial ability to implant in ectopic sites can be prevented by interleukin-12 in a murine model of endometriosis. Hum Reprod. 1999;14(12):2944-50.
15. Hirata T, Osuga Y, Yoshino O, Hirota Y, Harada M, Takemura Y, et al. Development of an experimental model of endometriosis using mice that ubiquitously express green fluorescent protein. Hum Reprod. 2005;20(8):2092-6.
16. Yao Z, Shen X, Capodanno I, Donnelly M, Fenyk-Melody J, Hausamann J, et al. Validation of rat endometriosis model by using raloxifene as a positive control for the evaluation of novel SERM compounds. J Invest Surg. 2005;18(4):177-83.
17. Rice VM. Conventional medical therapies for endometriosis. Ann N Y Acad Sci. 2002;955:343-52.
18. Rossi G, Somigliana E, Moschetta M, Santorsola R, Cozzolino S, Filardo P, et al. Dynamic aspects of endometriosis in a mouse model through analysis of implantation and progression. Arch Gynecol Obstet. 2000;263(3):102-7.
19. Schor E, Baracat EC, Simoes MJ, de Freitas V, Giannotti Filho O, de Lima GR. Effects of conjugated estrogens and progestogen in surgically induced endometriosis in oophorectomized rats. Clin Exp Obstet Gynecol. 1999;26(3-4):158-61.
20. Fang Z, Yang S, Lydon JP, DeMayo F, Tamura M, Gurates B, et al. Intact progesterone receptors are essential to counteract the proliferative effect of estradiol in a genetically engineered mouse model of endometriosis. Fertil Steril. 2004;82(3):673-8.
21. Kudoh M, Susaki Y, Ideyama Y, Nanya T, Mori M, Shikama H. Inhibitory effects of a novel aromatase inhibitor, YM511, on growth of endometrial explants and insulin-like growth factor-I gene expression in rats with experimental endometriosis. J Steroid Biochem Mol Biol. 1997;63(1-3):75-80.
22. Sakata M, Terakawa N, Mizutani T, Tanizawa O, Matsumoto K, Terada N, et al. Effects of danazol, gonadotropin-releasing hormone agonist, and a combination of danazol and gonadotropin-releasing hormone agonist on experimental endometriosis. Am J Obstet Gynecol. 1990;163(5 Pt 1):1679-84.
23. Jones RC. The effect of a luteinizing hormone releasing hormone (LRH) agonist (Wy-40,972), levonorgestrel, danazol and ovariectomy on

experimental endometriosis in the rat. Acta Endocrinol (Copenh). 1984;106(2):282-8.
24. Saito T, Yoshizawa M, Yamauchi Y, Kinoshita S, Fujii T, Mieda M, et al. Effects of the novel orally active antiestrogen TZE-5323 on experimental endometriosis. Arzneimittelforschung. 2003;53(7):507-14.
25. Yano S, Ikegami Y, Nakao K. Studies on the effect of the new non-steroidal aromatase inhibitor fadrozole hydrochloride in an endometriosis model in rats. Arzneimittelforschung. 1996;46(2):192-5.
26. Uchiide I, Ihara T, Sugamata M. Pathological evaluation of the rat endometriosis model. Fertil Steril. 2002;78(4):782-6.
27. Ingelmo JM, Quereda F, Acien P. Intraperitoneal and subcutaneous treatment of experimental endometriosis with recombinant human interferon-alpha-2b in a murine model. Fertil Steril. 1999;71(5):907-11.
28. Keenan JA, Williams-Boyce PK, Massey PJ, Chen TT, Caudle MR, Bukovsky A. Regression of endometrial explants in a rat model of endometriosis treated with the immune modulators loxoribine and levamisole. Fertil Steril. 1999;72(1):135-41.
29. D'Antonio M, Martelli F, Peano S, Papoian R, Borrelli F. Ability of recombinant human TNF binding protein-1 (r-hTBP-1) to inhibit the development of experimentally-induced endometriosis in rats. J Reprod Immunol. 2000;48(2):81-98.
30. Nothnick WB, Curry TE, Vernon MW. Immunomodulation of rat endometriotic implant growth and protein production. Am J Reprod Immunol. 1994;31(2-3):151-62.
31. Lebovic DI, Kir M, Casey CL. Peroxisome proliferator-activated receptor-gamma induces regression of endometrial explants in a rat model of endometriosis. Fertil Steril. 2004;82(Suppl 3):1008-13.
32. Matsuzaki S, Canis M, Darcha C, Dallel R, Okamura K, Mage G. Cyclooxygenase-2 selective inhibitor prevents implantation of eutopic endometrium to ectopic sites in rats. Fertil Steril. 2004;82(6):1609-15.
33. Efstathiou JA, Sampson DA, Levine Z, Rohan RM, Zurakowski D, Folkman J, et al. Nonsteroidal antiinflammatory drugs differentially suppress endometriosis in a murine model. Fertil Steril. 2005;83(1):171-81.
34. Dinulescu DM, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T. Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. Nat Med. 2005;11(1):63-70.
35. Otsuka J, Okuda T, Sekizawa A, Amemiya S, Saito H, Okai T, et al. K-ras mutation may promote carcinogenesis of endometriosis leading to ovarian clear cell carcinoma. Med Electron Microsc. 2004;37(3):188-92.
36. Elsheikh A, Milingos S, Loutradis D, Kallipolitis G, Michalas S. Endometriosis and reproductive disorders. Ann N Y Acad Sci. 2003;997:247-54.
37. Moon CE, Bertero MC, Curry TE, London SN, Muse KN, Sharpe KL, et al. The presence of luteinized unruptured follicle syndrome and altered folliculogenesis in rats with surgically induced endometriosis. Am J Obstet Gynecol. 1993;169(3):676-82.
38. Steinleitner A, Lambert H, Roy S. Immunomodulation with pentoxifylline abrogates macrophage-mediated infertility in an in vivo model: a paradigm for a novel approach to the treatment of endometriosis-associated subfertility. Fertil Steril. 1991;55(1):26-31.
39. Steinleitner A, Lambert H, Suarez M, Serpa N, Robin B, Cantor B. Periovulatory calcium channel blockade enhances reproductive performance in an animal model for endometriosis-associated subfertility. Am J Obstet Gynecol. 1991;164(4):949-52.
40. Berkley KJ, Dmitrieva N, Curtis KS, Papka RE. Innervation of ectopic endometrium in a rat model of endometriosis. Proc Natl Acad Sci U S A. 2004;101(30):11094-8.
41. Berkley KJ, Cason A, Jacobs H, Bradshaw H, Wood E. Vaginal hyperalgesia in a rat model of endometriosis. Neurosci Lett. 2001;306(3):185-8.
42. Parker CR Jr, Mahesh VB. Hormonal events surrounding the natural onset of puberty in female rats. Biol Reprod. 1976;14(3):347-53.
43. Singh KB. Persistent estrus rat models of polycystic ovary disease: an update. Fertil Steril. 2005;84(Suppl 2):1228-34.
44. Apter D, Butzow T, Laughlin GA, Yen SS. Accelerated 24-hour luteinizing hormone pulsatile activity in adolescent girls with ovarian hyperandrogenism: relevance to the developmental phase of polycystic ovarian syndrome. J Clin Endocrinol Metab. 1994;79(1):119-25.
45. Haning RV Jr, Hua JJ, Hackett RJ, Wheeler CA, Frishman GN, Seifer DB, et al. Dehydroepiandrosterone sulfate and anovulation increase serum inhibin and affect follicular function during administration of gonadotropins. J Clin Endocrinol Metab. 1994;78(1):145-9.
46. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The Androgen excess and PCOS Society criteria for the polycystic ovary syndrome: The complete task force report. Fertil Steril. 2009;91(2):456-88.
47. Roy S, Mahesh VB, Greenblatt RB. Effect of dehydroepiandrosterone and delta4-androstenedione on the reproductive organs of female rats: production of cystic changes in the ovary. Nature. 1962;196:42-3.
48. Shi D, Vine DF. Animal models of polycystic ovary syndrome: a focused review of rodent models in relationship to clinical phenotypes and cardiometabolic risk. Fertil Steril. 2012;98(1):185-93.
49. Beloosesky R, Gold R, Almog B, Sasson R, Dantes A, Land-Bracha A, et al. Induction of polycystic ovary by testosterone in immature female rats: Modulation of apoptosis and attenuation of glucose/insulin ratio. Int J Mol Med. 2004;14(2):207-15.
50. Carriere PD, Brawer JR, Farookhi R. Pituitary gonadotropin-releasing hormone receptor content in rats with polycystic ovaries. Biol Reprod. 1988;38(3):562-7.
51. Corbin CJ, Trant JM, Walters KW, Conley AJ. Changes in testosterone metabolism associated with the evolution of placental and gonadal isozymes of porcine aromatase cytochrome P450. Endocrinology. 1999;140(11):5202-10.
52. Diamanti-Kandarakis E. Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. Expert Rev Mol Med. 2008;10:e3.

53. Sanchez-Criado JE, Bellido C, Galiot F, Lopez FJ, Gaytan F. A possible dual mechanism of the anovulatory action of antiprogesterone RU486 in the rat. Biol Reprod. 1990;42(5-6):877-86.

54. Ajika K, Krulich L, Fawcett CP, McCann SM. Effects of estrogen on plasma and pituitary gonadotropins and prolactin, and on hypothalamic releasing and inhibiting factors. Neuroendocrinology. 1972;9(5):304-15.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1489656

Duzce Med J, 2024;26(S1):79-86
Düzce Tıp Fak Derg, 2024;26(S1):79-86

Experimental Animal Models in Heart Disease

Kalp Hastalıklarında Deney Hayvanı Modelleri

Ali GÖK
0000-0003-4103-9537
Ersin BEYAZÇİÇEK
0000-0002-6817-4512

Department Physiology, Düzce University Faculty of Medicine, Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Ersin BEYAZÇİÇEK
beyazcicek13@gmail.com

Received / Geliş Tarihi : 06.03.2024
Accepted / Kabul Tarihi : 24.04.2024
Available Online / Çevrimiçi Yayın Tarihi : 24.05.2024

ABSTRACT

Heart diseases constitute a significant global burden of mortality and morbidity. This encompassing word refers to a variety of illnesses, including coronary artery disease, heart failure, myocardial infarction, and valvular heart disease. Given the imperative need to comprehend and address these ailments, experimental studies are indispensable. Experimental animal models serve as indispensable tools in elucidating the mechanisms of heart disease. They are pivotal for developing novel treatments and assessing the efficacy of existing therapies. Among the commonly utilized animal models in heart disease research are mice, rats, rabbits, dogs, and pigs. Each model offers distinct advantages and limitations, allowing researchers to probe specific facets of cardiac pathology and unravel the intricate mechanisms involved in heart disease. In this comprehensive review, it was aimed to provide a succinct overview of the various animal models employed in heart disease research. The advantages and drawbacks of each model were delineated, the aspects of human heart disease they emulate were elucidated, and pivotal research findings facilitated by their utilization were highlighted. By synthesizing this information, it was the endeavor to provide researchers and clinicians with valuable insights into the diverse array of animal models available for investigating heart diseases, ultimately paving the way for enhanced understanding and treatment of these debilitating conditions.
Keywords: Coronary artery disease; heart failure; myocardial infarction; rodents; ischemia.

ÖZ

Kalp hastalıkları, dünya çapında önemli bir ölüm ve hastalık yükünü oluşturur. Bu genel terim, koroner arter hastalığı, kalp yetmezliği, miyokard enfarktüsü ve kapakçık kalp hastalığı gibi çeşitli durumları kapsar. Bu hastalıkları anlama ve ele alma gerekliliği göz önüne alındığında, deneysel çalışmalar kaçınılmazdır. Deneysel hayvan modelleri, kalp hastalıklarının mekanizmalarını açıklamada vazgeçilmez araçlar olarak hizmet eder. Yeni tedaviler geliştirmek ve mevcut tedavilerin etkinliğini değerlendirmek için kilit öneme sahiptirler. Kalp hastalığı araştırmalarında yaygın olarak kullanılan hayvan modelleri arasında fareler, sıçanlar, tavşanlar, köpekler ve domuzlar bulunmaktadır. Her model, araştırmacıların kalp patolojisinin belirli yönlerini incelemesine ve kalp hastalığında rol alan karmaşık mekanizmaları çözmesine olanak tanıyan farklı avantajlar ve kısıtlamalar sunar. Bu kapsamlı incelemede, kalp hastalığı araştırmalarında kullanılan çeşitli hayvan modellerinin kısa bir özetinin sunulması amaçlanmıştır. Her bir modelin avantajları ve dezavantajları belirlenmiş, insan kalp hastalığını taklit ettikleri yönleri açıklanmış ve kullanımlarına olanak tanıyan önemli araştırma bulguları vurgulanmıştır. Bu bilgileri sentezleyerek, araştırmacıların ve klinisyenlerin kalp hastalıklarını araştırmak için mevcut çeşitli hayvan modellerine sağladığı değerli bilgilerle, bu rahatsız edici durumların anlaşılmasının ve tedavisinin geliştirilmesine katkıda bulunmak amaçlanmıştır.
Anahtar kelimeler: Koroner arter hastalığı; kalp yetmezliği; miyokard enfarktüsü; kemirgenler; iskemi.

INTRODUCTION

There are numerous cardiovascular disorders; some affect the heart (myocarditis, coronary heart disease, hypertension), while others damage the arteries (atherosclerosis) or veins (thrombophlebitis). Myocardial infarction (MI), one of the most prevalent cardiovascular disorders, is an acute condition that causes necrosis of the heart muscle tissue (myocardium) as a result of a complete or partial blockage of blood supply to the heart (1). This condition affects the integrity of the cardiovascular system and can lead to serious complications or death of the patient. Such violations usually occur on the basis of atherosclerosis of the coronary arteries. Atherosclerosis leads to the narrowing of the coronary arteries and damage to the walls of blood vessels, which creates the basis for the formation of blood clots and arterial stenosis. Cardiovascular diseases account for 30% of deaths in Europe and America and 32% worldwide. According to the data of the Turkish Statistical Institute, approximately 505 thousand people died in 2022. Cardiovascular diseases accounted for 34.5% of these deaths. When deaths from circulatory system diseases were analyzed according to sub-clauses of death, it was reported that 42.3% of the deaths were due to ischaemic heart diseases, 23.5% were due to other heart diseases and 19.2% were due to cerebrovascular diseases (2). Among cardiovascular diseases, MI is still one of the leading causes of death and hospitalization in Turkey and worldwide. Today, the incidence of MI is increasing even in people under 40 years of age. MI is usually associated with diseases such as atherosclerosis, hypertension, and diabetes. In most cases, patients usually have a painful form of MI, which allows doctors to accurately diagnose the disease and start treatment quickly (1).

Animals utilized in research serve as crucial assets for comprehending the pathophysiology of diseases and for advancing therapeutic strategies. They are employed in fundamental medical and veterinary investigations. A diverse array of animals has been identified as effective models for studying diseases affecting both humans and animals alike. These research subjects encompass mice, rats, rabbits, guinea pigs, sheep, goats, cattle, pigs, primates, dogs, cats, birds, fish, and frogs (3). Our understanding of the cardiovascular system has substantially increased in recent decades; nonetheless, further study is needed to broaden our knowledge and give new therapeutic possibilities. There is also a need for a good animal model where cardiovascular function and illness may be researched efficiently and reliably, with possible translational applicability to humans (4). Animal models have contributed much to our understanding of the cardiovascular system. Small animals (Drosophila, Zebrafish, Xenopus, mice, and rats), medium-sized animals (Guinea pigs, rabbits, cats), and large animals (dogs, pigs, sheep, and non-human primates) continue to be important in preclinical research. However, criticism of animal-based platforms is emerging because, by definition, animal models represent inaccurate facsimiles of human diseases and disorders with differing genetic backgrounds and disease development mechanisms (5). Cardiac illnesses are frequently created in healthy animal models by genetic, pharmacological, or surgical modification. Certain animal species are better suited for specific induction procedures. Mice are good for creating genetic models, although surgical treatment is typically used on larger rodents and animals such as rats, rabbits, and dogs (4). The rat has been used as a basic model in cardiovascular research for many years (6). Experimental procedures have been developed to induce cardiovascular disease states in this species, such as cardiac hypertrophy and failure, MI, and systemic and pulmonary hypertension. Additionally, rat species that have these diseases spontaneously (congenitally) have also been bred. In order for an animal model developed for any cardiovascular disease in humans to be ideal, it is expected to have 5 important characteristics: a) it should resemble the disease observed in humans and enable chronic disease studies, b) it should produce predictable and controllable symptoms, c) it should be economic-technical, d) it should be acceptable in terms of animal ethics, and e) allow the measurement of heart-related biochemical or hemodynamic parameters.

There are also some difficulties with the models used in cardiovascular system diseases. For example, conditions such as hypertension or heart failure (HF) are slowly developing diseases accompanied by large-scale neuro-humoral adaptations in humans, whereas in animal models these diseases are induced acutely either with the help of surgical methods or drug administration. On the other hand, while cardiovascular diseases are not common in young people, they occur with aging, but the incidence is increasing. In contrast, young adult rats are used in many hypertension and HF studies. The animal model of aging is just beginning to be detailed. Moreover, despite the presence of high blood lipid levels, the development of atherosclerosis is not observed in most species of rats. However, animal experiments also have a very important place in advances in other areas of medicine. For example, almost all advances in cardiac surgery are based on animal experiments (7).

This review aimed to bring together the experimental animal models in heart disease that have been developed to date and to systematically present more understandable and comprehensive data.

ANIMAL TYPES USED IN EXPERIMENTAL MODELS OF HEART DISEASE

Small Rodent Models

Mus musculus (mice) and *Rattus norvegicus* (rats) are essential for animal models of heart illness, with each species providing distinct advantages. Mice and humans share genetic and physiological similarities, therefore genetically modified mouse models are thought to be useful in studying specific cardiac genes and signaling networks. Rats, with their bigger stature than mice, make surgical operations easier, allowing researchers to faithfully recreate human cardiac diseases such as ischemic heart disease and hypertension using techniques such as coronary artery ligation. Their anatomical similarities enable the study of surgical methods and interventional therapies applicable to human patients (8). These studies have helped develop new treatments and test the effectiveness of existing treatments. With thirty thousand protein-coding genes each, mice and rats are the most widely used animal models. Their genomes are highly similar to the human genome (9). Rodent models are frequently used in cardiovascular research because they are easier to handle and house, have a shorter gestation

period, can be genetically manipulated to produce transgenic strains, and have lower maintenance costs; thus, they are better suited for "high throughput" studies than large animal models (10). Because of these qualities, tiny rodent models are the most widely employed in investigations of heart physiology and illness, genetics, pharmacology, and long-term survival (4). However, because rodents are phylogenetically distant from humans, some pathophysiological aspects of disease and their responsiveness to pharmaceutical therapies may not be valid indicators (4,10). Rodent models serve an important role in laboratory-based cardiac research. They have a four-chamber heart structure similar to humans, with high genomic sequence similarity, and are relatively easy to handle, take up less space, and cost less than more evolved species. Mouse models have become the most common due to the widespread availability of genetically modified lines and established techniques for manipulating gene expression. Rats are also extensively employed in laboratories and have superior surgical manipulation and imaging capabilities than mice. Rat models that have been genetically engineered are increasingly being employed (11). The three main approaches to inducing heart disease in rodents are surgical, pharmacological, and gene manipulation (12). Technological advancements have enabled the measurement of many in vivo cardiac parameters in small rodents, complementing molecular, in vitro, and ex vivo functional research. These techniques include echocardiography, cardiovascular magnetic resonance imaging, electrocardiography, pressure-volume loops, and blood pressure measurement (4).

Medium Animal Model

The rabbit is a medium-sized animal with many cellular and molecular traits similar to humans, and it is a viable option for larger mammals. Several rabbit models are employed, including pressure or volume overload, ischemia, fast pacing, doxorubicin, drug-induced arrhythmias, transgenesis, and infection. These models also aid in the evaluation of therapy methods that may prove effective in human heart illness (13). Because of its medium size, rabbits have various potential benefits over other animals. Although the rabbit heart is smaller than that of a dog or a pig, it is large enough to allow for surgical and catheter-based therapies at a significantly reduced cost (5-15 times less expensive than those of dogs). At the same time, several 'adult human scale' therapies have been or are being scaled down for pediatric usage and assessment (e.g., pacemakers and CRT), allowing rabbit models to be useful. In rodents, surgical interventions remain more straightforward than microsurgical methods. More critically, rabbit cardiac physiology resembles human cardiac physiology more than mice or rats (14). Indeed, cellular electrophysiology and Ca++ transport in rabbits are more similar to those in humans than in rats or mice (15). This is especially important for research into HF and arrhythmias since changes in ion channel and Ca++ transporter function or expression are hypothesized to contribute directly to poor contractile performance and arrhythmogenesis (16).

Large Animal Models

The primary benefits of in vivo studies using large mammalian hearts are that: a) they can legitimately claim to be physiologically and/or clinically optimal, b) they allow for chronic studies, c) they allow for assessment of cardiac function and responses in the intact animal, and d) they are probably the best model for new drug screening and toxicity testing due to their conserved molecular mechanisms with humans rather than small animals such as rodents (17,18). However, it is important to recognize that these animal models are not representative of human ischemic heart disease. Most animal experiments use the abrupt closure of a coronary artery in previously healthy tissue. This is very different from the complex and progressive development of human cardiovascular disease, which includes underlying vascular disturbances as well as genetic and environmental components. There are also cost and logistical issues with employing large animal models. Most notably, large animal models are significantly more expensive to purchase and maintain in animal facilities than small animal models; daily housing fees for large animals are 30 to 90 times higher than those for mice (4,18).

Because of their close resemblance to human physiology, canine models are especially useful for studying conduction physiology and rhythm problems, as well as research based on heart rate, oxygen intake, and contractility. They have proven to be suitable candidates for long QT syndrome (LQTS) study as well as studies of Duchenne muscular hypertrophy, Brugada syndrome, and cardiac failure. Porcine models (*Sus scrofa domesticus*) are differentiated by their anatomical similarity to the human coronary circulation, making them ideal models for the study of myocardial ischemia and infarction. Their applications include the study of post-infarction remodeling, regenerative treatment methods, and interventional cardiology procedures. Ovine models (*Ovis aries*) play an important role in the advancement of cardiac surgery, cardiovascular interventions, medical device testing, hemodynamic studies, pharmacological research, and cardiovascular imaging due to their anatomical and physiological similarities to humans, as well as their manageable size. Less common models, such as non-human primates like macaques and baboons, are widely employed in atherosclerosis research to investigate the effects of dietary changes, innovative pharmacological regimens, and cardiac imaging studies (8).

THE MOST COMMON ANIMAL MODELS USED TO STUDY HEART DISEASE

Models of Myocardial Infarction

MI is the most severe clinical manifestation of coronary heart disease in particular and is the result of acute or chronic myocardial ischemia caused by the mismatch in oxygen demand and oxygen supply (19). MI is defined as “myocardial cell death due to pathologically prolonged ischemia”. Research models of infarction and myocardial ischemia are critical for investigating the acute and chronic pathobiological and pathophysiological processes in myocardial ischemia, as well as developing and optimizing future treatments (20). Animal models must meet specific requirements for testing cardioprotective treatments for MI. Animal models are very useful in understanding the underlying pathophysiology and progression of ischemia to MI and unblocking clinical studies. Preclinical research contributes to the development of new strategies for the diagnosis, prevention, and treatment of MI, as well as their

implementation in clinical settings (21). Currently, MI models can be split into two types. The first is an acute model, while the second is chronic. The acute model is often created either by coronary artery ligation or by medication induction. Both occlude the bloodstream, resulting in the pathological process of MI. However, none of these methodologies incorporate the pathological process of atherosclerosis development, which is the foundation of a true acute MI (22).

Surgical Ligation Model of Acute MI

The most common surgical technique for acute MI is closure of the left anterior descending coronary artery. Ligating different portions of the coronary artery might cause MI. Johns and Olson (23) established this approach in 1954, and with a few modifications, it is currently commonly employed in both small and large animals. The surgical process consists of multiple steps. Briefly, the animals are sedated and placed in the supine position. The left side of the sternum is incised laterally, as are the third and fourth intercostal muscles. The retractor separates the third and fourth ribs; the heart is revealed through squeezing; and the left anterior descending coronary artery is ligated with sterilized sutures. Electrocardiography is used to validate the process; ST segment elevation and white staining of the anterior wall of the left ventricle suggest the onset of MI. The main disadvantages of this model are the high death rate, postoperative infection, infarct size ranging from 4 to 59%, and the requirement for professional hands and an artificial ventilator (24,25).

Chemical Models of Acute MI

Isoproterenol-Induced MI Model

Isoproterenol (isoprenaline, ISO) is a synthetic sympathomimetic catecholamine. Although it is very similar in structure to adrenaline, it only stimulates β1 and β2 receptors and does not affect α receptors at all. It is the first and widely used agent to experimentally induce MI in rats (26,27). Rats with acute MI caused by ISO provide a well-established, non-surgical animal model (28). Compared to the surgical model, this one has a number of benefits, including low mortality, ease of use, non-surgical approach, and consequently no risk of post-operative infection. This paradigm works best with rats, although it has also been observed to work with mice and rabbits, among other species (29,30). The most advantageous aspect of the usability of ISO is that it has been stated that high doses of rats overlap with all biochemical, physiopathological, and histopathological changes of heart attack in humans, and therefore the ISO-induced heart attack model is well-standardized. This model is frequently used to investigate the beneficial effects of many drugs or their effects on cardiac functions (31). ISO administration is usually administered on the last two days of the study, regardless of the duration of the study. MI in rats is induced by subcutaneous injection of 65-150 mg/kg ISO hydrochloride dissolved in saline (26,29,32). Reactive oxygen species (ROS) are produced when ISO is oxidized. ROS modify membrane permeability, raise levels of cardiac-specific enzymes, cholesterol, and low-density lipoprotein, and lower levels of endogenous antioxidant enzymes (26,33). Potential pathways of ISO-induced MI include oxidative stress, ischemia, intracellular calcium loading, metabolic modifications, and changes in electrolyte concentration (34,35).

Adriamycin (Doxorubicin)-Induced MI Model

Adriamycin is a broad-spectrum anticancer medication used to treat hematologic malignancies and a variety of solid cancers. Adriamycin's primary side effects are cardiomyopathy and HF. According to studies, oxidative stress is a key factor in the development of adriamycin cardiotoxicity (36). Cytotoxic and cytostatic mechanisms of action of doxorubicin include topoisomerase II inhibition, non-radical-dependent mechanisms such as binding of doxorubicin-iron complex to DNA and interaction of DNA base pairs with the drug, and DNA damage by free radical production (37). Numerous additional mechanisms have been proposed as adriamycin's modes of action. Numerous investigations have documented changes in calcium metabolism following doxorubicin treatment, primarily in the area of calcium excess. Increased intracellular calcium, calcium buildup in the ventricular myocardium, calcium inclusions in the mitochondria, abnormalities in calcium transport in cardiac tissue, and modifications to the sarcoplasmic reticulum's ability to release calcium through effects on the Ca++-ATPase and Ca++ release channel are some of these changes. HF may cause calcium to build up inside heart cells, therefore the increase in calcium levels that have been seen is more likely a result of the action of adriamycin than a cause (38). Rats administered adriamycin are often used in research to understand the mechanism of cardiotoxicity and to prevent it. Adriamycin administered to rats at 2 mg/kg/week for 12 weeks causes decreased blood pressure and cardiac output, and the development of pleural effusion, ascites, and liver congestion. The advantage of this model is that it is simple, noninvasive, economical, and develops quickly (36).

Adrenaline-Induced Myocardial Infarction Models

Also known as epinephrine, adrenaline is primarily a stress hormone produced by the adrenal glands and released into the bloodstream. It also has medical uses, including the treatment of cardiac arrest, allergic reactions, and asthma. However, it has been shown that high doses of adrenaline can increase the formation of ROS and reactive nitrogen species (RNS), leading to tissue damage (39). MI induced by adrenaline in rats is considered a reliable experimental model for studying the cardioprotective effects of drugs (2). Additionally, it has been discovered that adrenaline promotes lipid peroxidation and depletes cellular antioxidants as a contributing factor to MI (40). When adrenaline is administered, it is applied on the last two days of the study, similar to the ISO model. MI in rats is induced by subcutaneous injection of 1 mg/kg adrenaline dissolved in physiological saline. At the end of the study, changes in the ST segment on electrocardiography as well as alterations in CK-MB, cTn-T, and cTn-I levels are observed. Moreover, increases in oxidative stress parameters such as MDA and total oxidative status are noted. Additionally, histopathological changes are observed (2).

MI Model Induced by Coronary Artery Embolization Method

In the coronary artery embolization method, the microsphere that causes intracoronary embolization is created by intracoronary injection of agarose or polystyrene beads or autogenous blood carrying thrombin or fibrinogen (24,41). This method is mostly preferred in large animal models. This approach was developed by

Sabbah et al. (42). It was created by applying embolization with a catheter 3-9 times at different times for 1-3 weeks, using a closed ribcage model on dogs.

Coronary artery embolization is induced percutaneously. For this reason, the risk of serious inflammation observed after surgical interventions such as thoracotomy is reduced. In addition, this model resembles the clinical conditions of patients with HF and acute coronary syndrome in whom atherosclerotic embolization and thrombolytic debris have entered the coronary microcirculation. The factor that limits the embolization method is the uncertainty of the area of coronary artery occlusion and whether it is in the desired location (24).

MI Model Induced by Cryonecrosis Method

This pattern is induced by cryosurgery. After intercostal thoracotomy, it was created by using a 0.18x1.2 cm^2 liquid nitrogen probe in the left ventricle 15 times for 20 minutes. However, with the cryodamage method, transmural lesion formation and therefore fibrosis may not always occur and aneurysm formation may not be observed. This model has generally been applied to rats and rabbits (24,43). The model is highly reproducible, easy to implement, and can be set up quickly and reliably. It produces a consistent transmural infarct lesion independent of coronary anatomy and eventually leads to HF. This method is particularly suitable for evaluating innovative pharmacological and tissue engineering-based strategies and studying the remodeling process (2).

Electrically-Induced Myocardial Infarction

The use of rodent MI models provides a fundamental basis for studies investigating MI processes and how they can be treated. The experimental MI model created by electrical stimulation is performed with echocardiography support (44,45). The advantages of this model include being minimally invasive in mice and providing high repeatability. Its disadvantages include requiring expertise in intervention and imaging, as well as the high cost of equipment. The anesthetized mouse is positioned in a supine position on the imaging platform. Subsequently, the mouse's legs are spread and secured with a band. The ventral neck area of the mouse is shaved and the shaved area is disinfected with a 10% povidone-iodine solution (2). The left anterior descending artery (LAD) is evaluated using high-frequency ultrasound. A neutral electrode is attached to the mouse's right leg. Then, a micro-manipulator-controlled monopolar needle is slowly inserted into the closed chest cavity. The needle is gradually directed towards the targeted area. While the needle is on the LAD, an electrosurgical unit is used to coagulate the area with electricity. Subsequently, the needle is slowly withdrawn. The formation of MI or occlusion, meaning the absence of blood flow distal to the occlusion, akinesia in the affected part of the left ventricle, and typical electrocardiography changes within seconds confirm the occlusion. Cardiac morphological changes are evaluated with electrocardiographic and echocardiographic parameters along with cTn-T or cTn-I (2).

MI Model Induced by Hydraulic Occluder and Ameroid Generator Methods

This method is used to occlude all or part of the coronary arterial branches, especially in large animal models. Therefore, these methods are suitable for the coronary stenosis model required for inducing MI and HF and for examining myocardium hibernation (27,46,47,55,56). A left anterolateral thoracotomy is required for occluder implantation. Following the pericardial incision, the LAD branch is dissected, and the hydraulic occluder is then positioned around the vessel. After that, the hydraulic occluder is inflated to produce either a full or partial occlusion. To ascertain the extent of the obstruction and document the flow rate in the LAD, an ultrasonic flow probe is positioned distal to the obstruction (24,47).

The ameroid constrictor is implanted using the same technique, but a different mechanism is used to apply the obstruction. Because casein polymeric material is hygroscopic, the ring surrounding the vein gradually narrows at body temperature. The ameroid constrictor has been employed in investigations involving big animal models of MI, just like the hydrolytic occluder (24,47).

MI Model Induced by Coiling/Gelfoam Methods

In this method, after the carotid artery is dissected and exposed, the coils are placed into the LAD. Gelfoam sponges are placed inside the coils to completely close the coronary artery (48,49). Coils and sponges are also placed at the source of the second diagonal artery. This method is a technique that eliminates suturing around diagonal branches and thoracotomy. It may induce inflammation and formation of collateral circulation after surgical ligation with sutures. The development of platinum coils in harmony with magnetic fields has facilitated the method of occlusion of the coronary artery with a percutaneous catheter (24).

MI Model Induced by the Cauterization Method

The first starting point of this method, also known as burning, is the MI model created with the use of a green laser (50). Briefly, the cauterization method was developed by being inspired by and modifying this model; 4-5. A one cm incision is made between the ribs after the skin is opened in the dorsoventral direction, the pectoral muscles are opened with the help of a retractor without cutting, and before the heart is taken out, slight pressure is applied to the rib cage to compress the heart, and cauterization is applied to the same point three times to induce MI from the distal end of the left coronary anterior descending artery extending along the anterior of the heart. The application is done once. The most important advantage of this method over ligation is that the heart is not removed during the surgical intervention and therefore there is no need for intubation.

Models of Chronic Myocardial Infarction

The chronic coronary artery disease (CAD) model can generally simulate the natural pathogenesis of CAD. It can be used to study the pathological processes of CAD. The methods of making these models include interventional oppression, high-fat diet, and high-fat diet combined with drugs or ligation. The high-fat diet method involves feeding the experimental animals with rich cholesterol foods for a relatively long time. It induces hyperlipidemia, atherosclerosis, and sclerotic plaques, which result in stenosis in the blood vessels and myocardial ischemia. This method is closest to the clinical pathological and physiological processes of CAD. It is better for observing the pathology of CAD and for comprehensive efficacy testing of drugs, but it requires longer preparing the model and the degree of ischemia is also difficult to control. At present, this method is successfully used with rats and

rabbits (22,51). A combination of medicines and ligation in conjunction with a high-fat diet can be used. The animal is first fed a high-fat or high-cholesterol diet for an extended length of time, resulting in lipid metabolic dysfunction and the progressive emergence of atherosclerosis. Myocardial ischemia can then be caused by a combination of medication injection and ligation. This technique allows for the convenient study of pathological changes and processes, as well as the treatment effect of medications on blood lipids, vascular lesions, and heart injury. The drug-induced effects are typically unstable, and ligation necessitates advanced surgical skills. Furthermore, this model cannot be employed in clinical settings to investigate autolysis recanalization, hence it is not optimal (22).

Animal Models of Heart Failure

HF is the number one cause of death worldwide. HF has a high death rate, with around 50% of patients dying within 5 years of diagnosis, which is higher than the mortality rate for most cancers (52). Furthermore, the prevalence of HF in industrialized nations is rising, resulting in a massive economic burden. The increase is due, at least in part, to improved treatment for acute MI, which has reduced mortality but not morbidity and is based on the number of surviving patients. Additional variables include an increased prevalence of comorbidities, which predispose to and accelerate the development of HF. As a result, there is an urgent need to change these risk factors and find new therapeutic approaches for HF patients (9).

To prevent and control HF caused by heart disease, it is vital to understand the pathophysiological mechanisms underlying these disorders and create innovative therapeutic techniques in response (5,8). Current treatments essentially halt the progression of this illness, highlighting the need for novel preventative and reparative therapy. The development of these innovative HF medicines necessitates testing of potential therapeutic techniques in relevant HF animal models (53).

Four clinical situations that can cause HF are described: Each will highlight essential characteristics of the clinical phenotype and recommend features of the clinical condition that should be included in an animal model designed to imitate the human state. Most animal models struggle to match the complexity of human illnesses that cause HF. HF models induced by myocardial ischemia: These are models created by coronary artery ligation and embolization (53,54). Tachycardia-induced HF model: Failure is created as a result of tachycardia with a fast atrial or ventricular pacemaker. In this model, due to technical difficulties, larger animals rather than small animals are generally used (54).

HF models created with pressure load and volume load: Pressure load with aortic valve stenosis and volume load with mitral valve insufficiency. HF models have been created with aortic stenosis in the supravalvular position in large animals such as cats, dogs, sheep, and pigs, and with transverse aortic stenosis in small animals such as mice. Mitral insufficiency and HF have been caused in dogs by cutting the chordae tendineae or by beta-adrenergic and angiotensin II pathways (53-55).

HF models are caused by hypertension and dilated, restrictive cardiomyopathy. Dilated cardiomyopathy is defined by ventricular dilation, systolic dysfunction, and diastolic filling abnormalities. The most important structural change is the increase in myocyte length and width. Additionally, interstitial fibrosis, decrease in extracellular matrix, progressive myocyte death, and decrease in capillary density are detected. In small and large animals, cardiomyopathy can be induced ischemically by surgical methods such as coronary artery ligation or damage, or by toxic agents (ISO, doxorubicin). Cardiomyopathy models can be used using spontaneously hypertensive rats, which develop spontaneous cardiomyopathy as they age, or in some cases genetically (53).

CONCLUSION

To summarize, the use of animal models in cardiovascular disease research is critical for improving our understanding of the pathophysiology of heart illnesses and evaluating new treatment options. From small animal models like mice and rats, which have provided valuable insights into genetic and molecular mechanisms, to larger animal models such as canines, swine, and sheep, which offer more similarities to human cardiovascular anatomy and physiology; each model has its advantages and disadvantages. However, the careful selection and design of animal models that mirror the specific pathophysiological mechanisms and characteristics of heart diseases in humans are essential for gaining crucial information to advance our understanding of cardiovascular diseases. These models not only allow for the study of anatomical, physiological, and cellular changes in cardiac disorders but also serve as a platform for the development of new therapeutic methods and the assessment of treatment efficacy. By considering the genetic homogeneity or heterogeneity, anatomical and physiological attributes, availability of background data, and practicality of using the animal model in research, researchers must ensure that the selected model accurately reflects the physiological and pathophysiological aspects of human cardiovascular disease. As research in cardiovascular disease continues to evolve, the role of animal models remains paramount in providing valuable insights into the pathophysiology of HF and the testing of potential therapies and interventions. Therefore, the careful consideration and selection of appropriate animal models for cardiovascular disease research are crucial in developing preventative and ameliorative treatments for this significant global health concern.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: AG, EB; Design: AG, EB; Data Collection/Processing: AG, EB; Analysis/Interpretation: AG, EB; Literature Review: AG, EB; Drafting/Writing: AG, EB; Critical Review: AG, EB.

REFERENCES

1. Jalolov NN, Sobirov OG, Kabilzhonova SR, Imamova AO. The role of a healthy lifestyle in the prevention of myocardial infarction. Neo Sci Peer Rev J. 2023;9:8-14.
2. Beyazcicek E, Beyazcicek O. Experimental myocardial infarction models and current approaches. In: İnandiklioglu N, editor. Current studies in basic medical sciences. Lyon: Livre de Lyon; 2023. p.55-76.
3. Leong XF, Ng CY, Jaarin K. Animal models in cardiovascular research: hypertension and atherosclerosis. BioMed Res Int. 2015;2015:528757.
4. Milani-Nejad N, Janssen PM. Small and large animal models in cardiac contraction research: Advantages and disadvantages. Pharmacol Ther. 2014;141(3):235-49.
5. van der Velden J, Asselbergs FW, Bakkers J, Batkai S, Bertrand L, Bezzina CR, et al. Animal models and animal-free innovations for cardiovascular research: current status and routes to be explored. Consensus document of the ESC Working Group on Myocardial Function and the ESC Working Group on Cellular Biology of the Heart. Cardiovasc Res. 2022;118(15):3016-51.
6. Bader M. Rat models of cardiovascular diseases. In: Anegon I, editor. Rat genomics: methods and protocols. Totowa, NJ: Humana Press; 2010. p.403-14.
7. Karasu Minareci E, Öğütman Ç. Experimental animal models for cardiovascular diseases. Turkiye Klinikleri J Cardiovasc Sci. 2011;23(1):65-74. Turkish.
8. van Doorn ECH, Amesz JH, Sadeghi AH, de Groot NMS, Manintveld OC, Taverne YJHJ. Preclinical models of cardiac disease: a comprehensive overview for clinical scientists. Cardiovasc Eng Technol. 2024;[Epub ahead of print]. doi: doi:10.1007/s13239-023-00707-w.
9. Riehle C, Bauersachs J. Small animal models of heart failure. Cardiovasc Res. 2019;115(13):1838-49.
10. Recchia FA, Lionetti V. Animal models of dilated cardiomyopathy for translational research. Vet Res Commun. 2007;31(Suppl 1):35-41.
11. Mouse Genome Sequencing Consortium; Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, et al. Initial sequencing and comparative analysis of the mouse genome. Nature. 2002;420(6915):520-62.
12. Alonzo M, Delgado M, Cleetus C, Kumar SA, Thakur V, Chattopadhyay M, et al. Methods for histological characterization of cryo-induced myocardial infarction in a rat model. Acta Histochem. 2020;122(7):151624.
13. Feng Y, Hemmeryckx B, Frederix L, Lox M, Wu J, Heggermont W, et al. Monitoring reperfused myocardial infarction with delayed left ventricular systolic dysfunction in rabbits by longitudinal imaging. Quant Imaging Med Surg. 2018;8(8):754-69.
14. Genoves P, Arias-Mutis OJ, Parra G, Such-Miquel L, Zarzoso M, Del Canto I, et al. Development and long-term follow-up of an experimental model of myocardial infarction in rabbits. Animals (Basel). 2020;10(9):1576.
15. Kobayashi T, Ito T, Yamada S, Kuniyoshi N, Shiomi M. Electrocardiograms corresponding to the development of myocardial infarction in anesthetized WHHLMI rabbits (Oryctolagus cuniculus), an animal model for familial hypercholesterolemia. Comp Med. 2012;62(5):409-18.
16. Bryant SM, Kong CH, Watson J, Cannell MB, James AF, Orchard CH. Altered distribution of ICa impairs Ca release at the t-tubules of ventricular myocytes from failing hearts. J Mol Cell Cardiol. 2015;86:23-31.
17. Hodges MM, Zgheib C, Liechty KW. A large mammalian model of myocardial regeneration after myocardial infarction in fetal sheep. Adv Wound Care (New Rochelle). 2021;10(4):174-90.
18. Camacho P, Fan H, Liu Z, He JQ. Large mammalian animal models of heart disease. J Cardiovasc Dev Dis. 2016;3(4):30.
19. Chapman AR, Adamson PD, Mills NL. Assessment and classification of patients with myocardial injury and infarction in clinical practice. Heart. 2017;103(1):10-8.
20. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth Universal Definition of Myocardial Infarction (2018). J Am Coll Cardiol. 2018;72(18):2231-64.
21. Liu J, Li X. Novel porcine models of myocardial ischemia/infarction-technical progress, modified electrocardiograms validating, and future application. In: Millis R, editor. Advances in electrocardiograms-clinical applications [Internet]. InTech; 2012. p.175-90.
22. Tang YP, Liu Y, Fan YJ, Zhao YY, Feng JQ, Liu Y. To develop a novel animal model of myocardial infarction: A research imperative. Animal Model Exp Med. 2018;1(1):36-9.
23. Johns TN, Olson BJ. Experimental myocardial infarction. I. A method of coronary occlusion in small animals. Ann Surg. 1954;140(5):675-82.
24. Akbay Çetin E, Onur M. A new modified myocardial infarction animal model. Cardiovasc Surg. 2013;1(3)69-71.
25. Yang XP, Sabbah HN, Liu YH, Sharov VG, Mascha EJ, Alwan I, et al. Ventriculographic evaluation in three rat models of cardiac dysfunction. Am J Physiol. 1993;265(6 Pt 2):H1946-52.
26. Beyazcicek E, Beyazcicek O. Protective effects of Lacticaseibacillus rhamnosus on isoprenaline-induced myocardial infarction in rats. J Appl Microbiol. 2023;134(1):lxac008.
27. Özgün E, Sayılan Özgün G, Usta U, Eskiocak S, Süer Gökmen S. Melatonin effects on serum paraoxonase and lactonase in experimental myocardial infarction. J Turk Clin Biochem. 2018;16(1):32-41. Turkish.
28. Brooks WW, Conrad CH. Isoproterenol-induced myocardial injury and diastolic dysfunction in mice: structural and functional correlates. Comp Med. 2009;59(4):339-43.
29. Ahmed MI, Abdelrazek HMA, Moustafa YM, Alshawwa SZ, Mobasher MA, Abdel-Wahab BA, et al. Cardioprotective effect of flibanserin against isoproterenol-induced myocardial infarction in female rats: role of cardiac 5-HT2A receptor gene/5-HT/Ca2+ pathway. Pharmaceuticals (Basel). 2023;16(4):502.
30. Neto JC, Paulino ET, Rodrigues AKBF, da Silva JCG, Bernardino AC, dos Santos Oliveira JM, et al. Cardioprotective effect of hydroalcoholic extract of Brazilian red propolis against isoproterenol-induced myocardial infarction in rats. Phytomedicine Plus. 2022;2(1):100190.

31. Oglakci-Ilhan A, Kusat-Ol K, Uzuner K, Uysal O, Sogut I, Yucel F, et al. Effect of chronic alcohol consumption on myocardial apoptosis in the rat model of isoproterenol-induced myocardial injury and investigation on the cardioprotective role of calpain inhibitor 1. Drug Chem Toxicol. 2022;45(6):2727-38.
32. Hesari Z, Kafshdoozan K, Kokhaei P, Bagheri B, Ghaffari Khaligh S. Lactobacillus paracasei has anti-inflammatory effect on the heart failure induced by isoproterenol in rats. Iran J Toxicol. 2022;16(3):195-202.
33. Haleagrahara N, Varkkey J, Chakravarthi S. Cardioprotective effects of glycyrrhizic acid against isoproterenol-induced myocardial ischemia in rats. Int J Mol Sci. 2011;12(10):7100-13.
34. Baraka SA, Tolba MF, Elsherbini DA, El-Naga RN, Awad AS, El-Demerdash E. Rosuvastatin and low-dose carvedilol combination protects against isoprenaline-induced myocardial infarction in rats: Role of PI3K/Akt/Nrf2/HO-1 signalling. Clin Exp Pharmacol Physiol. 2021;48(10):1358-70.
35. Chen Y, Peng L, Shi SQ, Guo G, Wen H. Boeravinone B alleviates gut dysbiosis during myocardial infarction-induced cardiotoxicity in rats. J Cell Mol Med. 2021;25(13):6403-16.
36. Kuloğlu T, Artaş G. The affects of adriamycin implementation to the immunreactivity of nesfatin-1 at rat heart tissue. Firat Med J. 2015;20(2):81-5.
37. Khonsary SA. Goodman and Gilman's: the pharmacological basis of therapeutics. Surg Neurol Int. 2023;14:91.
38. Quiles JL, Huertas JR, Battino M, Mataix J, Ramírez-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. Toxicology. 2002;180(1):79-95.
39. Aziz FTA, Sanad FA, Temraz A, El-Tantawy WH, Hassan MA. Study of cardioprotective activity of the methanolic extract of the aerial parts of Bauhinia madagascariensis compared to Bauhinia purpurea against adrenaline-induced myocardial toxicity in rats. Drug Chem Toxicol. 2022;45(5):2341-51.
40. Patil MP, Chaware VJ, Redasani VK. Potentiation of effects of propranolol and heparin by antioxidant in adrenaline induced myocardial infarction in rats. World J Pharm Res. 2022;11(12):1706-16.
41. Suzuki M, Asano H, Tanaka H, Usuda S. Development and evaluation of a new canine myocardial infarction model using a closed-chest injection of thrombogenic material. Jpn Circ J. 1999;63(11):900-5.
42. Sabbah HN, Stein PD, Kono T, Gheorghiade M, Levine TB, Jafri S, et al. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. Am J Physiol. 1991;260(4 Pt 2):H1379-84.
43. Li RK, Jia ZQ, Weisel RD, Merante F, Mickle DA. Smooth muscle cell transplantation into myocardial scar tissue improves heart function. J Mol Cell Cardiol. 1999;31(3):513-22.
44. Ghouri IA, Kelly A, Salerno S, Garten K, Stolen T, Smith GL. Characterization of electrical activity in post-myocardial infarction scar tissue in rat hearts using multiphoton microscopy. Front Physiol. 2018;9:1454.
45. Rossi C, De Senarclens O, Perrenoud JJ. Acute myocardial infarction and electrically induced left bundle branch block: is a diagnosis possible? Heart. 2013;99(4):288-9.
46. Harada K, Grossman W, Friedman M, Edelman ER, Prasad PV, Keighley CS, et al. Basic fibroblast growth factor improves myocardial function in chronically ischemic porcine hearts. J Clin Invest. 1994;94(2):623-30.
47. St Louis JD, Hughes GC, Kypson AP, DeGrado TR, Donovan CL, Coleman RE, et al. An experimental model of chronic myocardial hibernation. Ann Thorac Surg. 2000;69(5):1351-7.
48. Lee HW, Lee HC, Park JH, Kim BW, Ahn J, Kim JH, et al. Effects of intracoronary administration of autologous adipose tissue-derived stem cells on acute myocardial infarction in a porcine model. Yonsei Med J. 2015;56(6):1522-9.
49. Li RK, Weisel RD, Mickle DA, Jia ZQ, Kim EJ, Sakai T, et al. Autologous porcine heart cell transplantation improved heart function after a myocardial infarction. J Thorac Cardiovasc Surg. 2000;119(1):62-8.
50. Hirata Y, Umemura K, Uematsu T, Nakashima M. An experimental myocardial infarction model in the rat and its properties. Jpn J Pharmacol. 1995;67(1):51-7.
51. Fang ZY, Lin R, Yuan BX, Yang GD, Liu Y, Zhang H. Tanshinone IIA downregulates the CD40 expression and decreases MMP-2 activity on atherosclerosis induced by high fatty diet in rabbit. J Ethnopharmacol. 2008;115(2):217-22.
52. Bozkurt B, Ahmad T, Alexander KM, Baker WL, Bosak K, Breathett K, et al. Heart failure epidemiology and outcomes statistics: A report of the Heart Failure Society of America. J Card Fail. 2023;29(10):1412-51.
53. Houser SR, Margulies KB, Murphy AM, Spinale FG, Francis GS, Prabhu SD, et al. Animal models of heart failure: a scientific statement from the American Heart Association. Circ Res. 2012;111(1):131-50.
54. Torun N, Durmuş Altun G. Models of cardiovascular disease. Nucl Med Semin. 2019;5(1):96-101. Turkish.
55. Tsutsui H, Spinale FG, Nagatsu M, Schmid PG, Ishihara K, DeFreyte G, et al. Effects of chronic beta-adrenergic blockade on the left ventricular and cardiocyte abnormalities of chronic canine mitral regurgitation. J Clin Invest. 1994;93(6):2639-48.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1489639

Duzce Med J, 2024;26(S1):87-94
Düzce Tıp Fak Derg, 2024;26(S1):87-94

Design of Animal Experiments in Pharmacological Research

Farmakolojik Araştırmalarda Hayvan Deneyleri Tasarımı

Nuri Cenk COŞKUN
0000-0002-9202-1145

Department of Medical Pharmacology, Düzce University Faculty of Medicine, Düzce, Türkiye

ABSTRACT

Pharmacology, also known as pharmaceutical science, has made significant progress, especially in the 20th century, and has played a fundamental role in the development of today's modern drugs. Pharmacology uses in vitro, in vivo, and clinical research stages in drug development. Experimental animals are of great importance in in vivo research. The majority of the drugs used today were developed thanks to animal research. Research in which experimental animals will be used should be planned carefully, and a minimum number of animals should be used since the subject is a living being. In addition, one of the most important ethical principles is to avoid procedures that may cause unnecessary torture and pain to animals during experiments. The purpose of pharmacological research is to develop drugs for the treatment or diagnosis of diseases. For this reason, it is aimed at determining the effects of the substance you are researching in the presence of disease. Immediate use of a substance whose effects were previously unknown on humans may lead to various adverse events and even death. After many events in the past, drug development stages have been determined by accepted international rules. According to these rules, the effect of the substance being investigated must be investigated in experimental animals that have been used as disease models before humans. Many disease models have been developed for this purpose. Drugs developed in these disease models created in experimental animals are now successfully used in the treatment of humans.
Keywords: Pharmacology; experimental animal; disease models; medicine; experimental design.

ÖZ

İlaç bilimi olarak adlandırılan farmakoloji özellikle 20. yüzyılda çok önemli bir ilerleme kaydederek günümüz modern ilaçlarının geliştirilmesinde temel rol almıştır. Farmakoloji ilaç gelişiminde in vitro, in vivo ve klinik araştırma basamaklarından yararlanmaktadır. Bunların içerisinde bulunan in vivo araştırmalarda ise deney hayvanlarının önemi büyüktür. Günümüzde kullanılan ilaçların büyük çoğunluğu deney hayvanları araştırmaları sayesinde geliştirilmişlerdir. Deney hayvanlarının kullanılacağı araştırmalar, öznenin canlı bir varlık olması nedeniyle dikkatli planlanmalı ve asgari sayıda hayvan kullanımı sağlanmalıdır. Bunun yanı sıra deneyler sırasında da hayvanlara gereksiz yere eziyet ve acı verebilecek işlemlerden kaçınılması en önemli etik ilkelerdendir. Farmakolojik araştırmaların amacı hastalıklara karşı tedavi veya tanı amacıyla ilaç geliştirmektir. Bu nedenle araştırılan maddenin hastalık varlığında etkilerini tespit etmek amaçlanmaktadır. Daha önce insanlarda etkileri bilinmeyen bir maddenin hemen insanlarda kullanılması çeşitli olumsuzluklara hatta ölümlere yol açabilecektir. Geçmişte yaşanan pek çok olay sonrasında ilaç geliştirme aşamaları uluslararası kabul edilen kurallar ile belirlenmiştir. Bu kurallara göre araştırılan maddenin etkisinin insanlardan önce hastalık modeli oluşturulmuş deney hayvanlarında araştırılması gerekmektedir. Bu amaçla geliştirilmiş pek çok hastalık modeli oluşturulmuştur. Deney hayvanlarında oluşturulan bu hastalık modellerinde geliştirilen pek çok ilaç günümüzde başarı ile insanların tedavisinde kullanılmaktadır.
Anahtar kelimeler: Farmakoloji; deney hayvanı; hastalık modelleri; ilaç; deney tasarımı.

Corresponding Author
Sorumlu Yazar
Nuri Cenk COŞKUN
cenkcoskun@duzce.edu.tr

Received / Geliş Tarihi : 29.02.2024
Accepted / Kabul Tarihi : 22.04.2024
Available Online /
Çevrimiçi Yayın Tarihi : 24.05.2024

INTRODUCTION

The use of experimental animals in pharmacological scientific research has an important place among all pharmacological research. Especially in the last century, experimental animal research has played an important role in the development and introduction of modern medicines. In pharmacological scientific research, the design and planning of animal experiments are the most important steps for the success of the study. Each stage should be planned carefully, and waste of animals, time, and resources should not be allowed. The aim of this review was to give brief preliminary information on some issues that should be taken into consideration when using animal experiments in pharmacological scientific research and disease models in animals. Although some of the most used experimental animal models in the literature were included in this study, not all of them were mentioned.

ANIMALS IN PHARMACOLOGICAL RESEARCH

The use of experimental animals played an important role in the development of modern medicine. The reason for this is that although the substances whose effects are investigated on humans may differ in type, observing their effects in another living organism and physiological system produces more realistic results when applied to humans. That's why in vivo research has maintained its value for years. Studies with the first known experimental animal date back to 400 BC. Nobel Prize in Physiology between 1901 and 2020, or 186 scientists who won the Medicine (NPPM) award used experimental animals in their projects. 23 of these projects are directly related to pharmacology (1).

ANIMAL SPECIES USED IN SCIENTIFIC RESEARCH

Looking at the research, the preferred animal species in pharmacology research are mice, rats, rabbits, and guinea pigs. It is seen that there are pigs, pigs, dogs, and monkeys. Among these species, mice, rats, and rabbits are the most preferred. The reason for this is that these can be counted as their low body weight, their similarity to human anatomy (presence of similar organs), and their easy intervention. The type of animal to be used varies depending on the research goal. Even fish, pigeons, or helminths can be used (1). If surgery is to be performed in the research model, larger animals may be preferred. Although animal preferences vary depending on the disease model to be studied, many reasons should be taken into consideration, such as the amount and cost of the drugs to be administered, laboratory infrastructure, experience of the research, and compliance with the legislation. When we look at the percentage distribution of mammal species used in Nobel Prize-winning research, it is seen that the rodent species (mouse, rat) are the most common (42%), dogs (14%), rabbits (13%), and then other animal species (1).

PLANNING ANIMAL EXPERIMENTS IN PHARMACOLOGICAL RESEARCH

First of all, the problem should be determined through broad and comprehensive literature research. Afterward, a hypothesis regarding the problem should be put forward. The thing to consider in literature research is whether there has been a satisfactory publication on the subject you are researching. Trying to find an answer before is nothing but a waste of time and resources, except in some cases. After determining our hypothesis, it is necessary to determine the most accurate experimental method to prove it. This method may not always be an experimental animal research method in pharmacological research (Figure 1). If the best way to prove our hypothesis is through experimental animal research, we need to choose the most appropriate animal breed and disease model. When determining this choice, many issues such as our project budget, accessibility, the cost of the substance we will use for treatment, laboratory infrastructure, having a laboratory animal certificate, and the ability to apply the model should be considered. The 3R rule can help in this regard. The first R of the “3R” rule, one of today's bioethics rules, is “reduction”. This principle aims to keep the number of animals used in experiments as low as possible. Second R; “refinement” means to foresee and ensure the welfare and comfort of the animal. This principle aims to ensure that the living conditions of the animal are comfortable during the process, from the time it is selected for the experiment until its death. Third R: It is the principle of “replacement”. The purpose of this principle is that if the same results can be achieved with other experimental models other than experimental animals, they should be chosen first in the research. Today, “responsibility” has also been added to these principles. This principle can be summarized as conducting research by knowing the value of the experimental animal and complying with ethical rules (2). In light of these principles, research should be carried out by determining the minimum number of animals possible. Groups must be determined for the number of animals to be used. Statistical methods can be used for this. Reducing the number of animals in groups leads to less clarity as to whether the effect we are investigating is occurring. Groups with fewer than four animals each are considered non-consensual. If the therapeutic effect of a substance in a disease model is being investigated, it is generally recommended to use at least three different doses. This gives an idea about whether the drug-related effect is dose-dependent or not. Studies using a single dose are studies with less scientific competence. There must be control groups in studies. The group to which you applied the substance or method should be compared with groups to which no substance or method was applied. The purpose of this is to reveal whether the substance or method you are researching is different from normal or the effects of the research. If the substance or method you are researching does not reveal a significant difference compared to the control groups, it is considered to have no effect (3).

There are multiple types of control groups. Placebo control: In this group, animals are given a treatment that

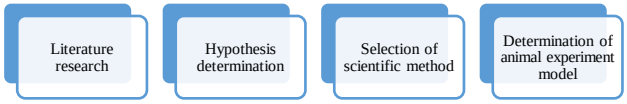

Figure 1. Experimental design algorithm

you think will not have any effect on the same treatment you give. For example, as a control of a drug administered to the gastrointestinal system via gavage, a 0.9% NaCl solution is given in the same way and the same amount. Here, it is investigated whether your medicine has any effect. Positive control: In this control group, a drug or substance that is currently used in the treatment of your disease model is administered to the control group. Here, it is investigated whether the effect of your medicine has a significant effect compared to the known treatment drug currently in use. Vehicle control: This control group, is aimed at investigating whether the substance used to make your medicine into a solution to administer it to animals has any effect on the disease model you are using and on the animals. Sham control: Here, if a serious surgical procedure is performed on the experimental animal during the formation of the disease model or sample collection (for example, abdominal operation and cecal ligation, carotid artery cannulation, etc.), it is aimed to reveal the effects of this operation on the experimental animal. The purpose of creating all these control groups is to reveal the pure and real effect of the treatment you apply (3).

To eliminate the gender effect in research, animals of the same sex are generally used in all groups. If your research includes hormonal effects that need to be avoided, male animals may be preferred. They also have to be the same genus and the same species. For example, while Wistar Albino male rats were used in one group of the same study, mice or rabbits could not be used in another group, and Spraque rats are not preferred in Dawley-type rats. Apart from this, animals of the same species and breed are distributed to groups randomly. Grouping animals with certain characteristics in a group creates a bias in the results of the experiment. Before starting your research, approval must be obtained from the Local Ethics Committee for Experimental Animals.

Information such as the purpose of your study, the experimental outline, treatments to be used, anesthetic drugs if used, and how euthanasia will be performed should be explained. The experiments must be carried out by a researcher who has a certificate for performing laboratory experiments on animals. If the researcher has no previous experience with the disease model to be used or has no experience with the substance to be used, pilot studies may need to be conducted before experiments. These studies play an important role in completing experiments in a shorter time and with less animal and material loss. These studies may take months (3).

Finally, when all permissions are obtained and the disease model is seen to be realized, the main experiments can be carried out. Experiments must be carried out meticulously, without any room for doubt, and every moment is recorded. The data obtained as a result of the experiment (such as EEG data, blood pressure data, hormone analysis data, genetic evaluation data, and pathological examination data) are recorded accurately and evaluated with statistical methods, and the results are obtained.

WAYS OF DRUG ADMINISTRATION

For the selection of drug administration methods, the disease model you will use in experimental animals and the type of experimental animal you will use are important. If the disease model you are planning is a systemic disease (diabetes, shock, etc.), the treatment you will apply must also be systemic. For example, local treatment methods come to the fore in models such as skin lesions or wound healing. Systemic drug administration routes are classified as enteral and parenteral (4). The enteral route can be summarized as the route used through the gastrointestinal system. Medicines used orally fall into this group. This group is defined as the most used and safest way to treat people today. In enteral drug use, the drugs can be mixed into the food and water of the animals, or the drug can be given directly into the animal's stomach by reaching the animal's stomach with various apparatus through the method we call gavage. The most important difference between these methods is that although it is natural to take the drug with food or drink, it is not possible to determine the exact amount of the drug administered. However, drugs given by gavage are given in a certain dose, and since it is ensured that it reaches the animal's stomach, there is no loss of drug dose. The gavage method is preferred, especially for drugs that will be used in low doses or have high costs. Gavage is the administration of medication through a syringe by placing a metal or plastic device orally into the animal's stomach. There are various sizes of gavage apparatus, depending on the breed and size of the animal. It is important to choose the appropriate apparatus according to the characteristics of the animal to avoid complications during application. Although the enteral route is mostly chosen for systemic treatments, it can also be used for local treatments in some disease (gastric ulcers, inflammatory bowel diseases, etc.) models (4). The parenteral route is the name given to drug administration routes other than enteral. Today, it is generally used for intravenous, intramuscular, or subcutaneous drug administration. These methods include percutaneous (on the skin) drug application, which is a local application method, as well as intraperitoneal (between the abdominal membranes) application, which is a systemic treatment application method that is more frequently used in experimental animals than in humans. In parenteral applications, drugs are generally used in specific and small doses via syringe. In parenteral administration, the duration of drug action is much shorter than in enteral administration. In intravenous administration, the effect of the drug can be seen within seconds. This period may take up to 1 hour for enteral applications (4).

EXPERIMENTAL ANIMAL DISEASE MODELS

The role of experimental animals has always been great in the development of drugs and treatments. In experimental animal research, to find the right treatments, the correct disease model must be created exactly in the experimental animal. Incomplete and inadequately created animal models cause both animal loss and research failure. For this reason, creating a disease model in experimental animals is the most important step of the research. This process can be long and challenging. The meticulousness of the researcher in creating a disease model will facilitate the success of the research. Especially in the last century, disease models created in experimental animals have developed considerably. Nowadays, an experimental animal model for almost every disease can be found (5).

Models of Central Nervous System Diseases

Models of common brain and spinal cord-related diseases as well as models of psychiatric diseases will be discussed in this section.

Epilepsy Models

Epilepsy models, which are disease models characterized by seizures, also known as "epilepsy" disease among the public, will be discussed. They are involuntary events that occur in the body because of pathologies experienced in the discharge of neurons. Symptoms such as convulsions and freezing are observed (6). Epilepsy models used in experimental animals were mentioned in Table 1.

Animal Models in Neurodegenerative Diseases

That may be seen as a result of degenerative changes in brain functions or that may progress with a decrease in the movement system or weakness in the muscles, such as amyotrophic lateral sclerosis (ALS) (11). Neurodegenerative disease models were mentioned in Table 2.

Table 1. Epilepsy models used in experimental animals

Method	Application Path	Mechanism of Effect
Focal penicillin model (7)	Systemic and application to the cortex surface in the brain	Stimulating effect of penicillin
Pentylenetetrazol, Bikukulin, Picrotoxin, Strychnine (8)	Systemic application	Glycine and Gaba receptor antagonism
Kainic acid (7)	Systemic application	Glutamate-like effect
Lithium-Pilocarpine (7)	Systemic application	Cholinergic-parasympathomimetic
Cobalt-Homocysteine (9)	Cobalt is placed in the brain	stimulating effect
Tetanus toxin (10)	Applied to certain parts of the brain	Glycine and Gaba release

Table 2. Neurodegenerative disease models used in experimental animals

Illness	Method
Alzheimer's (12)	Transgenic mice- Tg2576, PS1/APP, PDAPP
Alzheimer's (13)	Immune response model created by Aβ antibodies
Parkinson's (14)	6-hydroxydopamine, 1-methyl 4-phenyl, 1,2,3,6-tetrahydropyridine
Amyotrophic Lateral Sclerosis, ALS (15)	Transgenic mice- SOD1, TDP43, C21orf72

Mental Illnesses Models

These animal models are mostly used in the development of diseases such as depression and schizophrenia, which are considered within the scope of mental diseases.

Depression Patterns

This disease is characterized by symptoms such as unhappiness, reluctance, an inability to enjoy life and pessimism. Today, the diagnosis of this disease is made through a personal interview or examination. Since verbal communication is not possible in animals, the diagnosis is made based on findings such as a decrease in the daily movements of animals, a decrease in eating and drinking, and a decrease in communication with other animals. Models of this disease can be created with medication, behavioral tests, and genetic animal models (5).

Depression models used in experimental animals;

- **Drug Models**
  - Reserpine model (16)
  - Yohimbine toxicity model (17)
  - Apomorphine model (17)
  - Glucocorticoid/ Corticosterone model (18)
- **Behavioral Tests**
  - Forced buoyancy test (17)
  - Open field test (19)
  - Tail hanging test (16)
- **Genetic Models**
  - Finder's responsive array (18)
  - Holtzman albino strain (20)
  - Wistar-Kyoto type (21)
  - Transgenic models (18)

Schizophrenia Models

Schizophrenia is a chronic mental illness that distorts a person's perception of reality and causes delusions such as seeing or hearing things that do not actually exist. Experimental animals have an important place in research on this disease, for which no definitive treatment has yet been found.

Schizophrenia models used in experimental animals;

- **Pharmacological Models**
  - Amphetamine, Apomorphine-Dopaminergic agent application (22)
  - Phencyclidine, Ketamine- NMDA receptor antagonists (23)
- **Genetic Models**
  - DISC1 Deletion (24)
  - 22q11.2 Deletion (25)

Addiction Models

Addiction is among the most important health problems today. Experimental animals are used successfully in studies of drug-stimulant addiction as well as cigarette and alcohol addiction.

Addiction models used in experimental animals;

- **Patterns of Excessive Substance Use** (26)
  - Cocaine, Heroin
  - Fentanyl-Morphine
  - Nicotine-Cigarette
- **Animal Deprivation Models** (5,27)
  - Discontinuation of the addictive substance after becoming addicted
  - Antagonist drug administration
- **DSM-Based Animal Models** (28)

Models of Cardiovascular System Diseases

Hypertension Models

As is known, hypertension is a common disease among cardiovascular diseases. A lot of research is being done on the treatment of this disease, which progresses with an increase in blood pressure. Experimental animal models have an important place in these studies.

Hypertension models used in experimental animals;

- **Endocrine-Based Model**
  - Deoxycorticosterone acetate (DOCA) (29)
- **Diet-Induced Model**
  - High salt diet (30)
  - Obesity (31)
- **Model of Neurogenic Origin**
  - Hypothalamus and Rostral Ventero Lateral Medulla stimulation (32)
- **Genetic Model**
  - Spontaneously hypertensive rats - Inbred rat strain (33)

Myocardial Infarction Models

Today, the disease group that causes the most deaths in the world is still coronary artery disease. Despite the development of technology, the pharmaceutical industry, and the development of many new invasive treatments, it continues to have a high mortality rate. As a result, both acute myocardial infarction and the development of coronary artery stenosis are subject to extensive research. Rats are frequently used in these studies because the anatomy of the heart is very similar to the anatomy of the human heart. Among these models, some models require surgical skills.

Myocardial infarction models used in experimental animals;

- **Chemical Agent Application Model**
  - Isoprenaline: Synthetic sympathomimetic catecholamine (34)
- **Coronary Artery Ligation Model** (35)
- **Coronary Artery Embolization Model** (36)
  - Sponge foam, polystyrene microspheres, alcohol

Models of Respiratory System Diseases

Chronic obstructive pulmonary disease (COPD), which is common after smoking, and asthma, which occurs due to allergenic factors, are diseases that threaten a significant part of society. There is still no medicine or treatment that completely cures these diseases. However, many treatments have been developed to enable people with these diseases to continue their normal life comfort. Inhaled drug treatments, especially those developed in the last century, have both treated attacks and reduced the frequency of attacks.

COPD models used in experimental animals;

- Cigarette Smoke Model: Mouse, rat, Guinea Pigs, dog, monkey (5,37)
- Lipopolysaccharide Model: In aerosol form, in mice and rats (38)
- Elastase Model: Intranasal and tracheal administration in mice and rats (39)

Models of Gastrointestinal System Diseases

Gastrointestinal system diseases are among the most common diseases. These diseases, most of these, have definite treatments to be found despite still being treated and researched. A lot of illness has. These studies between inflammatory intestinal diseases and stomach ulcers are being conducted first.

Colitis and ulcer models used in experimental animals;

- **Colitis Models**
  - Dextran Sulfate Sodium (DSS) Model (40)
  - Trinitrobenzene Sulfonic Acid (TNBS) Model (41)
- **Ulcer Models**
  - Indomethacin (42)

Chronic Disease Models

Chronic diseases are diseases that last throughout people's lives. Since there is no definitive treatment for these diseases, the aim is to ensure the patient's life comfort as much as possible. The most prominent of these diseases is diabetes. Since much research has been done on diabetes, many animal models have emerged.

Diabetes models used in experimental animals;

- **Type 1 Diabetes Models**
  - Models Created with Chemical Substances - Alloxan (43)
  - Streptozocin - Antibiotic (43)
  - Non-obese diabetic (NOD) mouse (44)
  - Encephalomyelitis virus variant M (45)
- **Type 2 Diabetes Models**
  - Db/Db Diabetic Mouse (46)
  - Alloxan and Streptozocin (43)
  - Partial pancreatectomy (47)
- **Diet** (48)

Cancer Models

Today, most of the study was made on illness, none no doubt it is cancer. of this disease fatal to be, currently used of treatments side of the effects A lot and fatal possible new treatment find hoping made your studies to increase from where has happened. Most cancer types are caused by experimental animals. The models are also quite large.

Cancer models used in experimental animals;

- **Xenograft Models**
  - Transferring cancer tissue from a human to an animal (5)
  - With blood transfusion (5)
- **Syngenic Models**
  - Among creatures of the same species and genetics (5)
- **Transgenic Models**
  - Genetic models in which spontaneous neoplastic growth is stimulated (5)
- **Models Created with Carcinogenic Agents**
- **Leukemia Models**
  - High doses of gamma radiation or X-ray (49)
- **Lung Cancer Models**
  - Xenograph (50)
- **Thyroid Cancer Models**
  - Immunodeficiency syndrome mouse models with xenograft (51)
- **Breast Cancer Models**
  - N-Methyl-N- Nitrosourea injection in rats (52)
  - Transgenic model- Mouse mammary tumor virus (MMTV) (53)
- **Gastrointestinal System Cancer Models**
  - Hepatocellular Carcinoma Model: Diethylnitrosamine (DEN) (54)
  - Gastric Cancer Model: N-Methyl-N- Nitro -N-Nitrosoguanidine (MNNG) (55)
  - Colorectal Cancer Model: Azoxymethane (AOM) /1,2-Dimethylhydrazine (DMH) (56)

Other Models

Frequently used above and on a lot more study-made experimental illness from the models has been mentioned. In addition to these models, infectious diseases, eye, ear, and nose throat, dermatology, urology, nephrology, and orthopedics have successfully experimented with bestial illness models.

ANIMAL EXPERIMENTS IN TOXICOLOGY RESEARCH

One of the most important stages of drug development is toxicological testing. Toxicology tests are necessary to determine the toxic effects of new drugs and, therefore, the safety of drugs. These toxic effects are investigated first in vitro in cell cultures and then in vivo in experimental animals. Toxicology tests performed are classified according to the duration of drug exposure (57):

- Acute toxicity tests
- Subacute toxicity tests
- Subchronic toxicity tests
- Chronic toxicity tests
- Special toxicity tests

As a result of these tests, important parameters of the toxicity of a drug or substance are the LD50 value, which is the dose that kills 50% of the animals in the experimental group when given a single dose, and the concentration LC50 value of the drug or substance that kills 50% of the animals in the experimental group after exposure for a certain time. These results are the basic values for finding the therapeutic dose of the drug. LD50 and LC50 values of many substances have been found for both animals and humans (57).

SAMPLE COLLECTION METHODS

An important step in the design of animal experiments in pharmacological research is how to obtain the results of the substance for which you create a disease model and apply it for treatment. For this purpose, except for observational studies, samples must be collected from the experimental animal. These samples must be collected in a way that suits our purpose, does not affect the experimental results, and does not harm the welfare of the animal. When planning the research, it should be determined in advance which sample, in what quantity, at what time, and how you will collect it, and how and in what environment these samples will be stored until analysis. These preparations must be completed at the time of sample collection. For example, if a blood sample is taken, will it be serum? plasma? should be determined. Accordingly, it should be determined which blood collection tube should be used. If histopathological examination is to be performed, tissue samples should be placed in formalin solution without delay. Tissues that should not be exposed to chemicals should be immediately placed in a -800 C freezer or liquid nitrogen. If blood samples are to be stored for a short time, they can be stored in a -200 C deep freezer, but if they are to be stored for a long time, they can be stored in a -800 C deep freezer. In studies conducted with brain tissues, a hot water bath, and tissue oxygenation are required. There are also special sample collection methods. One of these is the microdialysis method. It is based on the principle of placing a specially prepared microdialysis apparatus in the brain region of the experimental animal to be examined and removing the neurotransmitters or substances in that region by the dialysis method. It is a very sensitive method and provides important data in brain research (58).

Main sampling types used in experimental animals;

- Taking a blood sample
- Collecting tissue samples
- Stool and urine collection
- Microdialysis
- Viewing and recording
- Bile
- Lymph fluid
- Cerebrospinal fluid
- Peritoneal ascitic fluid

SAMPLE ANALYSIS METHODS

The last and most important step of pharmacological research is the analysis of the data or samples you collect. At this stage, the data and samples obtained from the experimental animals should be analyzed. How this analysis will be carried out should be determined meticulously during the planning phase of your research. Because choosing the wrong analysis method can lead to your research being wasted. The analysis method may vary depending on the type of sample you collect. If you are going to measure biochemical parameters (AST, ALT, CRP, ALP, glucose, urea, etc.), you can get results with a blood sample on devices that investigate these parameters. If you are going to measure a substance that may be present in small amounts in the body, you can use analytical devices called HPLC, LC-MS/MS, or GC-MS to measure very small amounts (nmol, pmol). You can choose sensitive devices that can even analyze substances (e.g., etc.). If you want to examine the effects at the cellular level, you can take relevant tissue samples from the experimental animal and have a histopathological examination done. While some of these analysis methods are practical tests that can be performed by the researcher, some of them are methods that require expensive devices or experts in the field. For this reason, when planning at the very beginning of the research, it is necessary to take into account the budget of the research, the researcher's experience in the analysis, and the possibilities of accessing the analysis.

Some of the main analysis methods used in experimental animals;

- Histopathological Analyzes
- Analyzes with Analytical Devices- HPLC, LC-MS/MS, GC-MS
- Immunoassay Methods
- Imaging analytics

DISCUSSION

The use of experimental animals is of vital importance in pharmacological research. A drug planned to be used in humans must be tested in living systems like humans before it is offered to humans. Because drugs, which are chemical substances, can exhibit a very different behavior in a living system in vivo than in an in vitro environment, There are many reasons for this. Living systems are like machines with many gears intertwined. As a result of the malfunction of one of these wheels, the life of a living

creature may end or it may be seriously harmed. For this reason, whether a substance that does not belong to the body will harm these gears can only be tested in systems with similar gears. Today, despite all this technological progress, nothing like the human living system has been created. For this reason, the closest system we currently have is the system of experimental animals.

Considering all these stages, the design of experimental animals in pharmacological research must be prepared carefully and meticulously. Ultimately, the aim is to obtain real and accurate results.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: NCC; Design: NCC; Data Collection/Processing: NCC; Analysis/Interpretation: NCC; Literature Review: NCC; Drafting/Writing: NCC; Critical Review: NCC.

REFERENCES

1. Jota Baptista CV, Faustino-Rocha AI, Oliveria PA. Animal models in pharmacology: a brief history awarding the Nobel prizes for physiology or medicine. Pharmacology. 2021;106(7-8):356-68.
2. Altun A, Keskin İ. Ethical status and rules for selecting models in animal studies. Sakarya Med J. 2020;10(2):359-64. Turkish.
3. Johnson PD, Besselsen DG. Practical aspects of experimental design in animal research. ILAR J. 2002;43(4):202-6.
4. Yarsan E, editor. Veteriner hekimlikte ilaç uygulama yöntemleri. Ankara: Nobel Kitabevleri; 2019. Turkish.
5. Bora ES, Özlü C. Klinik bilimlerde deney hayvani modelleri. Ankara: Akademisyen Kitabevi. 2020. Turkish.
6. Senio M. Classification criteria of epileptic seizures and syndromes. Epilepsy Res. 2006;70(Suppl 1):S27-33.
7. Marangoz C. Experimental models of epilepsy. J Exp Clin Med. 1997;14(3):147-86. Turkish.
8. McCandless DW, FineSmith RB. Chemically induced model of seizures. In: Boulton AA, Baker GB, Butterworth RF, editors. Animal models of neurological disease, II. Neuromethods, Vol 22. Totowa, NJ: Humana Press; 1992. p.133-51.
9. Miromoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus model of epilepsy: rewiring the brain. Prog Neurobiol. 2004;73(1):1-60.
10. Pitkanen A, Schwartzkroin PA, Moshe SL, editors. Models of seizures and epilepsy. USA: Elsevier Academic Press; 2006.
11. Korkmaz Ü, Kaya M. Experimental models in neurodegenerative diseases. Nucl Med Semin. 2019;5(1):69-77. Turkish.
12. Do Carmo S, Cuello AC. Modeling Alzheimer's disease in transgenic rats. Mol Neurodegener. 2013;8:37.
13. Elçioğlu HK, Yılmaz G, İlhan B, Karan MA. Experimental animal models for Alzheimer disease. Nobel Med. 2018;14(1):5-13. Turkish.
14. Gubellini P, Kachidian P. Animal models of Parkinson's disease: An updated overview. Rev Neurol (Paris). 2015;171(11):750-61.
15. Martineau E, Di Polo A, Vande Velde C, Robitaille R. Dynamic neuromuscular remodeling precedes motor-unit loss in a Mouse model of ALS. eLife. 2018;7:e41973.
16. Hao Y, Ge H, Sun M, Gao Y. Selecting an appropriate animal model of depression. Int J Mol Sci. 2019;20(19):4827.
17. Kobeissy FH, editor. Psychiatric disorders: methods and protocols. Totowa, NJ: Humana Press; 2012.
18. Wang Q, Timberlake MA 2nd, Prall K, Dwivedi Y. The recent progress in animal models of depression. Prog Neuropsychopharmacol Biol Psychiatry. 2017;77:99-109.
19. Valvassori SS, Budni J, Varela RB, Quevedo J. Contributions of animal models to the study of mood disorders. Braz J Psychiatry. 2013;35(Suppl 2):S121-31.
20. Padilla E, Barret D, Shumake J, Gonzales-Lima F. Strain, sex and open-field behavior: factors underlying the genetic susceptibility to helplessness. Behav Brain Res. 2009;201(2):257-64.
21. Nam H, Clinton SM, Jackson NL, Kerman IA. Learned helplessness and social avoidance in the Wistar-Kyoto rat. Front Behav Neurosci. 2014;8:109.
22. Powell SB. Models of neurodevelopmental abnormalities in schizophrenia. Curr Top Behav Neurosci. 2010;4:435-81.
23. Tsai G, Coyle JT. Glutamatergic mechanisms in schizophrenia. Annu Rev Pharmacol Toxicol. 2002;42:165-79.
24. Clapcote S, Lipina TV, Millar JK, Mackie S, Christie S, Ogawa F, et al. Behavioral phenotypes of Disc1 missense mutations in mice. Neuron. 2007;54(3):387-402.
25. Basset AS, Chow EW. Schizophrenia and 22q11.2 deletion syndrome. Curr Psyciatry Rep. 2008;10(2):148-57.
26. Uzbay İT. Experimental animal models used in substance addiction studies. Madde bağımlılığı çalışmalarında kullanılan deneysel hayvan modelleri. MİSED. 2009;21-22:49-63. Turkish.
27. Becker JAJ, Kieffer BL, Le Merrer J. Differential behavioral and molecular alterations upon protracted abstinence from cocaine versus morphine, nicotine, THC and alcohol. Addict Biol. 2017;22(5):1205-17.
28. Vanderschuren LJMJ, Minnaard AM, Smeets JAS, Lesscher HMB. Punishment models of addictive behavior. Curr Opin Behav Sci. 2017;13:77-84.
29. Zicha J, Kunes J, Lébl M, Pohlová I, Slaninová J, Jelínek J. Antidiuretic and pressor actions of vasopressin in age-dependent DOCA-salt hypertension. Am J Physiol. 1989;256(1 Pt 2):138-45.
30. Prager-Khoutorsky M, Choe KY, Levi DI, Bourque CW. Role of vasopressin in rat models of salt-dependent hypertension. Curr Hypertens Rep. 2017;19(5):42.

31. Dobrian AD, Davies MJ, Prewitt RL, Lauterio TJ. Development of hypertension in a rat model of diet-induced obesity. Hypertension. 2000;35(4):1009-15.
32. Juskevich JC, Robinson DS, Whitehorn D. Effect of hypothalamic stimulation in spontaneously hypertensive and Wistar-Kyoto rats. Eur J Pharmacol. 1978;51(4):429-39.
33. Bomfim GF, Dos Santos RA, Oliveira MA, Giachini FR, Akamine EH, Tostes RC, et al. Toll-like receptor 4 contributes to blood pressure regulation and vascular contraction in spontaneously hypertensive rats. Clin Sci (Lond). 2012;122(11):535-43.
34. Shikalgar TS, Naikwade NS. Evaluation of cardioprotective activity of fulvic acid against isoproterenol induced oxidative damage in rat myocardium. Int Res J Pharm. 2018;9(1):71-80.
35. Wang J, Bo H, Meng X, Wu Y, Bao Y, Li Y. A simple and fast experimental model of myocardial infarction in the mouse. Texas Heart Inst J. 2006;33(3):290-3.
36. Isorni MA, Casanova A, Piquet J, Bellamy V, Pignon C, Puymirat E, et al. Comparative analysis of methods to induce myocardial infarction in a closed-chest rabbit model. Biomed Res Int. 2015;2015:893051.
37. Zhou S, Wright JL, Liu J, Sin DD, Churg A. Aging does not enhance experimental cigarette smoke-induced COPD in the mouse. PLoS One. 2013;8(8):e71410.
38. Vernooy JH, Dentener MA, van Suylen RJ, Buurman WA, Wouters EF. Long-term intratracheal lipopolysaccharide exposure in mice results in chronic lung inflammation and persistent pathology. Am J Respir Cell Mol Biol. 2002;26(1):152-9.
39. Pera T, Zuidhof A, Valadas J, Smit M, Schoemaker RG, Gosens R, et al. Tiotropium inhibits pulmonary inflammation and remodelling in a guinea pig model of COPD. Eur Respir J. 2011;38(4):789-96.
40. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990;98(3):694-702.
41. Neurath MF, Fuss I, Kelsall BL, Stüber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. J Exp Med. 1995;182(5):1281-90.
42. Souza MH, Mota JM, Oliveira RB, Cunha FQ. Gastric damage induced by different doses of indomethacin in rats is variably affected by inhibiting iNOS or leukocyte infiltration. Inflamm Res. 2008;57(1):28-33.
43. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26.
44. Mathews CE. Utility of murine models for the study of spontaneous autoimmune type 1 diabetes. Pediatr Diabetes. 2005;6(3):165-77.
45. Jun HS, Yoon JW. The role of viruses in type 1 diabetes: two distinct cellular and molecular pathogenic mechanisms of virus-induced diabetes in animals. Diabetologia. 2001;44(3):271-85.
46. Allen TJ, Cooper ME, Lan HY. Use of genetic mouse models in the study of diabetic nephropathy. Curr Diab Rep. 2004;4(6):435-40.
47. Pickup JC, Williams G, editors. Textbook of diabetes, 2nd ed. Cambridge, MA: Blackwell Science; 1997.
48. Kim JH, Saxton AM. The TALLYHO mouse as a model of human type 2 diabetes. Methods Mol Biol. 2012;933:75-87.
49. Almosailleakh M, Schwaller J. Murine models of acute myeloid leukaemia. Int J Mol Sci. 2019;20(2):453.
50. Hastings RH, Burton DW, Quintana RA, Biederman E, Gujral A, Deftos LJ. Parathyroid hormone-releated protein regulates the growth of orthotopic human lung tumors in athymic mice. Cancer. 2001;92(6):1402-10.
51. Jin Y, Liu M, Sa R, Fu H, Cheng L, Chen L. Mouse models of thyroid cancer: bridging pathogenesis and novel therapeutics. Cancer Lett. 2020;469:35-53.
52. Tsubura A, Lai YC, Miki H, Sasaki T, Uehara N, Yuri T, et al. Review: Animal models of N-methyl-N-nitrosourea-induced mammary cancer and retinal degeneration with special emphasis on therapeutic trials. In Vivo. 2011;25(1):11-22.
53. Akbari Bazm M, Naseri L, Khazaei M. Methods of inducing breast cancer in animal models: a systematic review. World Cancer Res J. 2018;5(4):e1182.
54. Zhang HE, Henderson JM, Gorrel MD. Animal models for hepatocellular carcinoma. Biochim Biophys Acta Mol Basis Dis. 2019;1865(5):993-1002.
55. Tatematsu M, Yamamoto M, Shimizu N, Yoshikawa A, Fukami H, Kaminishi M, et al. Induction of glandular stomach cancers in Helicobacter pylori-sensitive Mongolian gerbils treated with N-methyl-N-nitrosourea and N-methyl-N-nitro-N-nitrosoguanidine in drinking water. Jpn J Cancer Res. 1998;89(2):97-104.
56. Bobek P, Galbavy S, Ozdin L. Effect of oyster mushroom (Pleurotus ostreatus) on pathological changes in dimethylhydrazine-induced rat colon cancer. Oncol Rep. 1998;5(3):727-730.
57. Saygı Ş. Toxicity testings and the importance of test results in experimental toxicology. Gülhane Med J. 2003;45(3):291-8. Turkish.
58. İssi M. Removal techniques of blood from laboratory animals. J Bornova Vet Sci. 2008;30(44):43-48. Turkish.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1500615

Duzce Med J, 2024;26(S1):95-99
Düzce Tıp Fak Derg, 2024;26(S1):95-99

Senescence Model Theories from In Vitro through In Vivo

In Vitro'dan In Vivo'ya Yaşlılık Model Teorileri

Merve ALPAY
0000-0002-8782-9561

Department of Medical Biochemistry, Düzce University, Faculty of Medicine, Düzce, Türkiye

ABSTRACT

The theoretical equivalence of expressing that a cell is aging to its inability to perform the assumed function is not exactly accurate, it involves a gradual decrease in cell aging mechanisms. Factors such as genetics, lifestyle, and environmental effects maintain the biological change of the cell. The concept of cellular senescence was initially introduced by Hayflick and his collaborators in 1961 when they noticed that human diploid fibroblasts cultured in vitro could undergo only a limited number of cell divisions before their ability to proliferate was permanently halted. This phenomenon, known as the 'Hayflick limit', was subsequently linked to the gradual shortening of telomeres with each successive round of cell division. Throughout the aging process, senescent cells collect in different tissues. Their involvement in age-related health issues such as neurodegenerative disorders, heart problems, cancer, kidney-related changes, chronic lung diseases, and osteoarthritis suggests that targeting senescent cells therapeutically could be promising across various health conditions. This review will discuss the available data on which cell types may undergo aging based on biological aging and how these processes may impact age-associated tissue-specific pathologies. Additionally, the markers used to characterize the physiological transition of aging cells from in vitro to in vivo settings will be evaluated. The discussed data may serve as a significant starting point for an expanded definition of the molecular and functional characteristics of aging cells in different organs, thus supporting the development and enhancement of targeting strategies in vivo.
Keywords: Cell senescence; geriatric in vivo models; cellular aging theories; biological aging mechanism.

ÖZ

Bir hücrenin, varsayılan işlevini yerine getiremeyecek kadar yaşlanmasının karşılığı hücrenin biyolojik süreçlerinde her zaman tam bir yetersizliğe yol açmayabilir, hücre yaşlanma mekanizmalarında kademeli bir azalmayı içerir. Hücrenin biyolojik değişimini genetik, yaşam tarzı ve çevresel etkiler gibi faktörler sürdürür. Hücresel yaşlanma kavramı ilk olarak 1961 yılında Hayflick ve çalışma arkadaşları tarafından, in vitro kültüre alınan insan diploid fibroblastlarının çoğalma yetenekleri kalıcı olarak durdurulmadan önce yalnızca sınırlı sayıda hücre bölünmesine maruz kalabileceğini fark ettiklerinde ortaya atıldı. 'Hayflick sınırı' olarak bilinen bu fenomen, daha sonra, birbirini izleyen her hücre bölünmesi turunda telomerlerin kademeli olarak kısalması ile ilişkilendirildi. Yaşlanma süreci boyunca yaşlanan hücreler farklı dokularda toplanmaktadır. Nörodejeneratif ve kardiyovasküler bozukluklar, kanser, böbrekle ilgili değişiklikler, kronik akciğer hastalıkları ve osteoartrit gibi yaşa bağlı sağlık sorunlarına katılımları, yaşlanan hücreleri terapötik olarak hedeflemenin çeşitli sağlık koşullarında umut verici olabileceğini düşündürmektedir. Bu derlemede biyolojik yaşlanmaya bağlı olarak hangi hücre tiplerinin yaşlanabileceğine ve bu süreçlerin yaşla ilişkili dokuya özgü patolojileri nasıl etkileyebileceğine dair mevcut veriler tartışılacaktır. Ek olarak, yaşlanan hücrelerin in vitro ortamdan in vivo ortama fizyolojik geçişini karakterize etmek için kullanılan belirteçler de değerlendirilecektir. Tartışılan veriler, farklı organlarda yaşlanan hücrelerin moleküler ve fonksiyonel özelliklerinin genişletilmiş bir tanımı için önemli bir başlangıç noktası olarak hizmet edebilir, böylece in vivo hedefleme stratejilerinin geliştirilmesini ve artırılmasını destekleyebilir.
Anahtar kelimeler: Hücre yaşlanması; geriatrik in vivo modeller; hücresel yaşlanma teorileri; biyolojik yaşlanma mekanizması.

Corresponding Author
Sorumlu Yazar
Merve ALPAY
mervealpay@duzce.edu.tr

Received / Geliş Tarihi : 18.04.2024
Accepted / Kabul Tarihi : 20.05.2024
Available Online /
Çevrimiçi Yayın Tarihi : 13.06.2024

INTRODUCTION

The rapid aging or entry into an aging trend of advanced and developing societies, interest in aging, and old age studies have started to increase in recent years. This has led to a growing interest in the disciplines of gerontology and geriatrics, which encompass studies in the field. According to a report by the United Nations (UN) in 2022, approximately 10% of the world's population consists of older adults aged 65 and above. This percentage is expected to reach 16% by the year 2050 (1). Looking at Turkey, it is observed that the proportion of the elderly population has surpassed the world average, reaching 10.2% of the total population (2). This situation can lead to a concentrated focus on popular areas such as social, psychological, health, and care in aging and old age studies, potentially neglecting biogerontology. Biogerontology is a discipline that investigates why and how living organisms age (3). In the light of current knowledge, aging refers to the period from an individual's existence as an organism to death, while old age represents a unique stage of life like childhood and youth, and an older adult refers to a person above a certain age. The chronological age that distinguishes these categories is 60 according to the UN and 65 according to the World Health Organization (WHO). Therefore, it is understood that social conditions and environmental factors change the biological aging process, as the aging process is categorized differently. It is possible to build a society of ageless elderly individuals by optimizing external factors while the biological aging process is in progress (4). Based on all this information, biogerontology seeks answers to the following questions:

- Many aging models have been proposed to explain the aging process, in other words, why do we age?
- What are the biological processes associated with aging?
- Is aging genetically programmed or is it a multivariate random process?
- Are there biomarkers associated with aging?
- Is it possible to slow down or prevent aging? What chronic diseases occur with aging?

Cellular senescence is the result of a series of molecular and cellular changes that cells undergo throughout life (5). These changes can disrupt the function of cells and contribute to a number of diseases associated with aging (Figure 1). Throughout its historical development, many biological theories of aging have been proposed seeking answers to the causes of aging. These theories are discussed under two main headings: evolutionary theories of aging and physiological (mechanistic) theories of aging. While evolutionary theories of aging focus on the ultimate cause, mechanistic theories of aging focus on the convergent or apparent cause. According to stochastic theory, aging occurs as a result of the accumulation of randomly occurring errors in biomolecules. It is suggested that the accumulation of mutations in the genetic material of the cell due to external and internal factors over time and the advanced glycation products formed as a result of the glycation of biomolecules cause aging. According to the hereditary model, it is accepted that aging is a programmed process. The main cause of replicative senescence, defined as replicative cells losing their ability to divide after a certain number of divisions, is telomere shortening (6-9). Numerous processes associated with aging have been identified. These include genomic instability, epigenetic variations, and changes at the transcriptional level, as well as molecular damage, cellular aging and death, inflammation, and metabolic disorders (10,11). Although almost every aging model focuses on a single mechanism related to aging, it does not seem possible to explain it with a single mechanism since aging is a very complex event. This review aims to discuss the available data on which cell types may undergo aging based on biological aging and how these processes may impact age-associated tissue-specific pathologies.

CELLULAR PROCESSES ASSOCIATED WITH AGING

Telomere Shortening

Telomeres are repetitive DNA sequences located at the ends of chromosomes. With each cell division, telomeres shorten. The shortening of telomeres causes cells to lose their ability to divide and their genetic integrity, contributing to the cellular aging process (12).

Genetic Damage

Normally, the DNA molecule is stable and DNA replication is a very conservative process that tries to operate without errors. DNA damage has a significant impact on the aging of cells. Several threats such as ionizing radiation, oxidative stress, chemicals, and other environmental factors can cause DNA damage. With aging, oxidation in mitochondrial DNA (mtDNA) is much more prominent than in nuclear DNA. A range of genetic deteriorations can occur, from point mutation, which is the simplest form of mutation, to chromosome losses and gains. Complex DNA repair mechanisms have evolved to reduce these damages and keep the genome stable. However, these mutations, which are still rare, accumulate over time (age) and the genome becomes unstable. The accumulation of this damage is associated with the aging of cells and the development of aging-related diseases.

Oxidative Stress

Among the types of stress known to stimulate or accelerate cellular aging, oxidative stress is the accumulation of free radicals and other reactive oxygen species in cells. Oxidative stress can lead to a number of cellular damage

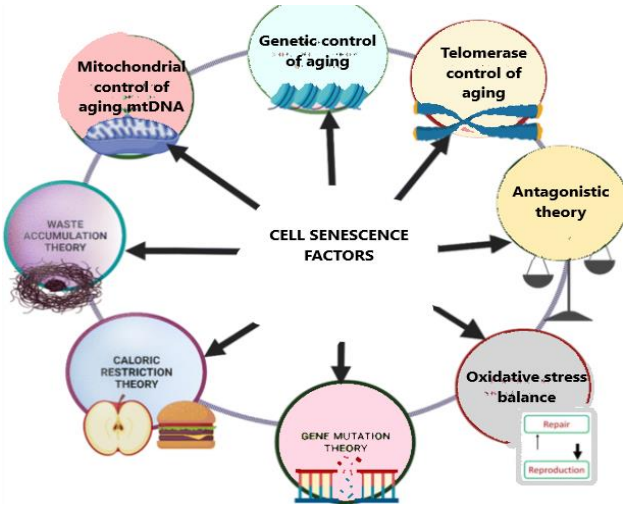

Figure 1. Variety arms of change cell structure

in cells, such as lipid peroxidation, protein damage, and DNA damage. This environment can lead to stress-induced cellular senescence (SIPS), which pushes cells to early aging with the effect of other molecular factors (13,14).

DNA Repair Mechanism Balance

They are segmental early aging (progeria) syndromes that occur as a result of defects in the DNA repair mechanism. It is characterized by defects in DNA helicase and DNA repair mechanisms as a result of mutation in the WRN gene. Lamin A production as a result of LMNA gene mutation. It has been determined that the rarity of the apolipoprotein E ε4 allele triggers cellular aging. It is characterized by disorder. Progeria syndromes shed light on some aspects of normal aging, but they do not include all the features of normal aging (15,16).

Cell Cycle Deregulation

The cell cycle is the process of division and proliferation of cells. Disturbances in the regulation of the cell cycle can prevent or accelerate the controlled growth and division of cells. This can contribute to the cellular aging process and the development of diseases such as cancer (17).

Disruption of Protein Homeostasis (Proteostasis)

Cells maintain protein homeostasis by ensuring the correct folding, processing, and degradation of proteins. With aging, the effectiveness of these mechanisms may decrease and problems such as protein misfolding and accumulation may occur (18,19).

Mitochondrial Dysfunction

Mitochondria are known as energy-producing structures in cells. Mitochondrial dysfunction can cause problems such as oxidative phosphorylation disorder, reactive oxygen species production, and decreased cellular energy production. This can contribute to the aging of cells (20).

Inflammation

Inflammation is increasingly linked to aging and chronic diseases. However, the observed increase in the basal inflammatory response with age causes a sustained low level of inflammation that promotes aging. The decline of the thymus gland, where T cells mature, is a much faster process than aging. Although proliferation decreases in the elderly, the number of T cells generally does not change. Apoptosis occurs with decreasing bcl-2 expression in maturing T cells. B cells also produce fewer antibodies. Senescent cells secrete many cytokines that initiate inflammation. In fact, in advanced ages, there are a small number of senescent cells in organs and tissues, but the inflammation-initiating factors secreted by them affect the functioning of autocrine, paracrine, and endocrine systems, also known as cellular signal transmission mechanisms (21,22).

Apoptosis

Elimination of damaged cells is also known as programmed cell death or apoptosis. The increase in nondividing cells with age has led to the acceptance that it contributes to aging. However, this mechanism can also be seen as an adaptive feature, as it prevents the proliferation of damaged cells and ensures their elimination by the immune system. In this way, it prevents the tissue from being damaged and turning into potentially cancerous cells. However, age-related decline in cell renewal and immune system capacity leads to an increase in the number of senescent cells. This increase contributes to loss of function in tissues and organs and eventually to aging (23,24).

These mechanisms indicate that cellular aging is a complex process and plays an important role in the development of aging-related diseases. Researchers are trying to understand these mechanisms and identify potential therapeutic targets to delay or prevent cellular aging.

Cell senescence and aging mechanisms are the result of a series of complex interactions that biological systems undergo throughout life. Many experimental animal models have been used to understand these mechanisms (25).

IN VIVO (EXPERIMENTAL MODEL) PROCESSES ASSOCIATED WITH AGING

Yeast (*Saccharomyces cerevisiae*)

Yeast is a simple eukaryotic organism and is a fundamental model organism in studies of cell aging. It is especially used to study mechanisms associated with aging, such as telomere length and DNA damage repair. In *S. cerevisiae* yeast, it has been shown that an increase in the copy of the Ras2 gene, mutations in the Ras1 gene, a decrease in the TOR signal, an increase in the AMPK signal, and the sirtuin gene called sir2 prolong lifespan (26,27).

Nematode (*Caenorhabditis elegans*)

Nematodes are a model organism frequently used in genetic and neurological research. In particular, it is a popular option for studying the genetic mechanisms of the aging process and the neurological effects of aging. Decrease in TOR signaling in *C. elegans* worm, It has been determined that an increase in AMPK signaling and the sirtuin gene called SIR-2.1 prolong lifespan (28).

Fruit Fly (*Drosophila melanogaster*)

Fruit flies are another model organism commonly used in aging research. Telomeres are often used to study aging mechanisms such as oxidative stress and metabolic effects. It has been determined that a decrease in the TOR signal in the *D. melanogaster* fruit fly, mutations in the Indy gene, which is important in the Krebs cycle, and the Methuselah gene, which encodes a G protein-related receptor, and the sirtuin gene called sir2, are associated with longevity. There is abundant evidence regarding the importance of diet and environmental temperature in the context of aging. In 1929, it was demonstrated that the lifespan of Drosophila species is inversely proportional to ambient temperature (29).

It has been shown in many studies conducted in yeast, worms, fruit flies, and rodents that calorie restriction at a level that does not cause malnutrition extends lifespan. As a result of calorie restriction, many changes have been observed in metabolism, cellular level, genetic structure, and neuroendocrine system. When metabolism slows down, ROU production, which is thought to play an important role in aging, also decreases (30).

Mammalian Cell Cultures

Mammalian models such as mice and mouse cell cultures are widely used in research on human aging. These models have biochemical and genetic properties similar to human cells and are used specifically to study mechanisms such as cellular aging, DNA damage repair, and apoptosis (31-33).

Aging Mouse Models

Some mouse strains show physiological and pathological characteristics similar to human aging when exposed to the

natural aging process (Figure 2). These mice are valuable models used to study aging mechanisms and aging-related diseases (34). These experimental animal models provide fundamental information on cell senescence and aging mechanisms and may help identify potential therapeutic targets for understanding and treating aging-related diseases. However, these models as well as research in humans are vital for a full understanding of human aging.
Aging mouse models are laboratory mice used to understand human aging and aging-related diseases. These mice show physiological and pathological characteristics similar to human aging when exposed to the natural aging process. More commonly used aging mouse models to understand aging-associated phenotypes and the mechanisms underlying the aging process will be described (35).

Senescence Accelerated Mouse (SAM)

SAM mice are a mouse model bred at Shizuoka University in Japan and are frequently used in aging research. SAM mice have a genetic predisposition to accelerate the normal aging process and display a number of aging-associated phenotypes. SAM mice constitute an important model for studying molecular and cellular mechanisms in the aging process. SAM mice are divided into two subtypes, senescence-accelerated prone mouse (SAMP) and senescence-accelerated resistant mouse (SAMR), depending on their genetic diversity. SAMP mice are more prone to signs of aging and diseases associated with aging, while SAMR mice are more resistant. Levels of CD4 and CD8 memory T cells and naïve T cells have been used to give good estimates of life expectancy of middle-aged mice. Differences between these two subtypes are examined to understand the genetic factors underlying the aging process and the mechanisms of aging-related pathologies (36,37).

Akkerman Health Aging Mice (*Akkermansia muciniphila*)

A bacterium called *Akkermansia muciniphila* plays an important role in the gut microbiota and has been associated with the aging process. Aging mouse models have been used to study the effects of this bacterium on mice. The effects of *Akkermansia muciniphila* on the aging process are studied to investigate issues such as changes in gut health and metabolic disorders associated with aging (38).

Transgenic Mouse

In a transgenic mouse model, without implementing calorie restriction, researchers achieved a prolonged median lifespan of 20% by consistently lowering body temperature by an average of 0.5-0.6°C through modulation of the hypothalamic 'central thermostat' over an extended period (39).

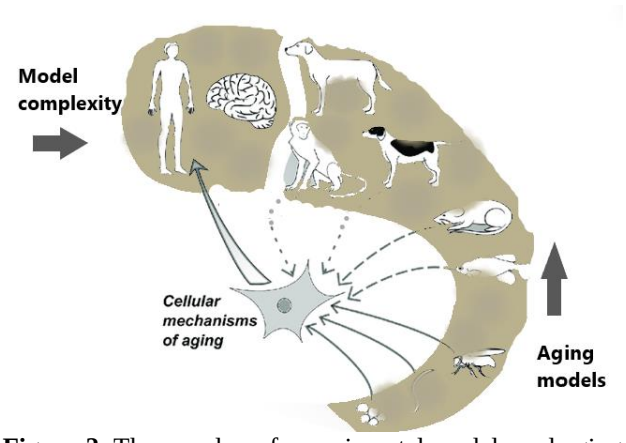

Figure 2. The paradox of experimental models and aging biology

CONCLUSION

Aging is a natural process and a natural part of life. However, it is a major risk factor for many diseases and can limit life expectancy. Interventions in model organisms have prevented or postponed the emergence of many chronic diseases as well as prolonging life. These interventions offer a promising approach to reducing aging-related health problems. Although our knowledge of aging and senescent cell types depicted in in vivo models and their roles in aging has increased significantly, the experimental models used to determine the molecular relationships of senescent cells in human tissues must be diversified and a comprehensive analysis of their roles in the aging phase is needed.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: MA; Design: MA; Data Collection/Processing: MA; Analysis/Interpretation: MA; Literature Review: MA; Drafting/Writing: MA; Critical Review: MA.

REFERENCES

1. Zheng Z. Twenty years' follow-up on elder people's health and quality of life. China Popul Dev Stud. 2020;3(4):297-309.
2. Adana F, Durmaz S, Özvurmaz S, Akpınar CV, Yeşilfidan D. Descriptors of living alone for elders: based on Turkey national data. BMC Geriatr. 2022;22(1):37.
3. Hayflick L. Theories of biological aging. Exp Gerontol. 1985;20(3-4):145-59.
4. Ersözlü M, Aydemir MA. Permanent, continuous and interested: ageless older adults. Senex: J Aging Stud. 2021;5(1):19-46. Turkish.
5. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res. 1965;37:614-36.

6. Di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol. 2021;22(2):75-95.
7. Zhang L, Pitcher LE, Yousefzadeh MJ, Niedernhofer LJ, Robbins PD, Zhu Y. Cellular senescence: a key therapeutic target in aging and diseases. J Clin Invest. 2022;132(15):e158450.
8. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-217.
9. Mahmoudi S, Brunet A. Aging and reprogramming: a two-way street. Curr Opin Cell Biol. 2012;24(6):744-56.
10. Rattan SIS. If aging is a disease, then it is your own fault. J Aging Sci. 2016;4(2):e120.
11. Sikora E, Bielak-Żmijewska A, Mosieniak G. What is and what is not cell senescence. Postepy Biochem. 2018;64(2):110-8.
12. Razgonova MP, Zakharenko AM, Golokhvast KS, Thanasoula M, Sarandi E, Nikolouzakis K, et al. Telomerase and telomeres in aging theory and chronographic aging theory (Review). Mol Med Rep. 2020;22(3):1679-94.
13. Slijepcevic P. DNA damage response, telomere maintenance and ageing in light of the integrative model. Mech Ageing Dev. 2008;129(1-2):11-6.
14. Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ. The central role of DNA damage in the ageing process. Nature. 2021;592(7856):695-703.
15. Stead ER, Bjedov I. Balancing DNA repair to prevent ageing and cancer. Exp Cell Res. 2021;405(2):112679.
16. Clarke TL, Mostoslavsky R. DNA repair as a shared hallmark in cancer and ageing. Mol Oncol. 2022;16(18):3352-79.
17. Petr MA, Tulika T, Carmona-Marin LM, Scheibye-Knudsen M. Protecting the aging genome. Trends Cell Biol. 2020;30(2):117-32.
18. Mc Auley MT, Guimera AM, Hodgson D, Mcdonald N, Mooney KM, Morgan AE, et al. Modelling the molecular mechanisms of aging. Biosci Rep. 2017;37(1):BSR20160177.
19. Vilchez D, Saez I, Dillin A. The role of protein clearance mechanisms in organismal ageing and age-related diseases. Nat Commun. 2014;5:5659.
20. Wolf AM. MtDNA mutations and aging-not a closed case after all? Signal Transduct Target Ther. 2021;6(1):56.
21. Sendama W. The effect of ageing on the resolution of inflammation. Ageing Res Rev. 2020;57:101000.
22. Pansarasa O, Mimmi MC, Davin A, Giannini M, Guaita A, Cereda C. Inflammation and cell-to-cell communication, two related aspects in frailty. Immun Ageing. 2022;19(1):49.
23. Wanner E, Thoppil H, Riabowol K. Senescence and apoptosis: architects of mammalian development. Front Cell Dev Biol. 2021;8:620089.
24. Hu L, Li H, Zi M, Li W, Liu J, Yang Y, et al. Why senescent cells are resistant to apoptosis: an insight for senolytic development. Front Cell Dev Biol. 2022;10:822816.
25. Kumar A, Bano D, Ehninger D. Cellular senescence in vivo: From cells to tissues to pathologies. Mech Ageing Dev. 2020;190:111308.
26. Ocampo A, Reddy P, Martinez-Redondo P, Platero-Luengo A, Hatanaka F, Hishida T, et al. In vivo amelioration of age-associated hallmarks by partial reprogramming. Cell. 2016;167(7):1719-33.e12.
27. Rabinowitz ZM, Cui L. Detecting cellular senescence in vivo: Imagining imaging better. Aging Cancer. 2023;4(3-4):97-110.
28. Mack HID, Heimbucher T, Murphy CT. The nematode Caenorhabditis elegans as a model for aging research. Drug Discov Today Dis Models. 2018;27:3-13.
29. Alpatov WW, Pearl R. Experimental studies on the duration of life. XII. influence of temperature during the larval period and adult life on the duration of the life of the imago of Drosophila melanogaster. Am Nat. 1929;63(684):37-67.
30. Conti B. Considerations on temperature, longevity and aging. Cell Mol Life Sci. 2008;65(11):1626-30.
31. Veronesi F, Contartese D, Di Sarno L, Borsari V, Fini M, Giavaresi G. In vitro models of cell senescence: a systematic review on musculoskeletal tissues and cells. Int J Mol Sci. 2023;24(21):15617.
32. Hartmann C, Herling L, Hartmann A, Köckritz V, Fuellen G, Walter M, et al. Systematic estimation of biological age of in vitro cell culture systems by an age-associated marker panel. Front Aging. 2023;4:1129107.
33. Minteer C, Morselli M, Meer M, Cao J, Higgins-Chen A, Lang SM, et al. Tick tock, tick tock: Mouse culture and tissue aging captured by an epigenetic clock. Aging Cell. 2022;21(2):e13553.
34. Brunet A. Old and new models for the study of human ageing. Nat Rev Mol Cell Biol. 2020;21(9):491-3.
35. Browder KC, Reddy P, Yamamoto M, Haghani A, Guillen IG, Sahu S, et al. In vivo partial reprogramming alters age-associated molecular changes during physiological aging in mice. Nat Aging. 2022;2(3):243-53.
36. Folgueras AR, Freitas-Rodríguez S, Velasco G, López-Otín C. Mouse models to disentangle the hallmarks of human aging. Circ Res. 2018;123(7):905-24.
37. Mitchell SJ, Scheibye-Knudsen M, Longo DL, de Cabo R. Animal models of aging research: implications for human aging and age-related diseases. Annu Rev Anim Biosci. 2015;3:283-303.
38. Yu Y, Lu C, Yu W, Lei Y, Sun S, Liu P, et al. B cells dynamic in aging and the implications of nutritional regulation. Nutrients. 2024;16(4):487.
39. Liu RK, Walford RL. The effect of lowered body temperature on lifespan and immune and non-immune processes. Gerontologia. 1972;18(5-6):363-88.

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1496949

Duzce Med J, 2024;26(S1):100-103
Düzce Tıp Fak Derg, 2024;26(S1):100-103

Some Alternative Methods to the Use of Laboratory Animals in Medical Research

Tıbbi Araştırmalarda Laboratuvar Hayvanlarının Kullanımına Alternatif Bazı Yöntemler

İlker KILIÇCIOĞLU
0000-0003-3367-9665
Görkem DÜLGER
0000-0002-9517-3830

Department of Medical Biology,
Düzce University Faculty of Medicine,
Düzce, Türkiye

Corresponding Author
Sorumlu Yazar
Görkem DÜLGER
gorkemdulger@yandex.com

Received / Geliş Tarihi : 26.03.2024
Accepted / Kabul Tarihi : 13.05.2024
Available Online /
Çevrimiçi Yayın Tarihi : 06.06.2024

ABSTRACT

Experimental animals have long been used for research and educational purposes. However, in recent years, ethical debates regarding the rights of animals to life have also become important. It is advocated that animal experiments can be carried out for scientific goals that can contribute to human and animal welfare in the long term and in cases where there are no alternative methods. The principles of "replacement", "reduction", and "refinement", known as the 3R rule, have been updated as 4R with the addition of the "responsibility" principle. These principles provide basic guidance for the ethical use of animals in scientific experiments. Techniques that can be alternatives to animal experiments in research and biological effect studies to test synthesized drug candidate compounds and various chemicals have been developed. In vitro cell culture techniques and bioimaging methods are very important alternatives to in vivo animal experiments. The micro-dose technique is another alternative method that reveals the effects of drug candidate compounds on volunteer individuals at very low doses without using animals. Software databases and in silico computer simulations are also gaining importance in research institutions as an alternative to animal experiments. In this review, the fundamentals of animal research ethics and emerging alternatives to reduce animal use in medical experiments were discussed.
Keywords: Experimental animals; ethics; alternative methods.

ÖZ

Deney hayvanları uzun süredir araştırma ve eğitim amaçlı olarak kullanılmaktadır. Ancak son yıllarda hayvanların yaşam haklarına ilişkin etik tartışmalar da önem kazanmaya başlamıştır. Uzun vadede insan ve hayvan refahına katkı sağlayabilecek bilimsel amaçlar doğrultusunda ve alternatif yöntemlerin bulunmadığı durumlarda hayvan deneylerinin yapılabileceği savunulmaktadır. 3R kuralı olarak bilinen replacement (değiştirme), reduction (azaltma) ve refinement (iyileştirme) ilkeleri, responsibility (sorumluluk) ilkesinin de eklenmesiyle 4R olarak güncellenmiştir. Bu ilkeler, hayvanların bilimsel deneylerde etik kullanımına ilişkin temel rehberlik sağlar. Sentezlenen ilaç adayı bileşiklerin ve çeşitli kimyasalların test edilmesine yönelik araştırma ve biyolojik etki çalışmalarında hayvan deneylerine alternatif olabilecek teknikler geliştirilmiştir. İn vitro hücre kültürü teknikleri ve biyogörüntüleme yöntemleri, in vivo hayvan deneylerine çok önemli alternatiflerdir. Mikro-doz tekniği ise ilaç adayı bileşiklerin gönüllü bireyler üzerindeki etkilerini hayvan kullanılmadan çok düşük dozlarda ortaya koyan bir diğer alternatif yöntemdir. Yazılım veritabanları ve in silico bilgisayar simülasyonları da araştırma kurumlarında hayvan deneylerine alternatif olarak önem kazanmaktadır. Bu derlemede, hayvan araştırma etiğinin temelleri ve tıbbi deneylerde hayvan kullanımını azaltmak için geliştirilen alternatif yöntemler tartışılmıştır.
Anahtar kelimeler: Deney hayvanları; etik; alternatif yöntemler.

INTRODUCTION

Laboratory animals are used in basic biological and medical research, in the development of diagnosis and treatment methods of diseases, in the reliability testing of drugs and chemicals, and for educational purposes in the fields of biology and medicine. However, in these areas where animals are used without their consent, animals are often exposed to interventions that may cause pain, distress, or permanent damage, and this causes serious ethical problems and debates. According to scientific society, it is advocated that in the presence of scientific goals that have a high probability of being achieved and that can contribute to human and animal welfare in the long term, experimental studies on animals can be carried out in cases where there is no alternative method (1,2).

Reports of scientific experiments on animals have existed for years, but their primary use has become more frequent in recent years. Animal welfare was an issue that was not taken into consideration for many years while these experiments were ongoing (3). In recent years, interest in ethical principles has increased in the scientific community. Within the scope of the legislation regarding animal experiments enacted within the framework of animal protection laws in developed countries, national and international scientific journals do not publish studies that are not approved by local ethics committees (4).

In vitro cell culture techniques and bioimaging methods are very important alternatives to in vivo animal experiments. In this review, the fundamentals of animal research ethics and emerging alternatives to reduce animal use in medical experiments were discussed.

THE 4R PRINCIPLES

The basic principles of scientific ethics originated from the Universities Federation for Animal Welfare (UFAW) project and were first put forward by Russell and Burch in 1959; it is briefly defined as 3R, due to the initials of the terms, "reduction", "replacement", and "refinement". These defined basic principles explain the qualities that can guide researchers in animal experiments (5). The reduction principle, in its original definition in Russel and Burch's study, is expressed as "reducing the number of animals used to obtain a certain amount and precision of information" (6). In terms of animal ethics, reduction means obtaining reliable data by using the least number of animals possible in experiments. The term refinement refers to the comfort of experimental animals in their living environments during the period between birth and death and to ensure that they are exposed to minimal pain and suffering in the procedures applied during experiments. In its original definition, this principle is defined as "reducing the incidence of violence in inhumane procedures applied to animals that have to be used" (6). For each animal species, the optimal living conditions must be defined throughout the experimental period. Replacement refers to the use of alternative materials instead of experimental animals, if possible, in scientific research. This principle is defined as "the use of insensitive materials that can replace methods using conscious living vertebrates" (6). In 1985, The International Foundation for Ethical Research (IFER) created the principle of responsibility and developed the principles in the form of 4Rs. This principle envisages the use of animal models for scientific courses and studies, increasing the society's level of awareness and awareness about the use of animals in scientific research, education, and testing of products, and increasing personal responsibility by developing new methods and realizing new technologies (7). In accordance with this principle, individuals and institutions that will conduct research with experimental animals must comply with legal regulations and ethical rules.

ALTERNATIVE METHODS

Some techniques have been developed that can be alternatives to animal experiments in research and biological effect studies where it is necessary to test synthesized drug candidate compounds and various chemicals. These alternative methods include cell and tissue cultures, the use of microorganisms, the use of plant materials, and non-invasive models such as human clinical studies. In addition, physicochemical techniques, various software and computer simulations, mathematical models, and nanotechnological methods can also be used. These techniques are cost-effective compared to animal use and can effectively replace animal models. The fact that these alternatives can address ethical concerns is an important advantage (2).

In-vitro testing with Animal Cell Cultures

In vitro tests allow the effects of drug candidate compounds and chemicals on the organism to be examined in laboratory conditions as an alternative to animals by monitoring their effects in the in vivo environment. In this method, cells are isolated from the target organ and grown in special flasks to create a growth environment for a specific tissue type (8). It is advantageous that this technique is repeatable and more economical. It is also easy to maintain and follow. These tests also allow preliminary screening as to whether further experiments will be required. Thus, the efficacy and toxicity of a drug candidate compound can be determined by these in vitro tests (9,10). Experiments based on cell culture may reduce the use of animals, but serum obtained from the animal is required for the continuity of the cultures of these cells. Cell culture models are performed in a growth medium containing fetal calf serum. Fetuses removed from pregnant cows are used to obtain serum from fetal blood. Fetal calf serum is used in cell culture media as it is a rich natural medium and significantly increases cell growth. Alternatively, there are advanced studies on the ability to culture many cell lines in a chemically synthesized medium without the use of animal products. Additionally, chemically synthesized media may be more effective than animal serum media because animal serum varies depending on the gender and genetic makeup of the animals. However, at least the cell culture method is an important alternative in terms of reducing animal use, and this point makes this technique important (11,12).

3D Cell-Culture Models and Organs-On-Chips

As a result of the advancement of known classical cell culture techniques, tissue models can be created with 3D cell culture technology. 3D models of multiple organ systems and skin and muscle are produced in microfluidic channels (13). In 3D cell culture models, cells are grown as 3D spheroids or aggregates on a matrix scaffold or without a scaffold. 3D cell culture modeling conditions can

be modified through the addition of proteins and other factors present in a tumor microenvironment or specific tissues. The cellular environment can be accurately mimicked in vivo through extracellular matrix components such as proteins and glycosaminoglycans, which are found in the structure of these matrices and play a role in communication and cell-to-cell contact through intercellular signaling pathways. It is thought that this method will accurately reflect drug screening experiments and reduce the use of laboratory animals for research purposes (14).

In Silico Methods

Computer-based tests use various advanced software and mathematical equations to create imitations of the real functioning of organ functions and metabolic processes in the human body. In this way, drugs can be designed and verified by simulation on special computer models and software programs. More importantly, these simulation models are used to predict the toxicity level of an experimental drug candidate chemical without harming any animals (15). In simulation studies, any biological effect is shown in an equational form, providing more accurate and predictable results compared to data obtained from experimental animals. The most successful example in this regard is the design of protease inhibitors for HIV patients. In these studies, for a more precise verification, protease inhibitors were designed on computers, and the necessary tests were carried out in a simulation environment. In this way, successful results were obtained without using animal models in drug efficacy trials (16). Various computer models have been created to examine atherosclerotic plaque formation, which is an important risk in the cardiovascular system (17).

To be mentioned again, the most important point in bioinformatics simulations is that they significantly reduce the use of animals in biological effect assessment studies. In addition, computer models developed specifically for the field of anatomy may make it possible to reduce or completely eliminate the need for animal dissection for educational purposes (18).

Micro-Dosing

Micro-dosing testing is an effective method developed to test the effectiveness level and usability of candidate drugs in drug development studies. In this method, metabolism data of the human body is obtained in the analysis of the drug that is in the testing phase. Studies have shown that the high failure rate observed in phase I clinical trials, the long duration of the trials, and their high cost have increased the importance of this technique. Micro-dosing can screen targeted drugs more quickly, and economically. Additionally, the accuracy of predicting metabolic effects is also quite high. In this method, the effectiveness at the metabolic level is investigated by applying the compound to be tested at a dose that is high enough to affect the cellular level but relatively low. However, full dosing in animals is still needed to obtain definitive data for drug use in humans (19).

Imaging Methods

With technological developments in the field of radiology, it has become more possible to reveal the functions of body parts, the structures of internal organs, and possible disease diagnoses compared to previous years. It is also possible to examine the biological effects of drugs and the changes that occur with new radiological equipment (20). Since it is possible to image animals multiple times in these methods, animal welfare is protected by reducing the number of animals used in research. Non-invasive imaging technologies such as X-rays or ultrasound allow observation of the skeletal system and organ functions of living animals. Thus, disease mechanisms or treatment methods can be studied in real time without harming the animal. Imaging is smaller than devices used for humans and is widely used for research purposes with equipment adapted to animals. During the imaging procedure, anesthetic drugs are used, unlike humans, to ensure that the animal is immobilized so that they do not panic and suffer. (21). Among these methods, computed tomography is functional in investigating bone structure, magnetic resonance imaging is functional in examining internal organs and soft tissues, and ultrasound is functional in monitoring organ movements and heartbeat (21,22).

CONCLUSION

The use of animals in education and research still causes many disagreements due to ethical concerns. However, it is clear that the development of vitally important drugs and vaccines in medicine will continue to develop thanks to these experiments. Despite this, the benefit level of the studies to be carried out should still be taken into account because animals have the right to life and this should always be taken into consideration.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: İK, GD; Design: İK, GD; Data Collection/Processing: İK, GD; Analysis/Interpretation: İK, GD; Literature Review: İK, GD; Drafting/Writing: İK, GD; Critical Review: İK, GD.

REFERENCES

1. Festing MFW, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J. 2002;43(4):244-58.
2. Kiani AK, Pheby D, Henehan G, Brown R, Sieving P, Sykora P, et al. Ethical considerations regarding animal experimentation. J Prev Med Hyg. 2022;63(2 Suppl 3):E255-66.
3. Franco AL, Nogueira MNM, Kalatzis Sousa NG, da Frota MF, Fernandes CMS, da Costa Serra M. Animal research. A bioethical reflection. Acta Bioethica. 2014;20(2):247-53. Portuguese.
4. de Aguilar-Nascimento JE. Fundamental steps in experimental design for animal studies. Acta Cir Bras. 2005;20(1):2-8.
5. Forni M. Laboratory animal science: a resource to improve the quality of science. Vet Res Commun. 2007;31(Suppl 1):43-7.

6. Russell WMS, Burch RL (1959). The principles of humane experimental technique - Special edition published by Universities Federation for Animal Welfare (UFAW), London, UK: Methuen & Co.; 1992.
7. Olfert ED, Cross BM, McWilliam AA. Guide to the care and use of experimental animals, vol 1. 2nd ed. Ottawa, Ontario: Canadian Council on Animal Care; 1993.
8. Pizzoferrato A, Ciapetti G, Stea S, Cenni E, Arciola CR, Granchi D, et al. Cell culture methods for testing biocompatibility. Clin Mater. 1994;15(3):173-90.
9. Shay JW, Wright WE. The use of telomerized cells for tissue engineering. Nat Biotechnol. 2000;18(1):22-3.
10. Steinhoff G, Stock U, Karim N, Mertsching H, Timke A, Meliss RR, et al. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits: in vivo restoration of valve tissue. Circulation. 2000;102(19 Suppl 3):III50-5.
11. Sharma S, Bhatt M, Dahiya N, Gupta AK, Brashier DBS, Sharma AK. Alternatives to animal experiments: an overview. Asian J Pharm Toxicol. 2015;3(7):1-6.
12. Rai J, Kaushik K. Reduction of animal sacrifice in biomedical science & research through alternative design of animal experiments. Saudi Pharm J. 2018;26(6):896-902.
13. Huh D, Torisawa YS, Hamilton GA, Kim HJ, Ingber DE. Microengineered physiological biomimicry: organs-on-chips. Lab Chip. 2012;12(12):2156-64.
14. Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol. 2014;12(4):207-18.
15. Roncaglioni A, Benfenati E. In silico-aided prediction of biological properties of chemicals: oestrogen receptor-mediated effects. Chem Soc Rev. 2008;37(3):441-50.
16. Arora T, Mehta AK, Joshi V, Mehta KD, Rathor N, Mediratta PK, et al. Substitute of animals in drug research: an approach towards fulfilment of 4R's. Indian J Pharm Sci. 2011;73(1):1-6.
17. Washio T, Okada J, Takahashi A, Yoneda K, Kadooka Y, Sugiura S, et al. Multiscale heart simulation with cooperative stochastic cross-bridge dynamics and cellular structures. Multiscale Model Sim. 2013;11(4):965-99.
18. Azer SA, Azer S. 3D anatomy models and impact on learning: a review of the quality of the literature. Health Prof Educ. 2016;2(2):80-98.
19. Tewari T, Mukherjee S. Microdosing: concept, application and relevance. Perspect Clin Res. 2010;1(2):61-3.
20. Hussain S, Mubeen I, Ullah N, Shah SSUD, Khan BA, Zahoor M, et al. Modern diagnostic imaging technique applications and risk factors in the medical field: A review. BioMed Res Int. 2022;2022:5164970.
21. Tremoleda JL, Sosabowski J. Imaging technologies and basic considerations for welfare of laboratory rodents. Lab Anim (NY). 2015;44(3):97-105.
22. Lauber DT, Fülöp A, Kovács T, Szigeti K, Máthé D, Szijártó A. State of the art in vivo imaging techniques for laboratory animals. Lab Anim. 2017;51(5):465-78.

AUTHOR GUIDELINES

SCIENTIFIC RESPONSIBILITY

In terms of scientific publishing standards, articles to be submitted should be prepared in accordance with the criteria of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME) and the Committee of Publication Ethics (COPE).

- All articles must be complied with the research and publication ethics. The responsibility of the articles belongs to the authors.
- Articles are required to have not been published in anywhere previously, and/or are not in the evaluation process for publication.
- Articles must be submitted with the Copyright Transfer Form signed by all authors to begin the evaluation process. For authors' order, the signature order in the Copyright Transfer Form is based on.
- The corresponding author is responsible for the final version of the article on behalf of all authors.

ETHICAL RESPONSIBILITY

- Compliance with The Principles of Helsinki Declaration (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) is required in all studies including "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken the approval from ethics committee of their institution and the "informed consent" from people participating the study, in the MATERIAL AND METHODS section.
- If "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and they have taken the approval from ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information of the ethics committee approval should be indicated together with the name of the committee, approval date and number, in the MATERIAL AND METHODS section.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
- The authors are responsible for reporting all personal and financial relationships that may be related with the study. It is necessary to state clearly whether there is any conflict of interest related to the submission and/or evaluation of the article.
- Compliance of the articles with the scientific and ethical rules is responsibility of authors.

SUBMISSION FILES

Articles must be uploaded to the system as separate files as described below.

Copyright Transfer Form: The Copyright Transfer Form to be obtained from the system during the submission must be signed by all authors in accordance with the authorship order in the article.

Cover Letter: Type of the article, the statement that has not been published previously in anywhere before, and/or not in the evaluation process for publication, if any, the people and institutions supporting the study financially and the relationship of these institutions with authors (if not, there is no relationship) must be stated. The names, academic titles, institutions, contact information and e-mail addresses of at least two reviewers suggested in relation to the subject of the article and not related to the authors and their institutions should be written. Editors' right to choose the reviewers are reserved.

Title Page: It must include the title of article (English and Turkish), short title not exceeding 40 characters, names, academic titles, ORCID® numbers, institutions, e-mail addresses of all authors, and also name, correspondence address, phone number, email address of the corresponding author. If the article has been presented previously in a scientific meeting; the name, date and place of the meeting (if not, not presented) should be stated.

Main Text: The title of the article (English and Turkish), short title not exceeding 40 characters, Abstract (English and Turkish), Keywords (English and Turkish), Main Text (sectioned according to the type of article submitted), References, Tables and Figures should be included.

Ethics Committee Approval Document: Ethics Committee Approval Document should be uploaded as a separate file for all research articles.

Note: If there are figures, pictures or photographs in the article, each of them must be uploaded as separate files.

SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading) and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be upload as separate files in .png, .jpg, etc. format and at least 300 dpi resolution.
- Captions of figure and photograph should be given on a separate page respectively, after the page including last table.
- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

- If any conflict of interest, financial support, donation and other editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES

- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

DÜZCE
ÜNİVERSİTESİ

Contact / İletişim

Düzce Üniversitesi Tıp Fakültesi Konuralp Yerleşkesi, Düzce
e-mail: duzcetipdergisi@duzce.edu.tr
web: https://dergipark.org.tr/en/pub/dtfd