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DERLEME MAKALESİ **REVIEW PAPER**

An Innovative Approach in The Field of Health: Nanoparticles/Nanomedicine

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Abstract: Since diseases began to play a role in human history, people have sought ways to heal and prevent disease. This struggle started in hunter-gatherer communities that lived tens of thousands of years ago and has survived to the present day. Nanotechnology is one of the current stops of today's modern medicine, which includes fine techniques that people with the mission of healing diseases in ancient times cannot even imagine. In this review, nanoparticles, which is product of nanotechnology, are classified according to various methods and the methods used during their preparation are mentioned. The underlying are principles of nanoparticles being used as drug delivery, imaging and vaccine adjuvants, and toxicity of nanoparticles have been investigated. Some of the nanoparticle applications that currently used in veterinary medicine and have the potential to be applied in the future are also mentioned.

Keywords: Nanoparticles, nanotechnology, vaccine delivery.

Sağlık Alanında Yenilikçi Bir Yaklaşım: Nanopartiküller/Nanotıp

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Öz: Hastalıklar insanlık tarihinde rol oynamaya başladığından beri insanlar hastalıkları iyileştirmenin ve hastalıklardan korunmanın yollarını aramışlardır. Bu mücadele on binlerce yıl öncesinde yaşamış olan avcı-toplayıcı topluluklarda başlamış, günümüze kadar gelmiştir. Eski çağlarda hastalıkları iyileştirme misyonunu yüklenmiş insanların hayal dahi edemeyeceği ince teknikleri barındıran günümüz modern tıbbının gelmiş olduğu güncel duraklardan birisi de nanoteknolojidir. Bu derlemede bir nanoteknoloji ürünü olan nanopartiküller çeşitli metotlara göre sınıflandırılmıştır ve hazırlanmaları sırasında kullanılan yöntemlerden bahsedilmektedir. Nanopartiküllerin ilaç iletimi, görüntüleme ve aşı adjuvanları olarak kullanılabilmelerinin altında yatan prensipler ile birlikte nanopartiküllerin toksisitesi konuları incelenmiştir. Nanopartiküllere yönelik veteriner hekimlikte günümüzde kullanılmakta olan ve gelecekte uygulanma potansiyeli bulunan uygulamalardan bazılarına da değinilmiştir.

Anahtar kelimeler: Aşı iletimi, nanopartiküller, nanoteknoloji.

INTRODUCTION

The goal of humanity since ancient times has been to protect themselves against life-threatening diseases. Accordingly, everything started with the thought that the Indians and Buddhists believe, anyone who drinks snake venom can protect themselves from being poisoned. Following the lead of Edward Jenner's idea that cowpox

should protect people from smallpox, a new era was opened and the field of medicine became closely interested in the concept of improving immunity against potentially deadly diseases (Plotkin et al., 2013). However, with the spread of infectious diseases, the need of developing new effective vaccines emerged. Most vaccines are formulated to mimic pathogens, thereby stimulating an individual's immunity to disease. Nevertheless, vaccine development has not always been such a simple task. Therefore, many studies have been initiated to obtain detailed information on the recognition of the immune system against antigens, which has led to the synthesis of many vaccines with antibody and cell-mediated protection mechanisms (Li et al., 2013).

The impact of mass vaccination practices on global health has been great. Thanks to this, infectious diseases such as smallpox have been eliminated, and diseases like polio and diphtheria have become less life-threatening. Despite the overall success of vaccination, there is still an urgent need to develop new vaccines against diseases such as cancer, tuberculosis and malaria that cause deaths globally. It is also necessary to develop conventional vaccines for populations with immune system disorders. In vaccine development studies, there has been a shift in direction from whole cell vaccines and live attenuated vaccines to safer subunit vaccines. However, new vaccine candidates alone cannot produce an adequate immune response. Therefore, adjuvants are required that can enhance, accelerate and/or prolong the specific immune response. Vaccines designed to produce a long-lasting and protective antibody response against pathogens primarily consist of antigen and adjuvant. Conventionally, antigens have been obtained from inactivated microorganisms. However, this situation has started to decrease and nowadays synthetic peptides and recombinant proteins have become preferred because they are safer (Nordly et al., 2009).

Structures considered as new vaccine candidates are generally weakly immunogenic and susceptible to degradation. Therefore, they need new adjuvants that optimize their immunogenicity. These novel nanoparticle-based adjuvants can be designed to reduce dosage frequency by an appropriate route of administration to induce a specific immune response (Scheerlinck et al., 2006; Wang et al., 2011).

Nanotechnology is a science that has its origins in precision engineering. Nano is a Latin word meaning dwarf, and the idea of nanotechnology was first proposed by Nobel laureate physicist Richard Feynman in 1952 in Southern California (Kakade, 2003; Buzea et al., 2007). Feynman, at conferences long before nanotechnologies were designed, pointed out that objects consisting of only a few atoms are small enough to affect the physical properties of quantum mechanics, and the importance of miniaturization in this context. The famous quote he said in 1959, "There's plenty of room down there", also draws attention to this issue.

According to the definition of the International System of Units (Système international d'unités, SI), a nanometer (nm) is a unit of length 10^{-9} meters long. In general, nanomaterials (NM) are defined as materials with at least one dimension, 1-100 nm in diameter, 1-1000 nm in length. Today, the European Union (EU) and the United States of America (USA) have different definitions of NMs and there is no single internationally accepted definition. It is known that different organizations also have disagreements on the definition of NMs (Boverhof et al., 2015; Jeevanandam et al., 2018). For over 30 years, nanoparticles have been defined as ordered structures with diameters less than 1000 nm (Brigger et al., 2002). However, a definition has been made recently by the British Standards Institute. Nano objects with three external nanoscale dimensions are defined as nanoparticles (BSI, 2011). When the longest and shortest axis lengths of a nano-object are different, the terms nanorod or nanoplate can be used instead of nanoparticle (NP) (Jeevanandam et al., 2018).

In the literal sense, the term nanotechnology was popularized by Eric Drexler in the 1980s. Nanotechnology is the technology of experimenting and manipulating particles called nanoparticles (Verma et al., 2012). Nanotechnology offers the opportunity to design nanoparticles on parameters that vary in composition, size, shape and surface properties for use in fields such as medicine and veterinary medicine (Moghimi et al., 2005; Couvreur & Vauthier, 2006). Due to their similarity in size to cellular components, nanoparticles can enter living cells by the cellular endocytosis mechanism, especially using pinocytosis (Treuel et al., 2013). Nanoparticles have revolutionized the diagnosis of diseases, the use of biological-active substances in preventive medicine or the treatment of diseases. The emergence of virus-like particles (VLPs) and the re-understanding of the importance of nanoparticles such as quantum dots and magnetic nanoparticles indicates the convergence of protein biotechnology with inorganic nanotechnology, which promises a significant advance for the field of nanomedicine (Pankhurst et al., 2003; Tissot et al., 2008). Approved nanotechnology vaccine and drug delivery systems highlight the revolution in disease prevention and treatment (Maurer et al., 2005; Couvreur & Vauthier, 2006; Roldão et al., 2010).

CLASSIFICATION OF NANOPARTICLES

There are many classifications of nanoparticles depending on their origin, shape, structure and application purpose. They are classified under four main categories: according to their structure and usage, origin, shape and various other classifications (Table 1).

Table 1. Classifications of nanoparticles (El-Sayed & Kamel, 2018).

Classification	Nanoparticles
According to their structure and usage	Polymeric Nanoparticles
	Liposomes
	Fullerenes and Bucky tubes
	Microbivores and Respirocytes
	Nano Shells
	Quantum Dots
	Solid Lipid Nanoparticles
	Magnetic Iron Oxide Nanoparticles
	Dendrimers
	Nano Emulsion
	Nano Bubbles
	Aluminosilicate Nanoparticles
	Polymeric Micelles
	Polymer-coated Nanocrystals
	Polymeric Nanospheres
	Metallic Nanoparticles
	Ceramic Nanoparticles
According to their origin	Inorganic Nanoparticles
	Organic Nanoparticles
	Hybrid Nanoparticles
According to their shape	They are classified as spheres, tubes or drops depending on the application purposes such as therapeutic, diagnostic, vaccine administration, nutrition
Various other classifications	Structures that strengthen the immune system
	Virus-like particles
	Self-assembling structures/proteins

NANOTECHNOLOGY APPLICATIONS

Nanotechnology is seen as an area that can provide solutions to numerous problems in terms of improving animal health and production.

Nanoparticles as Drug Delivery Systems: The use and efficacy of many currently available pharmacological agents are limited by their low bioavailability and undesirable side effects. Approximately 95% of all potential therapeutic agents have poor bioavailability and pharmacokinetics (Brayden, 2003). Nanoparticle-based drug delivery systems can be designed to overcome such problems and improve the therapeutic index and safety profile of the substances transported by these systems. Nanoparticles have shown remarkable efficacy in many studies targeting the delivery of antimicrobials (Cordeiro et al., 2000), analgesics (Rose et al., 2005), anti-inflammatory (Metselaar et al., 2003) and anti-neoplastic agents (Hofheinz et al., 2005). Until about 10 years ago, there are at least 30 nanoparticle-based therapeutic products and more than 200 nanoparticle drug delivery systems, which were approved for clinical use in humans and are still in clinical studies (Wagner et al., 2006; Bawa, 2008). Today, it is known that there are at least 50 nanoparticle-based therapeutic products approved for clinical use in humans, and this number is increasing. Many of these therapeutics are prohibitively expensive for use in veterinary medicine, but several nanoparticle formulations are currently available in the veterinary field. As nanoparticle production facilities develop, these therapeutics will become more suitable for use in the veterinary field (Sainz et al., 2015; Ventola, 2017; Agrahari & Agrahari, 2018).

Nanoparticles improve the therapeutic index of the pharmaceutical agents they carry through four key mechanisms. First, they ensure the use of drugs that would be insoluble or unstable under other conditions when used. Secondly, they increase the concentration of the pharmaceutical at the site of action targeted by the pharmaceutical, resulting in greater efficacy. Thirdly, they reduce systemic toxicity and drug concentration in healthy tissues, primarily because the accumulation occurs at target sites. Lastly, the lower clearance of nanoparticles compared to the parent drug allows nanoparticles to be used as a sustained and controlled release method over long periods of time (Sahoo & Labhasetwar, 2003; Bakker-Woudenberg et al., 2005; Fahmy et al., 2005).

As a result of these mechanisms, nanoparticle formulations require a lower dose compared to the free drug. This is especially true in veterinary medicine in two ways: It can allow the use of expensive human drugs that were previously impossible due to the high cost of dosing, and reduces the levels of residues first directly in the carcass, then indirectly in the environment and food. To create a successful drug delivery system, nanoparticles must be loaded with a sufficient amount of pharmaceutical agent, transport it to the target tissue, and then release the pharmaceutical agent at the target site. Nanoparticles can be loaded with drugs by encapsulating the drug into the particle or by attaching the drugs to the surface of the particle (Lu et al., 2007). The method of drug loading depends on the nanoparticle type, the type of drug and the target. When a drug is loaded into a nanoparticle, the drug takes on the external properties of the nanoparticle until the particle is destroyed or the drug is released to its target. The targeting of nanoparticles to specific sites is carried out passively (based on the fundamental properties of the nanoparticle) and/or actively (by binding a functional group or part of the targeted molecule to the nanoparticle). The archetypal mechanism of passive targeting is achieved by the "enhanced permeability and retention (EPR) effect" demonstrated by nanoparticles almost ubiquitously due to their small size (Maeda, 2010). This effect is based on the ability of intravenous (IV) nanoparticles to extravasate in regions of increased vascular permeability, but if they are not extravasated, the nanoparticles remain in circulation. Due to this effect, nanoparticles accumulate in areas of increased vascular permeability such as areas of inflammation, tumors, and infections. In other words, this effect causes the active substances carried by the nanoparticles to target these regions (Ishihara et al., 2010).

With the involvement of the reticuloendothelial system, the nanoparticles are first opsonized and then taken up into the cell, so their circulating number is reduced and they can undergo extravasation (Laverman et al., 2000). To overcome this, nanoparticles can be coated with hydrophilic

materials such as polyethylene glycol (PEG), which is often used, which reduces opsonization and prolongs the circulation time (Arulsudar et al., 2004).

In addition to coating with PEG, nanoparticle properties such as size, surface charge, hydrophobicity, and structural design can be designed to passively target specific tissues or cell types. On the other hand, the neglect of hydrophilic coating causes nanoparticles to be rapidly taken up by cells of the mononuclear phagocytic system, making them ideal for targeting intracellular parasitic, bacterial, fungal, and viral infections. Passive targeting does not require the addition of a functional group or part of the molecule to be targeted, therefore less expensive than active targeting, thus potentially more useful for veterinary use (Schiffelers et al., 2001; Adiseshaiah et al., 2010).

In addition to passive targeting, active targeting may be necessary to increase the interaction between nanoparticles and target tissues. Active targeting is achieved by binding a functional group or part of the molecule to the nanoparticles, which causes the resulting structure to bind to a specific receptor/cell type, thereby increasing the concentrations in the targeted region. Ligand-mediated binding is particularly valuable for therapeutics that are not readily taken up by cells and require facilitated fusion, endocytosis, or some other type of uptake process to access intracellular active sites. Significant advances have been made in the active targeting of nanoparticles to inflammatory markers, adhesion molecules, and abnormal cell surface receptors, enabling high levels of pharmaceutical delivery to tumors and vascular diseases such as atherosclerosis (Guccione et al., 2004; Winter et al., 2010).

Using and developing antibodies and antibody fragments to target nanoparticles to a particular tissue or cell type can be expensive; however, targeting specific tissues by changing the charge of nanoparticles or coating them with a substance naturally uptake by that tissue is a more cost-effective approach for developing targeted nanoparticles for veterinary use. This method has been successfully used in the treatment of neurological diseases, with the aim of increasing the binding and uptake of nanoparticles to the blood-brain barrier (Lu et al., 2006).

When it comes to the target area, the next step is the release of the drug. Numerous drug release/nanoparticle uptake mechanisms can be created depending on the properties and surface characteristics of the nanoparticle in question. Possible drug release mechanisms include (1) drug molecule release due to nanoparticle degradation or enzymatic degradation; (2) diffusion from intact nanoparticle; (3) release from the surface of the nanoparticle; (4) fusion of the nanoparticle with the cell surface membrane followed by release of the contents into the cell; (5) the release of the content into the endoplasmic reticulum following endocytosis of the nanoparticle, and (6) the triggered release, triggered by the application of an external factor such as a magnetic field, temperature, or pH change. Often a combination of these processes coexists and particles can be engineered to have optimal and controllable release kinetics targeting them to specific intracellular pathways (Couvreur & Puisieux, 1993; Liu et al., 2007).

Nanoparticles have found widespread use in drug delivery with indications such as cancer, infection and analgesia. Nanoparticular delivery systems increase drug concentration and retention time in the tissue by increasing drug penetration into the target tissue, thus improving the pharmacokinetics of intravenous, ocular, inhalation, intra-articular, perineural, epidural, oral and topically applied pharmaceuticals (Shek et al., 1994; Gershkovich et al., 2008; Cai et al., 2010). Although there are still many areas of nanotechnology that have not yet been explored within the veterinary field, a number of nanoparticle formulation studies including veterinary field preparations have been tabulated (Table 2). More studies will emerge as the development costs of nanoparticle formulations decrease. Some of the pharmaceuticals designed for human use also have the potential to be applied in the veterinary field, especially anti-cancer drugs for pet animal use.

Nanoparticles in the Field of Diagnostics: Recently, extensive research has been conducted to develop nanoparticle systems for in vivo diagnostic imaging and laboratory-based diagnostic methods. The aim is to enable the identification of subclinical diseases through the use of refined nanoparticle systems in diagnostic analyses using high-resolution imaging methods that are sensitive enough to detect even small aggregates of atypical cells within an entire organism and in direct, rapid, precise diagnostic analyses for early detection of biomarkers/pathogens. Nanoparticle platforms that utilize similar application principles for nanoparticle drug delivery systems can be loaded with imaging agents to detect pathological tissues and specific cell types. Nanoparticles are used to visualize, characterize, and measure cellular processes in living organisms, and this imaging process can be performed at the macroscopic or molecular level. Some nanoparticles, such as quantum dots, gold nanoparticles, and perfluorocarbon nanoparticles, have imaging properties as structural characteristics, while other nanoparticles, such as liposomes, can be loaded with contrast media used in imaging to enable the detection of pathological tissues. These nanoparticles are either based on conduction mechanisms involving passive targeting, or are conjugated into various ligands (such as monoclonal antibodies, peptides, polysaccharides or aptamers) to direct them to a specific cell type or pathway (Matteucci & Thrall, 2000; Bentolila et al., 2009).

Table 2. Overview of some nanoparticle systems involved in drug and vaccine delivery used in veterinary medicine. All the listed studies have been carried out in species where the actual use of the nanoparticle is intended (Underwood & Van Eps, 2012).

Species	Nanoparticle	Agent	Disease	Delivery Pathway	Findings
Horse	Liposome	99 m-technetium	Imaging	IV	
	Liposome	DNA	Transfection of equine spermatozoa	N/A	◇,▲
	Liposome	Diamidine	Treatment of babesiosis	IM	●,■,▲
	Micellar microemulsion	Propofol	Anesthesia	IV	△
	Micelle	Ivermectin	*Strongylus vulgaris*	IM	▼
	Polymer nanospheres	*Streptococcus equi* antigens	Strangles vaccine	Intranasal	●,◇,▲
	Water based nanoparticle adjuvant	*Rhodococcus Equi* Vap peptides	*R. equi* pneumonia vaccine	IM	●,◇
Pigs	Dendrimer	Foot and mouth disease vaccine	Foot and mouth disease	IM	◇,▲
	Liposome	α-tocopherol	Vitamin E supplementation	PO	△,◇,▲
	Chromium nanocomposite	Chromium	Chromium supplementation	PO	◇,▲
	Polymeric	*E. coli* fimbriae vaccine	*E. coli*	PO	◇,▲
Birds	Liposome	Butorphanol	Arthritis in parrots and conures	SC	▲,◇,▲
	Liposome	Avian pathogenic E-coli vaccine	Avian colibacillosis vaccine	Intraocular	●,■,▲
	Liposome	Fimbriae antigens SEF14 and SEF 21	Salmonella enteritidis vaccine	Intraocular/nebulized	●,▲
	Liposome	DNA vaccine	Newcastle disease virus and infectious bursal disease	Transdermal	●,◇,▲,▲
	Polymer nanoparticle	*Chlamydophila psittaci* vaccine	*C. psittaci* vaccine	Nebulized	●,◇,▲
	Chitosan nanoparticles	Copper	Copper supplementation	PO	▲
Cat	Emulsion	Propofol	Anesthesia	IV	●,◇,■,▼
	Liposome	Muramil tripeptide	Mammary Adenocarcinoma	IV	●,▼
	Liposome	Photosensitizer	Squamous cell carcinoma	IV	●,■,▲●,■,△,◇,▼
	Liposome	IL-2 DNA	Chronic rhinitis	Intraperitoneal	●,■,▲
	Liposome	Technetium-99m	Imaging of sarcomas	IV	●,▲
	Liposome	Doxorubicin	Soft tissue sarcoma	IV	●,■,◇,▼
	Liposome	Ribavirin	Infectious peritonitis of cats	PO, IM, IV	◇,▼
	magnetic nanoparticle	Granulocyte–macrophage colony stimulating factor, IL-2, IFN-γ	Fibrosarcoma	Intratumoral	●,■,▲
Sheep	Liposome	Bovine leukemia virus	Bovine leukemia virus vaccine	IM	●,▲
	Liposome	Staphylococcal antigens	Staphylococcal mastitis vaccine	IM	●,◇,▲
	Polystyrene nano beads	Ovalbumin	Evaluation of nanobeads as an adjuvant	SC, IM, Intradermal	◇,■,▲
	Polystyrene nano beads	Foot and Mouth disease antigens	Foot and Mouth disease vaccine	Intradermal	◇,▲
	Micelle	*Fasciola hepatica* antigen Fh12	*F. hepatica*	SC	●,▲
	DNA Chitosan nanospheres	Newcastle disease vaccine and IL-2 gene	Newcastle disease vaccine	IM	●,▲
Dog	Emulsion	Cyclosporine	Immunosuppressive to prevent tissue/organ transplant rejection	PO	■,▲
	Liposome	Hydromorphone	Analgesia	SC	■,◇,▲
	Liposome	Clodronate	Malignant histiocytosis	IV	●,▲
	Liposome	Trifluralin	Leishmaniosis	IV	▲
	Liposome	Meglumine antimoniate	Leishmaniosis	IV	●,■,▲●,△,▲△,◇,▲
	Liposome	IL-2 DNA	Osteosarcoma lung metastasis	IV	●,■,▲
	Liposome	DNA	Hemangiosarcoma	Intraperitoneal	●,■,▲
	Liposome	Amphotericin B	Leishmaniosis	IV	●,■,▲
	Liposome	Muramyl tripeptide	Oral melanoma	IV	●,▲
	Liposome	Muramyl tripeptide	Spleen hemangiosarcoma	IV	●,▲
Cattle	Liposome	Streptomycin	Brucellosis	Intramammary	◇,▲
	Liposome	Gentamicin	*S. aureus* mastitis	Intramammary	◇,△,▼
	Liposome	Adriamycin	Leukemia	IV	●,◇,▲
	Niosome	Flurbiprofen	Analgesic	IV	△,◇,▲
	Ring shaped	Respiratory Syncytial Virus	Cattle respiratory system	Intranasal, IM	▲
	Nanoparticle	Virus nucleoprotein	syncytial virus		
	Liposome	Diclofenac	Anti-inflammatory and analgesic	Transdermal	●,▲△,▲△,▼▲

● Clinical trial/evaluated in animals with clinical disease, △ pharmacokinetic study, ◇ compared with non-nanoparticle formulation, ▲ The effect of nanoparticle formulation was considered beneficial or the nanoparticle formulation fulfilled the objectives of the study, ▼ The nanoparticle formulation has no beneficial effect / there is no difference in the non-nanoparticle formulation compared to the free drug, ■ side effect was seen

The preliminary phase of the study focused on the macroscopic use of nanoparticles such as radiography, magnetic resonance imaging (MRI), scintigraphy, positron emission tomography (PET) and computed tomography (CT) for the diagnosis of tumors and foci of inflammation. Due to the increased permeability in areas such as tumor and inflammatory foci, nanoparticles are extravasated from there. The EPR effect causes more signals to be received from the contrast medium of the imaging agent that the nanoparticles carry to the area in question. Some nanoparticle formulations, such as radiolabeled liposomes, have advanced to the clinical trial stage in human medicine. However, concerns about toxicity and safety, particularly complement-mediated hypersensitivity reactions that occur in 5-45% of human patients during liposome administration, have restricted the diagnostic use of such substances (Szebeni et al., 2007). It may prove that lesion localization may be more challenging and imaging using nanoparticles would be very useful in animal species where there are fewer alternative agent/imaging methods. For example, radiolabeled liposomes have the potential to find tumors or septic foci in farm animals, where it is unlikely to use conventional imaging methods due to their size. Although this area of research lags behind other nanotechnology applications in terms of its readiness for clinical use and applicability, the investigation of nanoparticle systems in diagnostic imaging has gained significant momentum and labeled nanoparticles are becoming an extremely valuable tool in terms of being the subject of research. Ligand-guided nanoparticles, such as fluorescent quantum dots, provide unprecedented information about the pathophysiology of disease by identifying diseased tissues and molecular processes through intravital microscopy (Bentolila et al., 2009). In models of animal diseases on a larger scale, nanoparticle-based imaging techniques are widely used to assess the bio distribution of potential nanoparticle-drug delivery systems. Much of the available information on nanoparticle clearance, the degree of uptake by the mononuclear phagocytic system, and tissue localization is based on the results of studies with imaging agents conjugated into potential nanoparticle conduction systems. The new diagnostic analyses have been successfully created thanks to the binding of functional nanoparticles to biological molecules such as antibodies, peptides, proteins and nucleic acids (Luchini et al., 2010). Spectroscopy, combined with flow cytometry and histological methods, provides a new platform that can be used as a powerful, highly sensitive and amplification-free pathogen detection method to map molecular profiles associated with disease and infection. The ultimate goal of this technology is to develop a simple, sensitive panel for biomarker proteins that enables early detection of diseases such as cancer. While more work needs to be done before this complex

system becomes a clinical reality, there are numerous proven nanoparticle-based detection systems for the detection of viral, parasitic, and bacterial pathogens in the veterinary field (Kumanan et al., 2009; Yuan et al., 2009). In addition to disease diagnosis, conjugated nanoparticles to the monoclonal antibodies of the veterinary drug molecule are integrated into immune assays to determine drug levels in foodstuffs with a fast, precise, and simple analytical method (Zhang et al., 2008). These techniques have great potential for the detection of drug residues and the early diagnosis of diseased animals.

Vaccine Delivery: Vaccines designed to create a long-lasting and protective antibody response against a pathogen are basically composed of antigens and adjuvants. Traditionally, antigens have been provided from inactivated microorganisms. However, this situation has begun to decrease and synthetic peptides and recombinant proteins have become preferred today because they are safer (Nordly et al., 2009). These structures, which are seen as new vaccine candidates, are usually weakly immunogenic and susceptible to degradation. For this reason, it needs new adjuvants that make their immunogenicity appropriate. The effect of traditional adjuvants cannot be adjusted, but with the advent of nanotechnology, there has been a drastic increase in the number of new antigen delivery strategies. The new nanoparticle-based adjuvants can be designed to reduce dosage frequency through an appropriate administration pathway to stimulate specific immune response. Providing a better mucosal immunity by choosing the intranasal route can be shown as an example of this (Morein et al., 2004; Scheerlinck et al., 2006; Wang et al., 2011). In a situation where more than one animal needs to be treated together in a commercial enterprise, in comprehensive agricultural practices such as wildlife, where accessibility is difficult or where a small amount of manpower is used in proportion to the size of the area, the use of new generation vaccination systems in the veterinary field is more reasonable due to the inconvenience of vaccination by conventional means (Nordly et al., 2009).

Nanoparticle adjuvants can increase the immunogenicity of a vaccine in five potential ways (Nordly et al., 2009). First, nanoparticle adjuvants activate pattern recognition receptors such as Toll-Like Receptors by mimicking pathogen-associated molecular patterns. This triggers the intracellular signaling cascade and initiates the formation of a natural immune response. Natural immune response formation also results in increased adaptive immune response. Secondly, nanoparticle adjuvants activate co-stimulator molecules in antigen-presenting cells, leading to increased activation by the antigen of T cells. Thirdly, nanoparticle adjuvants can control the location of the released antigen, the duration of its stay and the dose of the antigen, thus preserving the existing level of immunity and increasing the translocation of the antigen to the lymph nodes. Fourth, nanoparticle adjuvants act as reservoirs to ensure long-term antigen transmission. Finally, nanoparticles can be designed to produce virus-like particles with morphology similar to virus capsids that stimulate an immune response without containing the genetic material that causes infection in the host (Nordly et al., 2009).

Nanoparticle adjuvants approved for use in veterinary medicine (or in clinical trials) include emulsions, liposomes, polystyrene nanobeads, immunostimulant complexes (ISCOMs) and inorganic particles (Scheerlinck et al., 2006; Nordly et al., 2009; Vandamme et al., 2011). In order for nanoparticle adjuvants to be practically usable in veterinary medicine, they must be stable, easy to apply, biodegradable and inexpensive. The principle of cheapness is especially important in species that are offered to human consumption. Although the use of formulations of expensive nanoparticles, such as DNA-coated gold nanoparticles, can be cost-prohibitive, the flexible and adjustable nature of nanoparticle-based vaccine delivery systems holds great promise for the development of veterinary vaccines that can be administered in a more convenient way at low frequency compared to their conventional counterparts.

The nanoparticle vaccine delivery system has been successfully developed in more than 40 animal diseases such as equine influenza and *Streptococcus equi.* var. *equi* infections in horses (Morein et al., 2004; Florindo et al., 2009); foot and mouth disease virus, BVD virus and *Toxoplasma gondii* infections in ruminants (Harpin et al., 1999; Cubillos et al., 2008; Hiszczyńska-Sawicka et al., 2011); Newcastle and H5N1 influenza in poultry (Rimmelzwaan et al., 1999; Zhang et al., 2010); enterotoxigenic *E. coli* infections and atrophic rhinitis in pigs (Kang et al., 2008); parvovirus infections and atopic dermatitis in dogs (Morein et al., 2004; Mueller et al., 2005; Vandamme et al., 2011).

ALTERNATIVE MEDICATION DELIVERY METHODS

The term compliance refers to the patient's willingness and degree to comply with the prescribed medications or protocol. Non-compliance, on the other hand, means non-submission and non-obedience and is associated with the degree of unwillingness and disobedience with the drugs or protocol prescribed by the physician to the patient (Vermeire et al., 2001). Research in the UK suggested that only 37 per cent of clients has shown full compliance with the medicines or follow-up

practices recommended by the veterinarian. It has been revealed that approximately 70-85 percent of patient owners give only one-third of the required doses on time (Loftus, 2012).

Most vaccines are still administered by injection. In recent years, however, there has been a greater focus on alternative routes of administration to increase patient compliance. Among these alternative routes of application, mucosal and transdermal routes come to the fore. Since most pathogens infect the host through the mucosal or dermal epithelium, these pathways are seen as attractive ways in which vaccines can be administered to control and prevent disease at the site of infection. The advantage of alternative administration is that vaccines target local immune-active tissue. Langerhans cells in the epidermis or mucosa-associated lymphoid tissue are shown as an example. Mucosal immunization results in the stimulation of local immune responses. IgA secretion is an important immune effector mechanism in this type of immunity. Some of the studies that apply alternative application pathways that are being investigated for lipid-based conduction systems can be shown as examples of this issue. Mucosal (intranasal) administration of MF59-adjuvanted influenza subunit vaccines is one of the studies reported. As a result of the study, mucosal IgA response to influenza was developed. The percentage of subjects whose antibody response in their serum was developed by mucosal administration was only slightly lower compared to intramuscular administration. The immune response with the adjuvanted vaccine was not significantly different from the immune response with the unadjuvanted vaccine. This suggests that MF59 adjuvation is of no use. Both vaccines produced more responses than those seen in placebo recipients, suggesting a potential benefit of intranasal administration (Agrawal et al., 2003).

Lipid-based nanoparticle delivery systems are seen as promising alternatives, according to Alum. The MF59 emulsion adjuvant is already on the market, and other types of lipid-based nanoparticle systems are in clinical development. Furthermore, more clinical trials are needed to fully explain the potential of lipid-based nanoparticles. Lipid-based systems are biocompatible and biodegradable, versatile in that they have the possibility of including other immunostimulatory agents that optimize the immunological properties of the specific adjuvant. Further research on their adjuvant mechanisms is needed to fully understand the immunostimulatory properties of lipid-based adjuvants and to ensure rational separation of vaccines into specific immunological profiles. Alternative routes of administration are attractive in terms of ensuring adherence, but there is still not enough information on important factors such as optimal dosing and repeatability of administration (Nordly et al., 2009)

CONCLUSION

Today, thanks to the researches, treatment methods have been developed for diseases that were not cured in the past, and even thanks to the vaccines produced, it has been possible to protect against some diseases to a great extent. However, despite the developing technology and medical techniques, new diseases and new problems continued to emerge, and new solutions were tried to be offered to new problems. Nanotechnology and nanoparticles are thought to be a field with the potential to bring new solutions to today's problems. The fact that nanoparticles can be designed with the preferred size, shape, charge, porosity, efficiency and stability and that they can be adjusted to cross biological barriers at any time shows promise in many areas. These properties have made both cellular and humoral immune responses more efficient in some improved vaccine formulations and are open to improvement if more extensive research is conducted (Hajizade et al., 2014; Yue & Ma, 2015). Similarly, in research on antibiotic formulations synthesized with nanoparticles, it has been determined that antibiotics given at once can be effective for a long time under harsh ambient conditions and suppress the growth of bacteria used in the study by realizing the desired antimicrobial effect (Carmona et al., 2018). The field of study of nanotechnology is not limited to these, but it can be used for diagnostic purposes in veterinary medicine (Mishra et al., 2010), as a biocide (Hu & Hsieh, 2015), as a topical shampoo for pet animals (Bansod et al., 2015), as nutraceutical (Sirirat et al., 2013) or for sperm purification (Pawar & Kaul, 2014).

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