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Experimental Inflammation Models Created in Laboratory Animals

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Abstract: Inflammation is the organism's vascular, humoral, and cellular response against various endogenous and exogenous harmful effects. Inflammation is a physiopathological process that both removes the causes of cell injury and starts tissue restructuring and healing by eliminating necrotic cells and tissues resulting from cellular injury. Inflammation has two phases which are acute and chronic. In the short acute phase, it is seen that leukocytes accumulate in the area of inflammation and mediate the secretion of chemical mediators in addition to vascular changes such as increased permeability and exudation of protein-rich fluid to extravascular tissues. In the longer chronic phase, vascular changes, edema, and massive neutrophil infiltration occur. Many steroid and non-steroid anti-inflammatory drugs are widely used in the treatment of inflammatory diseases in the world, and many of them have significant adverse effects. They cause serious adverse effects, especially in long-term users. Therefore, safer alternative drugs are needed. Experimental animal models are used in anti-inflammatory effects studies. The most commonly used model among them is paw edema in rats with carrageenan, which measures the effects of drugs on acute inflammation. In this study, information was given related to the experimental animal models used to investigate the effects of drugs on acute and chronic inflammation.

Keywords: Acute, Chronic, Experimental animal models, Inflammation, Rat.

Laboratuvar Hayvanlarında Oluşturulan Deneysel İnflamasyon Modelleri

Öz: İnflamasyon endojen ve eksojen çeşitli zararlı etkilere karşı organizmanın gösterdiği vasküler, hümoral ve hücresel yanıttır. İnflamasyon hem hücre zedelenmesinin nedenlerini ortadan kaldıran hem de hücresel zedelenme sonucu oluşan nekrotik hücreleri ve dokuları ortamdan uzaklaştırarak dokunun yeniden yapılanmasını ve iyileşmesini başlatan fizyopatolojik bir süreçtir. İnflamasyonun akut ve kronik iki fazı vardır. Kısa süren akut fazda; permeabilite artışı ve ekstravasküler dokulara proteinden zengin sıvı eksüdasyonu gibi vasküler değişikliklerin görülmesinin yanında, lökositlerin inflamasyon alanında birikip kimyasal mediyatörlerin salgılanmasına aracılık ettikleri de görülür. Daha uzun süren kronik fazda; vasküler değişiklikler, ödem ve büyük miktarda nötrofil infiltrasyonu gerçekleşir. Dünyada inflamatuvar hastalıkların tedavisinde oldukça yaygın kullanılan steroid ve nonsteroid yapıda çok sayıda antiinflamatuvar ilaç mevcuttur ve bunların birçoğunun da önemli yan tesirleri vardır. Özellikle uzun süre kullananlarda ciddi yan tesir oluştururlar. Dolayısıyla daha güvenli alternatif ilaçlara ihtiyaç vardır. Antiinflamatuvar etki araştırmalarında deneysel hayvan modelleri kullanılır. Bu modellerden en sık kullanılanı ilaçların akut inflamasyondaki etkilerini ölçen karagenin ile sıçanlarda oluşturulan pençe ödemidir. Bu çalışmada ilaçların akut ve kronik inflamasyondaki etkilerini araştırmak için kullanılan deneysel hayvan modelleri hakkında bilgi verilmiştir.

Anahtar Kelimeler: Akut, Deneysel hayvan modelleri, İnflamasyon, Kronik, Sıçan.

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INTRODUCTION

Inflammation is the organism's vascular, humoral, and cellular response against various endogenous and exogenous harmful effects. Inflammation is a physiopathological process that both removes the causes of cell injury and starts tissue restructuring and healing by eliminating necrotic cells and tissues resulting from cellular injury (1). Inflammation is caused by various stimuli. They are trauma, surgical interventions, infectious agents and their toxic products, physical factors such as extreme heat and cold, caustic chemicals, immune response, and tissue ischemia (2,3).

Inflammation consists of two main phases. Acute inflammation is short-term, ending in a few minutes to a few days. It is observed with redness, warmth, pain, swelling, and loss of function. It is characterized by vascular and cellular changes. Vascular changes begin right after getting injured. Arterioles and venules dilate, and blood flow increases. It causes increased permeability in the earliest phase of inflammation and exudation of protein-rich fluid into extravascular tissues. One of the important events in acute inflammation is the significant accumulation of leukocytes at the injury site. Here, neutrophils are dominant in the first 6 to 24 hours, while this picture changes in favor of monocytes in the following 24 to 48 hours. Leukocytes in the area of inflammation kill microorganisms in this area, break up necrotic tissue and foreign antigens, and release chemical mediators, causing tissue damage. Plasma and chemical mediators of cellular origin, such as vasoactive amines, plasma proteases, arachidonic acid metabolites, and cytokines, are responsible for inflammation symptoms and tissue damage (4-7).

Chronic inflammation is considered a long-term (weeks, months, years) inflammation in which active inflammation and healing processes occur together. Vascular changes are characterized by edema and massive neutrophil infiltration. Chronic inflammation cells are macrophages, lymphocytes, and plasma cells. Macrophages activated secrete a large number of mediators. These mediators produce tissue destruction, angiogenesis, and fibrosis, which are the characteristic features of chronic inflammation. Granulomatous inflammation is a different pattern of chronic inflammation. It is characterized by a cluster of activated macrophages that resemble an enlarged squamous epithelial cell (5-9).

Steroid anti-inflammatory drugs and non-steroid anti-inflammatory drugs (NSAIDs) are the most commonly used drugs with anti-inflammatory activity in treating inflammatory diseases.

Steroids show their anti-inflammatory effects by inhibiting;

-Phospholipase A2 activity and synthesis of prostaglandins, thromboxanes, and leukotrienes,

-Synthesis of binding integrin molecules on the surface of leukocytes, endothelial adhesion molecule-1, and intracellular adhesion molecule-1,

-Acute phase reactants synthesis,

-Synthesis and release of cytokines,

-Expression of TNF-alpha and interleukin-1 genes (10-12).

NSAIDs have analgesic and antipyretic effects as well as anti-inflammatory effects. However, their anti-inflammatory activity is weaker than glucocorticoids, and their analgesic activity is weaker than narcotic analgesics. The anti-inflammatory mechanism of action of this group of drugs has not been fully elucidated. It is because that inflammation occurs from a wide variety of events, and meanwhile, many mediators and endogenous modulatory substances that play an important role in inflammation are released. Reduction prostaglandin synthesis, IL-1, and IL-6 production and secretion, activation of neutrophil leukocytes, the release of lysosomal enzymes, and formation of active oxygen radicals by inhibiting the cyclooxygenase enzyme are their most accepted mechanisms (13-16).

Anti-inflammatory drugs are widely used in the world, and there are a large number of anti-inflammatory drugs. However, they have significant adverse effects; especially when used for a long time,

they create serious side effects. Thus, there is a need for safer, stronger, and more economical alternative drugs. The purpose of this review is to give information about paw edema models, auricular edema models, peritoneal vessel permeability increase test induced by acetic acid, pleurisy test, cotton pellet test used in chronic inflammation, and formalin-induced paw edema test used to investigate the effects of drugs on acute inflammation.

EXPERIMENTAL ANIMAL MODELS IN INFLAMMATION

1.Models of Acute Inflammation

These models are those that investigate the anti-inflammatory effects of many natural and synthetic substances in acute inflammation. In acute inflammation models, edema formation is followed by various measures of change in volume, thickness, or weight of the inflamed organ. At the same time, it is also examined by measuring the permeability of staining that is administered intravenously and has plasma protein binding properties in the inflamed area. Sprague-Dawley rats, Wistar rats, and Swiss albino mice are the most commonly used experimental animals. Generally, male animals are preferred so that the hormonal cycle in female animals does not affect the experiment. However, if desired, equal numbers of male and female animals can also be used.

In the experiments, carrageen, histamine, serotonin, bradykinin, dextran, lipopolysaccharide, arachidonic acid, croton oil, oxazolone, and acetic acid are used as inflammatory agents (17,18).

1.1 Carrageenan-Induced Paw Edema

This model is the most commonly used to measure the anti-inflammatory effect in acute inflammation. Carrageen is a phlogistic agent with no antigenic properties and no obvious systemic adverse effects (17). Carrageenan injection initiates the early phase of inflammation by stimulating the phospholipase A2 enzyme, while sulfated sugars in their chemical structure activate the complement system and inflammatory mediators. In this process, postcapillary venules dilate, which results in the exudation of fluid and cells into the extravascular tissues. edema caused by carrageen is initially due to injection trauma, the release of mediators such as histamine and serotonin, while the Phase 2 reaction that occurs 3 hours later is due to cyclooxygenase activation and prostaglandin synthesis. The mentioned model is a very suitable one for evaluating drugs that have anti-inflammatory effects by inhibiting cyclooxygenase. Carrageen can cause local inflammation where it is administered with a single dose subcutaneous injection without creating a systemic effect (18). Sprague-Dawley and Wistar rats are most commonly used as experimental animals in carrageen and other paw edema models. Before starting the experiment, animals should be housed for at least one week in a 12-hour light and 12-hour dark environment, at normal room temperature (22 °C), and fed with standard feed and water to allow them to get used to the environment. In addition, if paw edema is to be measured with a hydro-plethysmometer, researchers should also receive training on this subject. Before starting the experiment, animals are weighed and their weights determined. If the anti-inflammatory effect of a single substance will be considered, animals are divided into 5 groups.

1. The control group, physiological saline was given
2. Anti-inflammatory group, NSAIDs was given
3. The 4th and 5th groups are the groups in which three different doses of the substance for its anti-inflammatory effect are tested.

After the rats are divided into 5 groups, their normal right hind paw volumes are recorded by measuring with a hydro-plethysmometer. Thirty minutes after the substance is administered intraperitoneally and whose anti-inflammatory effect is investigated, and 1 hour after the substance is used orally, acute inflammation is created by injecting 0.1 ml of 1% carrageen solution prepared with saline or distilled water into the subplantar region of the right hind paws of animals in all groups. The volume increase caused by the carrageenan is

measured with a hydro-plethysmometer 5 times in one hour periods one hour after the carrageenan injection. Thus, both the maximum effect 3 hours after the injection of carrageenan and the course of inflammation are monitored (18,19).

1.2 Histamine-Induced Paw Edema

It causes an increase in permeability at the level of postcapillary venules by shrinking endothelial cells through histamine H1 receptors, widening the spaces between cells. As a result, plasma fluid and proteins leak into the extracellular space, and edema occurs. However, edema is minimal and temporary. It shows a proinflammatory effect by increasing the release of inflammatory cytokines and lysosomal enzymes from macrophages via histamine H1 receptors and activating basophil and eozinophilic leukocytes and fibroblasts (20). Histamine-induced paw edema is a suitable method to evaluate, especially the acute anti-inflammatory effects of drugs that act by inhibiting histamine. It is not suitable for evaluating drugs that act on prostaglandins without inhibiting the effect of histamine. This model is also used to confirm the efficacy of agents acting on phase 1 of carrageenan-induced paw edema. To create paw edema, 0.1% solution of histamine of 0.1 ml prepared with distilled water is injected into the subplantar region of the right hind paw of the animals. The volume increase caused by histamine is measured 30 minutes after the injection and 6 times in 30-minute periods (21).

1.3 Bradykinin-Induced Paw Edema

Since bradykinin stimulates the phospholipase A2 enzyme, bradykinin-induced paw edema is mediated by prostaglandins. Paw edema induced by bradykinin is mild and short-term (22).

1.4 Lipopolysaccharides-Induced Paw Edema

Lipopolysaccharides cause paw edema by increasing TNF-alpha and interleukin-1 expression and activating myeloperoxidase. This model is used to evaluate the anti-inflammatory activity of drugs that inhibit the activity of cytokines such as TNF-alpha and interleukin-1 (23).

1.5 Dextran-Induced Paw Edema

Administration of dextran to the rat paw causes the release of mediators such as histamine and serotonin in that area and increases vascular permeability.

Paw edema is caused by administering 0.1 ml of 1% dextran (24,25).

Measuring the Paw Edema

a- Measurement with Hydro-Plethysmometer

A hydro-plethysmometer device is used to measure paw edema in acute inflammation models. They are commonly used sensitive devices with a low margin of error. In order to reduce the measurement error, researchers need to be trained in using the device beforehand. Before the drugs are given, the right hind paw volumes of the experiment animals are measured and recorded. After the drugs are given, and edema occurs (1 hour after carrageenan, 30 minutes after histamine), right hind paw volumes are measured again, and the first measurements are compared with the measurements obtained. The inflammation and anti-inflammatory effect in the paws are determined according to the formulas below. The amount of inflammation in the paws was calculated for each subject with the following formula;

Percent inflammation rate (%) = (Vt – V0) / V0 × 100

V0: Paw volume before injections of inflammatory substances such as carrageenan or histamine (ml)

Vt: Paw volume (ml) t hours after injections of inflammatory substances such as carrageenan or histamine

The anti-inflammatory effect rates of the drugs were calculated with the following formula by taking the average of the % increase compared to the normal paw volumes of the rats in the groups;

Anti-inflammatory effect (%) = [1-(D / C)] x 100

D: average % increase in rat paw volume in the drug group

C: average % increase in paw volume of rats in the control group (19).

b- Measuring the Paw Weight

After the study, the animal of the experiment is sacrificed, and both hind paws are cut off over the lateral malleolus, removed, and weighed. The right hind paw is used to study the anti-inflammatory effect, and the left hind paw is used for control. The anti-inflammatory effect is calculated by taking the weight differences between both paws. It has no advantage over the measurement made with a plethysmometer, it is used less frequently, and it is a method that is not considered ethically appropriate (26).

1.6 Arachidonic Acid-Induced Auricular Edema

It is a test used to evaluate substances having the potential to be used to treat inflammatory diseases of the skin. Topically-applied arachidonic acid rapidly converts to cyclooxygenase and lipoxygenase products, which cause local erythema, edema, and neutrophil accumulation at the application site. Therefore, the reduction of edema is due to inhibition of the synthesis of cyclooxygenase and lipoxygenase products (27).

1.7 Oxazolone-Induced Auricular Edema

Oxazolone is an allergenic substance that causes delayed-type hypersensitivity and increases the CD8+ lymphocyte count. Topically applied oxazolone causes an increase in arachidonic acid metabolites such as prostaglandins and leukotrienes and interferon and produces local inflammation (28).

1.8 Craton Oil and TPA-Induced (12-O-tetradecanoylphorbol-13-acetate) Auricular Edema

It is a model that evaluates the effects of systemic and locally-used steroids and NSAIDs. Topically applied craton oil causes histamine and serotonin secretion, increased vascular permeability, neutrophil migration, and increased synthesis of eicosanoids. PGI2 and LTB4 are eicosanoids responsible for increased vascular permeability in the TPA-induced inflammation model. Substances that inhibit cyclooxygenase and lipoxygenase pathways also inhibit TPA-induced inflammation (18,29).

Measuring Auricle Volume

Auricular thickness is measured by measuring 0.01 mm unit length by means of an Oditest calipers or thickness gauge device. Before and after the study, the right and left ear thicknesses are measured with this device and subtracted from each other, and the resulting edema volume is calculated (24,25).

1.9 Acetic Acid-Induced Peritoneal Vascular Permeability Increase Test (Whittle Method)

It is a model developed by Whittle. It is a model in which substances that have the potential to inhibit the effects of phlogistic agents such as acetic acid, which increases vascular permeability and causes plasma proteins and fluid to leak out of the vessel, are evaluated. In the experiment, a model is created by giving IV after acetic acid intraperitoneally administration and staining substances such as 1% sodium thioglycolate, Pontamine Sky Blue, or Evans Blue, which have a high binding capacity to plasma proteins. The degree of penetration of these stainings from the vessels into the peritoneal fluid is measured by a spectrophotometer. In addition, data on macrophage migration is obtained by counting the macrophages in the fluid samples (30,31).

1.10 Pleurisy Model

Pleurisy represents an exudative inflammation. Inflammation is induced by injecting solutions such as carrageenan and dextran into rats through the 3rd to 5th intercostal space. In carrageenan-induced pleurisy, it is possible to measure fluid extravasation, leukocyte migration, and biochemical parameters in post-study pleural fluid and evaluate its anti-inflammatory activity (32).

2. Subacute Inflammation Models

2.1 Subcutaneous Air Sac Model

In the repair phase of inflammation, the proliferation of endothelial cells and fibroblasts penetrating the inflammatory fluid forms the granulation tissue. In subacute inflammation models, the aim is to measure the capacity of anti-inflammatory agents to inhibit granuloma tissue. In this model, air sacs are formed by injecting 20 ml of air under the skin in the back area of the experimental animal. One day later, an irritant substance such as carrageenan or a 1% solution of croton oil in 0.5 ml sesame oil is injected into the sacs by preventing air leakage and creating inflammation. After injecting the irritant substance, the substance to be measured for its anti-inflammatory effect is given to the animals for five days. At the end of the 5th day, the backs of the animals are opened under anesthesia, and the fluids formed in the air sacs are collected and compared with the control group. The anti-inflammatory effect is calculated, and biochemical analyzes are performed (33).

3. Chronic Inflammation Patterns

3.1 Cotton Pellet Granuloma Test

It is the most commonly used test to evaluate the efficiency of anti-inflammatory drugs considered effective in the chronic phase of inflammation. Animals are anesthetized with 25mg/kg thiopental sodium 30 minutes after administering the anti-inflammatory agent to be tested. Cotton pellets prepared under sterile conditions, weighing 10 ± 1 mg and autoclaved at 120 °C for 30 minutes, are placed under the skin in the interscapular region of the animals under anesthesia. Following this operation, the anti-inflammatory drug is appropriately given to the animals for one week. On the eighth day, the animals are sacrificed, and the cotton pellets surrounded by granuloma tissue are carefully removed. The extracted pellets are wet-weighed and recorded. The wet weight of the pellets is related to the amount of transudate. Then, the cotton pellet and the granuloma tissue formed around it are dried together at 60°C for 18 hours and weighed. Dry weights are associated with the formation of granulomatous tissue. The degree of chronic anti-inflammatory effect is measured by comparing the weights of cotton pellets in all study groups.

Exudate inhibition (%) = (1 – Exudate in treated group/ Exudate in controls) x 100.

Granuloma inhibition (%) = (1 - Granuloma in the treated group/granuloma in the control group) x 100 (34).

3.2 Formalin-Induced Paw Edema

This pattern closely resembles human arthritis. It is a suitable model for measuring the chronic anti-inflammatory effects of various substances.20 μL of formalin (2%) is injected into the subplantar region of the right hind paws of animals. Formalin-induced oedema, which causes severe irritation and pain in experimental animals, is biphasic. While the early neurogenic phase is associated with p-substance and bradykinin, the later inflammatory phase is associated with serotonin, histamine, prostaglandin, and bradykinin (18,26).

CONCLUSION

Inflammatory diseases are common diseases in the world. NSAIDs are more effective in acute inflammation, and steroids are more effective in chronic inflammation. However, both drug groups have significant adverse effects. Thus, there is a need for safer and more effective drugs. In many parts of the world, natural herbal substances are used in the treatment of inflammatory diseases, as well as existing drugs. In evaluating these substances and available drugs, the experimental animal models of inflammation described in this review remain valuable.

Conflict of interest

The author declare that they have no conflict of interest.

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