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Review

Nanomedicine and veterinary science: The reality and the practicality

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ABSTRACT

Nanomedicine is a rapidly expanding field with a promising future that is already permeating veterinary science. This review summarises the current applications for nanoparticles in human medicine and explores their potential applicability for veterinary use. The principles underlying the use of nanoparticles in drug delivery, imaging and as vaccine adjuvants are explored along with the unique issues surrounding nanoparticle toxicity and regulatory approval. A brief overview of the properties of different nanoparticle systems including, liposomes, micelles, emulsions and inorganic nanoparticles, is provided, along with a description of their current and potential future applications in veterinary medicine.

Introduction

Nanotechnology is revolutionizing human medicine, particularly in the fields of imaging and drug delivery. For over 30 years, nanoparticles, defined as ordered structures with diameters smaller than 1000 nm (Brigger et al., 2002), have been engineered to develop novel diagnostic methods and targeted therapies (Fig. 1). In the medical field, nanoparticle research has focused primarily on targeted delivery of therapeutic agents, vaccine development and novel diagnostic methods. In some of these areas, nanoparticles have delivered effective and scientifically validated solutions, leading to their incorporation into marketable products that are already extending to veterinary species. However, 'over-speculation' on the potential of futuristic nano-inventions that are in the early stages of development can cloud the realistic progress that is being made.

This review will focus on the basic principles behind the use of nanoparticles for drug delivery, diagnostics and vaccine formulation. Common forms of nanoparticle are discussed, along with their clinical applications and limitations, providing the reader with a realistic synopsis of the practical applications of nanoparticles to veterinary medicine at present and in the near future.

Nanoparticles as precise drug delivery systems

The use and efficacy of many currently available pharmacological agents are limited by low bioavailability and unwanted side effects. Approximately 95% of all potential therapeutic agents have poor bioavailability and pharmacokinetics (Brayden, 2003). Nanoparticle drug delivery systems can be engineered to overcome these issues and thus improve the therapeutic index and safety profile of the substances they carry. In multiple studies, nanoparticles have already demonstrated remarkable efficacy for targeting delivery of anti-neoplastic agents (Hofheinz et al., 2005), antimicrobials (Cordeiro et al., 2000), analgesics (Rose et al., 2005) and anti-inflammatory agents (Metselaar et al., 2003). There are over 200 nanoparticle drug delivery systems in development, with at least 30 nanoparticle based therapeutic products approved for clinical use in humans and a similar number in clinical trials (Wagner et al., 2006; Bawa, 2008). Many of these preparations are prohibitively expensive for veterinary use, but, several nanoparticle formulations are already available on the veterinary market, and as nanoparticle production facilities are scaled up for commercialisation, these preparations will increasingly become more affordable for veterinary application.

Nanoparticles improve the therapeutic index of the pharmaceutical agents they carry through four key mechanisms. Firstly, they enable the use of drugs that would otherwise be insoluble or unstable. Secondly, they increase the concentration of pharmaceutical at its intended site of action, resulting in increased efficacy. Thirdly, because of preferential accumulation at target sites, they lower systemic toxicity and drug concentration in healthy tissues. Fourthly, nanoparticles have reduced clearance compared to the

Abbreviations: IV, intravenous; SC, subcutaneous; IM, intramuscular; SLN, solid lipid nanoparticles; IL, interleukin; MRI, magnetic resonance imaging; PET, positron emission tomography; CT, computed tomography; PEG, polyethylene glycol; EPR, enhanced permeability and retention; FDA, US Food and Drug Administration.

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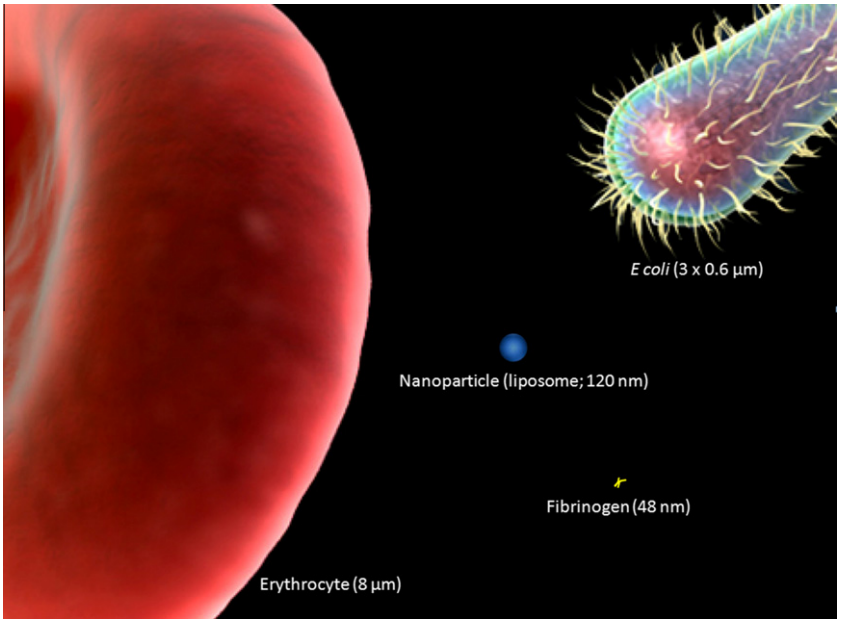

Fig. 1. A schematic diagram depicting the relative size of a typical nanoparticle compared to an erythrocyte, an *E. coli* bacterium and a molecule of fibrinogen. Whilst nanoparticles are defined as being less than 1000 nm in diameter, most nanoparticles used in medical applications are between 50 and 200 nm in diameter.

parent drug and thus provide a method of sustained controlled release over a period of days or even weeks (Sahoo and Labhasetwar, 2003; Bakker-Woudenberg et al., 2005; Fahmy et al., 2005).

As a consequence of these mechanisms, nanoparticle formulations require a reduced dose compared to free drug. This is particularly pertinent to veterinary medicine as it may (1) allow the use of expensive human pharmaceuticals whose application has previously been precluded by the cost of dosing and (2) reduce the levels in carcasses leading to lower environmental impact and lower residues in food.

To form a successful drug delivery system, the nanoparticle must be loaded with a sufficient amount of pharmaceutical, carry this to the target tissue and then release the pharmaceutical at the target site. Nanoparticles can be loaded with drugs via encapsulation within the particle or via surface attachment (Lu et al., 2007). The method of drug loading depends upon the type of nanoparticle as well as the drug type and the target. By loading a drug into the nanoparticle, the drug essentially takes on the external properties of the nanoparticle until the particle is destroyed or the drug is released at its target.

Targeting of nanoparticles to specific sites is achieved passively (based on the innate properties of the nanoparticle) and/or actively (the coupling of a targeting moiety to a nanoparticle). The archetypical mechanism of passive targeting occurs via the 'enhanced permeability and retention (EPR) effect', which is demonstrated almost ubiquitously by nanoparticles due to their small size (Maeda, 2010). This effect relies on the ability of intravenous (IV) nanoparticles to extravasate at sites of increased vascular permeability, but otherwise be retained in the circulation. This results in accumulation of nanoparticles at sites of increased vascular permeability (e.g. tumours, infections and areas of inflammation), hence targeting of the agents they carry to these sites (Ishihara et al., 2010).

Opsonisation and subsequent uptake of nanoparticles by the reticuloendothelial system reduces the number remaining in circulation and able to extravasate (Laverman et al., 2000). To overcome this, nanoparticles can be coated with hydrophilic substances, the most commonly used being polyethylene glycol (PEG), which reduces opsonisation and prolongs circulation time (Arulsudar et al., 2004).

In addition to a PEG coating, nanoparticle characteristics, such as size, surface charge, hydrophobicity and architecture can be engineered to passively target certain tissues or cell types (Adiseshaiah et al., 2010). In fact, omission of a hydrophilic coating results in rapid uptake of nanoparticles by cells of the mononuclear phagocyte system, rendering them ideal for targeting intracellular parasitic, bacterial, fungal and viral infections (Schiffelers et al., 2001). Passive targeting does not require the addition of a targeting moiety and so is often less expensive than active targeting and is potentially more useful for application to veterinary medicine.

In addition to passive targeting, active targeting may be necessary to increase the interaction between nanoparticles and target tissues. This is achieved via attachment of a targeting moiety to the nanoparticles, causing them to adhere to a particular receptor/cell type so increasing their concentration at the site of interest (Goutayer et al., 2010).

Ligand-mediated binding is particularly valuable for therapeutics that are not easily taken up by cells, and require facilitated fusion, endocytosis or another uptake processes to access intracellular active sites. Significant progress has been made by actively targeting nanoparticles to inflammatory markers, adhesion molecules and abnormal cell surface receptors enabling delivery of high levels of pharmaceutical to vascular diseases (e.g. atherosclerosis) and to tumours (Guccione et al., 2004; Winter et al., 2010).

The use and development of antibodies and antibody fragments to target nanoparticles to a particular tissue or cell type can be expensive; however, targeting nanoparticles to specific tissues simply by altering their charge, or coating them in a substance that is naturally taken up by that tissue is a more cost-effective approach in developing targeted nanoparticles for veterinary use. This method has been successfully used to enhance nanoparticle adherence to and uptake across the blood–brain barrier for treatment of neurological diseases (Lu et al., 2006; Weiss et al., 2008).

Once at the target site the next essential step is drug release. A myriad of mechanisms for drug release/nanoparticle uptake may occur based on the properties and surface characteristics of the nanoparticle in question (Georgieva et al., 2010). Possible mechanisms of drug release include (1) liberation due to nanoparticle disintegration, or enzymatic breakdown; (2) diffusion from the intact nanoparticle; (3) release from the surface of the nanoparticle; (4) fusion of the nanoparticle with the cell surface membrane and subsequent release of contents into the cell; (5) endocytosis of the nanoparticle with subsequent release of contents into the endoplasmic reticulum, and (6) triggered release initiated by application of an external factor, such as a magnetic field, a change in temperature or pH (Couvreur, 1993; Liu et al., 2007). Often a combination of these processes coexists, and particles can be engineered to have optimal and controllable release kinetics that target them to specific intracellular pathways.

Nanoparticles have found widespread use in drug delivery, with indications including cancer, infection and analgesia. In addition to IV administration, nanoparticle delivery systems improve the pharmacokinetics of ocular, inhalational, intra-articular, perineural, epidural, oral and topical pharmaceuticals, primarily via increasing drug penetration to the target tissue, increasing the concentration of drug and its retention time in that tissue (Shek et al., 1994; Aly, 2008; Gershkovich et al., 2008; Cevc and Vierl, 2009; Cai et al., 2010). Although there are still large areas of nanotechnology not yet explored for veterinary application, a number of studies have evaluated nanoparticle formulations specifically for veterinary use (Table 1) and more work will be undertaken as development costs fall. In addition to those listed in Table 1 many nanoparticle based therapeutics have been studied in veterinary species in pre-clinical studies of pharmaceuticals targeted for the human market. Some of these pharmaceuticals also have potential for veterinary application particularly anti-cancer drugs for companion animal use.

Nanoparticles in diagnostics

Recently, extensive research has been performed to develop nanoparticle systems for in vivo diagnostic imaging and laboratory-based diagnostics. The aim is to enable sub-clinical disease identification via the use of refined nanoparticle systems in both sensitive high-resolution imaging modalities that can detect small aggregates of atypical cells within an entire organism, and in direct, rapid and sensitive diagnostic assays for early detection of biomarkers and pathogens.

Harnessing similar delivery principles as those for nanoparticle drug delivery systems, nanoparticle platforms can be loaded with imaging agents to detect pathological tissues and specific cell types. Imaging can be macroscopic or at a molecular level where nanoparticles are used to visualise, characterise and measure cellular processes in living organisms. Some nanoparticles, such as quantum dots, gold nanoparticles and perfluorocarbon nanoparticles, have innate imaging properties, whereas others (such as liposomes) can be loaded with imaging contrast agent to enable detection of pathological tissues (Matteucci and Thrall, 2000; Bentolila et al., 2009). These nanoparticles rely on passive delivery mechanisms or are conjugated to various ligands (e.g. monoclonal antibodies, peptides, polysaccharides or aptamers) to direct them to a certain cell type or pathway.

Initial work focused on the macroscopic use of nanoparticles with conventional imaging modalities, such as radiography, magnetic resonance imaging (MRI), nuclear scintigraphy, positron emission tomography (PET) and computerised tomography (CT), for the diagnosis of tumours and inflammatory foci. Due to the increased permeability at these sites, nanoparticles extravasate and demonstrate the EPR leading to an increased signal from the imaging contrast agent they carry at that site.

Some nanoparticle formulations, such as radiolabelled liposomes, have progressed to clinical trials in human medicine. However, concerns over toxicity and safety, in particular the complement mediated hypersensitivity reactions that occur in 5–45% of human patients during liposome administration, have restricted their use for diagnostic purposes (Szebeni et al., 2007). In the veterinary species, where lesion localisation may be more challenging and fewer alternative agents/imaging modalities exist, nanoparticle imaging may prove to be very useful. For examples, of radiolabelled liposomes have the potential to locate tumours or septic foci in large animals where size prohibits the use of conventional imaging (Fig. 2).

Whilst this field of research does lag behind other nanotechnology applications in its readiness and applicability for clinical use, investigation of nanoparticle systems in diagnostic imaging has gained considerable momentum and labelled nanoparticles are an extremely valuable tool from a research standpoint. Ligand directed nanoparticles, such as fluorescent quantum dots can identify diseased tissues and molecular processes via intravital microscopy providing unprecedented information on disease pathophysiology (Bentolila et al., 2009). On a larger scale, nanoparticle based imaging techniques have been used extensively in animal disease models to evaluate the biodistribution of potential nanoparticle-drug delivery systems. Much of the information available regarding nanoparticle clearance, degree of uptake by the MPS and tissue localisation is based on the results of studies with imaging agents conjugated to the potential nanoparticle delivery system.

Novel diagnostic assays have been successfully formed by linking functionalised nanoparticles to biological molecules such as antibodies, peptides, proteins and nucleic acids (Driskell et al., 2005; Luchini et al., 2010). Coupled with spectroscopy, flow cytometry and histological methods these provide a powerful novel platform for mapping the molecular profiles associated with disease and infection and a highly sensitive, amplification-free method of pathogen detection (Halfpenny and Wright, 2010). The ultimate aim for this technology is to develop a simple, sensitive panel for biomarker proteins that enables early detection of diseases such as cancer.

Whilst further work is necessary before this complex system becomes a clinical reality, there are a multitude of nanoparticle based detection systems successfully validated 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, nanoparticles conjugated to monoclonal antibodies of veterinary drug have been integrated into immunoassays to provide a fast, sensitive and simple analytical method for determining drug levels in foodstuffs (Shen et al., 2007; Zhang et al., 2008). These techniques have great potential for the detection of drug residues and early identification of diseased animals.

Vaccine delivery

Vaccines, designed to stimulate a long lasting and protective antibody response to a pathogen, are comprised primarily of antigen and adjuvant. Traditionally, inactivated microorganisms provided the antigen, but recently there has been a shift towards the use of safer synthetic peptides and recombinant proteins (Nordly et al., 2009). Alone, these new vaccine candidates are often poorly immunogenic and sensitive to degradation, and they require an optimised adjuvant that improves immunogenicity (Nordly et al., 2009). Conventional adjuvants are not tuneable, but with the advent of nanotechnology a plethora of novel antigen carrying

Table 1
An overview of some of the nanoparticle systems evaluated for drug and vaccine delivery for veterinary use. All studies listed have been performed in the species for which the nanoparticle is intended. ● Clinical trial/evaluated in animals with clinical disease, △ pharmacokinetic study, ◇ compared to non-nanoparticle formulation, ▲ beneficial effect of nanoparticle formulation or nanoparticle formulation fulfilled aims of study, ▼ no beneficial effect of nanoparticle formulation/no difference to non-nanoparticle formulation where compared with free-drug, ■ adverse effects.

Species	Nanoparticle	Agent	Disease	Administration route	Reference
Cats	Emulsion	Propofol	Anesthesia	IV	Wiese et al. (2010)●◇■▼ Cleale et al. (2009)●◇△■▼
	Liposome	Muramyl tripeptide	Mammary adenocarcinoma	IV	Fox et al. (1995)●▼
	Liposome	Photosensitiser	Squamous cell carcinoma	IV	Buchholz et al. (2007)●■▲ Buchholz et al. (2005)●■△◇▲
	Liposome	IL-2 DNA	Chronic rhinitis	Intraperitoneal	Veir et al. (2006)●■▲
	Liposome	99 m-technetium	Imaging of sarcomas	IV	Matteucci et al. (2000)●▲
	Liposome	Doxorubicin	Soft tissue sarcoma	IV	Poirier et al. (2002) ●■ ◇▼ Kleiter et al. (2010)●■▲
	Liposome	Ribavirin	Feline infectious peritonitis	PO, IM, IV	Weiss et al. (1993)◇▼
	Magnetic nanoparticle	Granulocyte–macrophage colony stimulating factor, IL-2, IFN-γ	Fibrosarcoma	Intratumoural	Huttinger et al. (2008)●■▲ Jahnke et al. (2007)●■▲
Dogs	Emulsion	Cyclosporine	Immunosuppressive to prevent transplant rejection	PO	Bernsteen et al. (2003)■▲
	Liposome	Hydromorphone	Analgesia	SC	Wunsch et al. (2009)■◇▲ Smith et al. (2008)△▲
	Liposome	Clodronate	Malignant histiocytosis	IV	Hafeman et al. (2009)●▲
	Liposome	Trifluralin	Leishmaniosis	IV	Marques et al. (2008)▲
	Liposome	Meglumine antimoniate	Leishmaniosis	IV	Ribeiro et al. (2008)●■▲, Schettini et al. (2005)●△▲ and Valladares et al. (2001)△◇▲
	Liposome	IL-2 DNA	Osteosarcoma lung metastases	IV	Dow et al. (2005)●■▲
	Liposome	DNA	Haemangiosarcoma	Intraperitoneal	U'Ren et al. (2007)●■▲
	Liposome	Clodronate	Immune mediated haemolytic anaemia	IV	Mathes et al. (2006)●■▲
	Liposome	Doxorubicin	Various canine tumours	IV	Hauck et al. (2006)●△■▲ Vail et al. (1997)●■▲
	Liposome	Endostatin DNA	Soft tissue sarcoma	IV	Kamstock et al. (2006)●■▲
	Liposome	Immunotherapy	Atopic dermatitis	Intradermal	Mueller et al. (2005)●■▲
	Liposome	Cisplatin	Various tumours and osteosarcoma	IV	Marr et al. (2004)■▲ Vail et al. (2002)●■▼
	Liposome	IL-2	Pulmonary neoplasia	Inhaled	Khanna et al. (1997)●■▲
	Liposome	Amphotericin B	Blastomycosis	IV	Krawiec et al. (1996)●■▲
	Liposome	Amphotericin B	Leishmaniosis	IV	Oliva et al. (1995)●■▲
	Liposome	Muramyl tripeptide	Oral melanoma	IV	MacEwen et al. (1999)●▲
	Liposome	Muramyl tripeptide	Osteosarcoma	IV	Kurzman et al. (1995)●▲
	Liposome	Muramyl tripeptide	Splenic haemangiosarcoma	IV	Vail et al. (1995)●▲
Pigs	Dendrimer	Foot and mouth disease vaccine	Foot and mouth disease	IM	Cubillos et al. (2008)◇▲
	Liposome	α-tocopherol	Vitamin E supplementation	PO	Bontempo et al. (2000)△◇▲
	Chromium nanocomposite	Chromium	Chromium supplementation	PO	Wang et al. (2009)◇▲
	Polymeric	*E. coli* fimbriae vaccine	*E. coli*	PO	Vandamme et al. (2011)◇▲
Cattle	Liposome	Streptomycin	Brucellosis	Intramammary	Nicoletti et al. (1989)◇▲
	Liposome	Gentamicin	*S. aureus* mastitis	Intramammary	MacLeod and Prescott, 1988◇△▼
	Liposome	Adriamycin	Leukaemia	IV	Onuma et al. (1989)●◇▲
	Niosome	Flurbiprofen	Analgesic	IV	Confalonieri et al. (2010)△◇▲
	Ring shaped	Human respiratory syncytial	Bovine respiratory	Intranasal, IM	Riffault et al. (2010)▲
	Nanoparticle	Virus nucleoprotein	Syncytial virus		
	Liposome	Diclofenac	Anti-inflammatory and analgesic	Transdermal	Lynn et al. (2004)●▲ Caldwell et al. (2004)△▲, Schleining et al. (2008)△▼, and Levine et al. (2009)▲
Horses	Liposome	99 m-technetium	Imaging	IV	Underwood et al. (2012)■▲
	Liposome	DNA	Transfection of equine spermatozoa	N/A	Ball et al. (2008)◇▲
	Liposome	Diamidine	Treatment of babesiosis	IM	Timofeev et al. (1994)●■▲
	Micellar microemulsion	Propofol	Anaesthesia	IV	Boscan et al. (2010)△ Boscan et al. (2006)■▲
	Micelle	Ivermectin	*Strongylus vulgaris*	IM	Klei et al. (1984)▼
	Polymer nanospheres	*Streptococcus equi* antigens	Strangles vaccine	Intranasal	Florindo et al. (2009)●◇▲
	Water based nanoparticle adjuvant	*Rhodococcus Equi* Vap peptides	*R. equi* pneumonia vaccine	IM	Cauchard et al. (2006)●◇
	Liposome	*Toxoplasma gondii* antigen	*T. gondii* vaccine	IM	Hiszczynska-Sawicka et al. (2011)▲
Sheep	Liposome	Bovine leukaemia virus	Bovine leukaemia virus vaccine	IM	Usui et al. (2003)●▲

(*continued on next page*)

Table 1 (*continued*)

Species	Nanoparticle	Agent	Disease	Administration route	Reference
	Liposome	Staphylococcal antigens	Staphylococcal mastitis vaccine	IM	Amorena et al. (1994)●◇▲
	Polystyrene nano-beads	Ovalbumin	Evaluation of the nanobeads as an adjuvant	SC, IM, Intradermal	Scheerlinck et al. (2006)◇■▲
	Polystyrene nano-beads	Foot and Mouth Disease antigens	Foot-and-Mouth disease vaccine	Intradermal	Greenwood et al. (2008)◇▲
	Micelle	*Fasciola hepatica* antigen Fh12	*F. hepatica*	SC	Martinez-Fernandez et al. (2004)●▲
	DNA chitosan nanospheres	Newcastle disease vaccine and IL-2 gene	Newcastle disease vaccine	IM	Zhang et al. (2010)●▲
Birds	Liposome	Butorphanol	Arthritis in parrots and conures	SC	Paul-Murphy et al. (2009a)▲ and Paul-Murphy et al. (2009b)◇▲
	Liposome	Avian pathogenic E-coli vaccine	Avian colibacillosis vaccine	Intraocular	Yaguchi et al. (2009)●■▲
	Liposome	Fimbriae antigens SEF14 and SEF 21	*Salmonella enteritidis* vaccine	Intraocular/ nebulised	Li et al. (2004)●▲
					Fukutome et al. (2001)●◇▲
	Liposome	DNA vaccine	Newcastle disease virus and infectious bursal disease virus vaccine	Transdermal	Heckert et al. (2002)▲
	Liposome	*M. gallisepticum* vaccine	*M. gallisepticum* vaccine	SC	Barbour and Newman, 1990▲●
	Micelle	Newcastle disease vaccine	Newcastle disease vaccine	PO	Wambura, 2010●▲
	Polymer nanoparticle	*Chlamydophila psittaci* vaccine	*C. psittaci* vaccine	Nebulised	Verminnen et al. (2010) and Verminnen et al.●◇▲
	Chitosan nanoparticles	Copper	Copper supplementation	PO	Wang et al. (2011a)▲

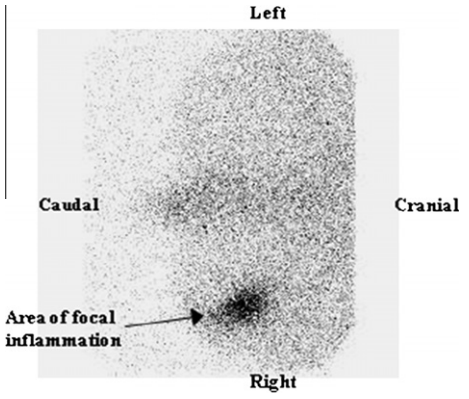

Fig. 2. Dorsal scintigraphic image of the caudal thigh muscles of a horse with an area of focal inflammation in the right thigh. This image was acquired 21 h post injection with 99 m-technetium labelled PEG-liposomes. Increased radiopharmaceutical uptake is present at the site of focal inflammation consistent with liposome accumulation at that site.

strategies are now available. These novel nanoparticle-based adjuvants are highly tuneable and can be engineered for reduced dosage frequency via a convenient administration route in order to provoke a specific immune response, e.g. the intranasal route to better target mucosal immunity (Morein et al., 2004; Scheerlinck et al., 2006; Wang et al., 2011b). This makes them highly amenable to engineering for veterinary species where large numbers of animals may need to be treated at once in a commercial unit, or when vaccination by conventional means is inconvenient due to extensive management systems or poor accessibility (e.g. wildlife).

Nanoparticle adjuvants increase the immunogenicity of a vaccine in five (potential) ways (Nordly et al., 2009). Firstly, by mimicking pathogen-associated molecular patterns they can activate pattern recognition receptors, such as Toll-like receptors, and trigger intracellular signalling cascades that initiate the innate immune response, resulting in enhancement of the adaptive immune response. Secondly, by upregulating co-stimulatory molecules on antigen presenting cells, nanoparticle adjuvants increase the activation of T cells by antigen. Thirdly, nanoparticle adjuvants can control the residence time, location and dose of antigen released so as to maintain immunity levels and enhance translocation of antigen to lymph nodes. Fourthly, they act as a depot to provide prolonged delivery of antigens. Finally, nanoparticles can be engineered to produce virus like particles that have similar morphology to virus capsid, and stimulate immune responses without the infectious genetic material that is responsible for host infection (Nordly et al., 2009).

Nanoparticle adjuvants that are approved for veterinary use (or in clinical trials) include emulsions, liposomes, polystyrene nanobeads, immune-stimulating complexes (ISCOMs) and inorganic particles (Scheerlinck et al., 2006; Nordly et al., 2009; Vandamme et al., 2011). For practical veterinary use, nanoparticle adjuvants need to be inexpensive, stable, easy to administer and biodegradable in species used for human consumption (Aucouturier et al., 2001). Although use of the more expensive nanoparticle formulations (such as DNA-coated gold nanoparticles) may be cost prohibitive, the flexibility and adjustable nature of nanoparticle based vaccine delivery systems holds great promise for the development of veterinary vaccines that can be administered via a more convenient route at reduced frequency compared to conventional counterparts.

To date, more than 40 diseases of animal species including equine influenza and *Streptococcus equi* var. *equi* infection in horses (Florindo et al., 2009; Morein et al., 2004), foot-and-mouth disease, bovine virus diarrhoea virus and *Toxoplasma gondii* in ruminants (Harpin et al., 1999; Cubillos et al., 2008; Hiszczynska-Sawicka et al., 2011); Newcastle disease and H5N1 influenza in poultry (Rimmelzwaan et al., 1999; Zhang et al., 2010), enterotoxigenic E. coli and atrophic rhinitis in swine (Kang et al., 2008), and parvovirus and atopic dermatitis in dogs (Morein et al., 2004; Mueller et al., 2005; Vandamme et al., 2011) have nanoparticle vaccine delivery systems that are either successfully developed or under development. A detailed review of nanoparticle based veterinary vaccines is provided by Scheerlinck and Greenwood (2008).

Nanoparticle toxicity and safety issues

The unique physicochemical properties of nanoparticles that potentiate their usefulness for veterinary applications can also result in adverse effects. Careful and thorough evaluation of these effects and their implications, not only for the animals receiving the nanoparticle formulation but also on people in contact with them, the environment, and of course any residues in food-producing livestock, is an essential part of nanoparticle development (O'Brien and Cummins, 2010). Just as the biological properties of nanoparticles vary with their physicochemical properties, the potential toxicity of nanoparticles can also be affected by a wide range of factors including chemical composition, charge, size and shape (Card et al., 2011). Of particular concern are the non-biodegradable nanoparticle formulations, particularly inorganic nanoparticles, which are generally associated with more significant adverse effects and have greater potential for accumulation than biodegradable biocompatible nanoparticles such as liposomes and micelles.

Evaluation of the safety of nanoparticle formulations requires a combination of in vitro and in vivo studies. The preliminary step is to evaluate in vitro cytotoxicity, genotoxicity and the influence of nanoparticles on cell signalling and other cellular functions using cell culture models and gene expression analyses (Schrand et al., 2010). Common mechanisms of nanoparticle toxicity at this level include DNA damage resulting in mutagenesis and potential carcinogenesis, oxidative damage, interactions with proteins and enzymes, cellular apoptosis, up-regulation of inflammatory genes, mitochondrial damage and cytoskeletal and extracellular matrix disruption (You et al., 2005; Pernodet et al., 2006; Park et al., 2008; Zhao and Castranova, 2011).

As different cell types show varying sensitivity to nanoparticle formulations (Schrand et al., 2010) it is important to use a variety of cell types with which the nanoparticle is likely to interact based on biodistribution studies. Whereas in vitro studies provide a better understanding of the comparative toxicity of nanoparticles, the results rarely translate directly into in vivo systems. Therefore in vivo studies are also essential to characterise fully the biodistribution and potential adverse effects of nanoparticles.

The absorption, distribution, metabolism, accumulation and excretion of each nanoparticle formulation must be defined by pharmacokinetic and toxicokinetic studies, and at each stage the interactions of nanoparticles with fluids, cells and tissues needs to be considered. In addition to direct toxic effects such as anaphylaxis, hepatotoxicity, nephrotoxicity, inflammation and granuloma formation (Chen et al., 2006; Ji et al., 2007; Szebeni et al., 2007; Ho et al., 2011), the persistence and accumulation of non-biodegradable nanoparticles, such as magnetic silica, in tissues over prolonged periods of time (Kim et al., 2006), is a concern in food producing species and raises the potential for environmental contamination. Moreover, the reproductive and developmental consequences of nanoparticle formulations must also be considered in breeding animals. Nanoparticle-induced disruption of endothelial barriers such as the blood–testis barrier can have reproductive consequences (Kim et al., 2006; Sharma and Sharma, 2007; Rahman et al., 2009) and developmental defects have been reported in association with metallic nanoparticles and dendrimers (Heiden et al., 2007; Asharani et al., 2008; Browning et al., 2009).

In addition to the direct effects of intentional nanoparticle administration, their increasing use in commercial and industrial applications results in nanoparticle accumulation in the environment, which can have significant impact on veterinary species, particularly fish (O'Brien and Cummins, 2010, 2011; Van Hoecke et al., 2011). Recently there has been increased cohesion between nanoresearchers to address the issue of nanotoxicity, with several web-databases and forums available to aid selection of approved nanomaterials and to provide information on the environmental impact and safety of nanoparticles. These include the EU Nanoforum,[1] the Nano Health Environment Commented Database project,[2] 'The Good Nano Guide'[3] and Nanowerk.[4]

In order to address potential safety issues arising from the use of nanotechnology, effective regulation of the nanotech industry is essential. Historically, this has lagged behind nanoparticle application not only in the medical field, but also in the food, construction, energy and automotive industries. At present nanoparticle formulations for veterinary and medical use are approved by the appropriate regulatory bodies in their respective countries, such as the FDA.[5] The regulatory frameworks are often based primarily on the properties of substances in bulk rather than nanoparticle form. However, effective nano-regulation requires an understanding and consideration of the unique properties of nanoparticle formulations (Eifler et al., 2011). The Royal Society was one of the first bodies to initiate a paradigm shift in the attitude towards regulation of the nanotechnology industry with their 2004 report 'Nanoscience and nanotechnologies: opportunities and uncertainties'.[6] But, even by that time many nanotech formulations were already being marketed commercially. It was only in October, 2011 that The Nanotechnology Regulatory Science Act was introduced in the US Senate, to address the health and safety risks of nanomaterials, by which time over 1300 consumer nanotechnology products were available (Pryor, 2011).

Recently, a number of international bodies have also been formed to address concerns over nanotoxicity and safety. These include the OECD Working Party on Manufactured Nanomaterials[7] and ObservatoryNANO.[8] Whilst some progress has been made, the stances of the various regulatory bodies worldwide need to be refined to provide a harmonised, dynamic and inclusive approach to nanoregulation so that they are able to adapt quickly to change in this rapidly expanding field. Detailed information on current nanoregulatory bodies is provided in the 2011 ObservatoryNANO report (ObservatoryNANO, 2011) and the 2011 OECD Working Party on Manufactured Nanomaterials report (OECD, 2011).

Nanoparticle formulations

This section provides a brief synopsis of the most prominent nanoparticle systems, their applications and limitations, with a view to familiarising the reader with the basic details of those formulations available for veterinary application. A complete overview of each of these systems is beyond the scope of this review and references to articles that provide a comprehensive analysis of each nanoparticle system are provided within the text.

Polymeric nanoparticles

Polymeric nanoparticles are prepared by combining the active substance/drug with a polymer. The active components are dissolved in, entrapped in, or adsorbed to the surface of the polymer nanoparticle. Polymeric nanoparticles exist in a variety of forms ranging from nanospheres to dendrimers (Fig. 3a), and utilise both natural and synthetic polymers. Polymer delivery characteristics, surface properties, morphology and composition can be readily tai-

[1] See: www.nanoforum.org.
[2] See: http://nhecd.jrc.ec.europa.eu/.
[3] See: http://goodnanoguide.org.
[4] See: http://www.nanowerk.com/phpscripts/n_dbsearch.php.
[5] US Food and Drug Administration; see: www.fda.gov/nanotechnology/.
[6] See: http://royalsociety.org/uploadedFiles/Royal_Society_Content/policy/publications/2004/9693.pdf.
[7] See: http://www.oecd.org/document/26/0,3746,en_2649_37015404_42464730_1_1_1_1,00.html.
[8] See: http://www.observatory-nano.eu/project/.

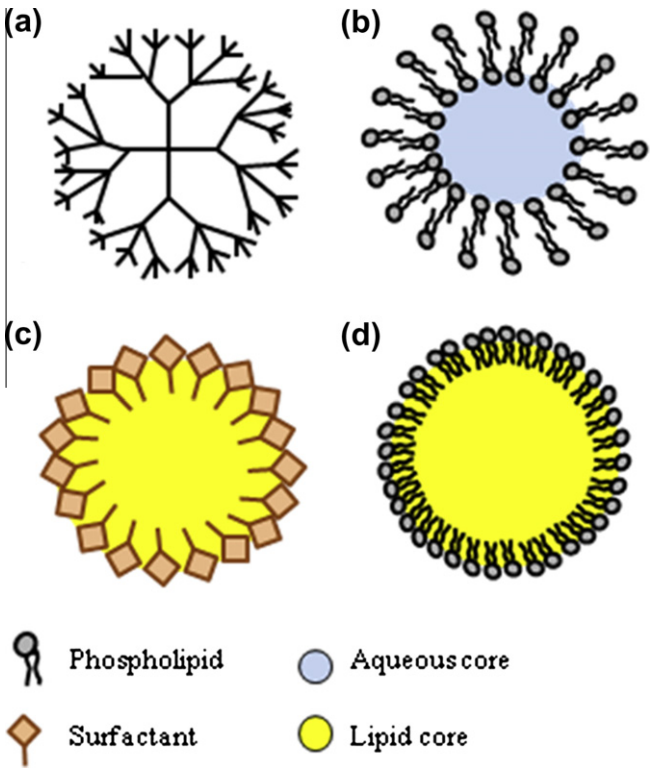

Fig. 3. A schematic diagram of some of the forms of nanoparticle systems discussed in this article: (a) a polymeric dendrimer typical size range 100–200 nm; (b) a liposome, typical size range 50–200 nm; (c) a nanoemulsion, typical size range 50–500 nm; (d) a phospholipid micelle typical size range 5–50 nm.

lored and optimised to achieve the desired drug loading, biocompatibility, targeting, degradation and controlled release kinetics (Yang, 2000).

The primary limitations to the development of polymeric nanoparticles include toxicity, irritancy, the need for a biodegradable soluble material and a lack of large scale production methods (Muller and Keck, 2004). Several reviews have focused on the application of polymer materials in drug delivery (Uhrich et al., 1999; Yuk and Bae, 1999; Paleos et al., 2007) and gene delivery (Morille et al., 2008; Paleos et al., 2009).

Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are composed of lipids that are solid at room temperature, stabilised by surfactant and suspended in an aqueous solution. The pharmaceutical agent is dissolved or dispersed within the lipid. SLNs exhibit several advantages over polymeric nanoparticles. For example, they have comparatively higher drug entrapment efficiency and can be administered by multiple routes (orally, topically, and IV). Moreover, hydrophobic drugs are stable in their lipid matrix, they protect sensitive drugs from the external environment, they have minimal toxicity and they do not require the use of organic solvents in their production (which can be easily scaled up to commercial level) (Blasi et al., 2007; Mishra et al., 2009). Additionally SLNs can provide controlled release formulations lasting up to several weeks, they adhere to mucosal surfaces, promote the absorption of orally administered drugs and have particular potential for drug delivery to the brain as they are capable of transporting pharmaceuticals across the blood brain barrier (Muller and Keck, 2004; Blasi et al., 2009). A thorough review on SLN formulations for drug delivery is provided by Souto and Doktorová (2009).

Liposomes

Liposomes are vesicles in which an aqueous core is encapsulated within one or more phospholipid bilayers (Fig. 3b). They have been used as drug delivery systems since the 1960s, with their first market appearance in 1986 in a cosmetic formulation by Dior, and are considered by some to be the archetypical nanoparticle (Muller and Keck, 2004). Liposomes are highly flexible delivery systems, able to carry both hydrophobic and hydrophilic substances. They can be conjugated to antibodies or ligands and have their size, the number of phospholipid bilayers, lipid composition, surface characteristics and charge engineered to optimise their suitability for an intended use.

Liposomes are suitable for topical, IV and IM administration, but because they are susceptible to degradation in the gastrointestinal tract they are rarely suitable for oral use. They have been investigated for targeted drug, imaging agent, vaccine and gene delivery with promising results (Dams et al., 2000; Bakker-Woudenberg et al., 2005). Several commercially available liposome formulations are on the market and there are multiple studies on their application in the veterinary species (Table 1). However, their application has been slowed somewhat by stability issues, difficult manufacturing techniques and expense. In recent years, the scaling up of liposome production for commercial applications has resulted in the availability of liposomes that have a reasonable shelf life and are an affordable, practical option for use in veterinary medicine.

Liposomes have biodegradable constituents so toxicity is rarely an issue; however, complement-mediated hypersensitivity reactions may occur in response to IV liposome administration (Muller and Keck, 2004; Szebeni et al., 2007). The clinical picture of these complement mediated reactions includes cardiovascular, respiratory and cutaneous symptoms, as well as chest and back pain, hypersalivation, increased urination and defaecation, and even death (Szebeni et al., 2007). Such reactions only occur in a small proportion of individuals and can be prevented by slow infusion and the administration of anti-histamines and anti-inflammatories, but they have inhibited the successful application of liposomes in human medical imaging.

Nanoemulsions

Nanoemulsions are dispersions of oil and water where the dispersed droplets are stabilised with a surface film composed of surfactant and co-surfactant. Most commonly, drugs are loaded into the dispersed phase where the droplet size is typically 20–200 nm. An oil-in-water emulsion consists of dispersed oil droplets within an aqueous solution. Water-in-oil emulsions and water-in-oil-in-water emulsions have also been formulated for biomedical application.

The advantages of nanoemulsions include simple, inexpensive preparations, high stability, the ability to solubilise lipophilic substances and to protect from degradation. Nanoemulsions are versatile and can be prepared by many different aqueous solutions, surfactant and oil constitutes. According to the properties of their constituents, they exist in a wide range of compositions and thus perform a wide variety of tasks.

Low-cost, solvent free nanoemulsions have been produced for use in veterinary species (Vandamme and Anton, 2010) and promising results have been achieved using nanoemulsions for drug delivery, particularly via the oral and transdermal routes (Kawakami et al., 2002; Kang et al., 2004; Ke et al., 2005). However, nanoemulsions are relatively new nanoparticles and a considerable amount of fundamental work needs to be performed to fully establish their physiochemical behaviour. Additionally the high concentrations of solvents, surfactants and co-surfactants in some nanoemulsion formulations can be toxic to the tissues where they

accumulate or are applied, resulting in haemolysis, cellular damage, and tissue inflammation (Karasulu et al., 2007; He et al., 2010). The potential toxicity of the solvents and emulsifiers that are suitable for use have been published by various regulatory bodies including the World Health Organization[9] and the FDA, and any nanoemulsion formulation for veterinary application would have to meet similar regulatory standards. Detailed reviews of the use of nanoemulsions in drug delivery can be found elsewhere (Lawrence, 2000; Karasulu, 2008; He et al., 2010).

Micelles

Polymeric micelles have a unique structural composition characterised by a hydrophobic core sterically stabilised by a hydrophilic shell. Due to their hydrophilic shell they are highly water soluble and evade MPS clearance. Thus, micelles have long circulation times and exhibit the EPR effect. Hydrophobic substances are stored in the micelle core, where they are solubilised and protected from enzymatic degradation.

Micelles are divided into four classes, based on the constituents of their hydrophilic shell: (1) phospholipid micelles, whose shell is comprised of phospholipids; (2) pluronic micelles, where the shell is a block copolymer comprised of hydrophilic polyethylene oxide and hydrophobic polypropylene oxide blocks; (3) poly(L-amino acid) micelles, and (4) polyester micelles with a shell composed of biocompatible polymers (Koo et al., 2005).

Micelles can be easily prepared, have low toxicities and have the potential to be a versatile system for effective delivery of different classes of therapeutic agent, with at least six micelle formulations currently being studied in clinical trials (Matsumura and Kataoka, 2009; Saif et al., 2009). However, careful engineering of the micelle is necessary to ensure long-term stability and drug loading capacity (Blanco et al., 2009). Further information on micelles and their clinical application can be found in the reviews by Blanco et al. (2009), Kim et al. (2010) and Yokoyama (2010).

Inorganic nanoparticles

The most intensively studied inorganic nanoparticles are reviewed in brief below. In early studies, inorganic nanoparticles demonstrated great potential as nanocarriers for therapeutic agents, vaccines and imaging agents. However, their clinical application is limited by concerns over toxicity, lack of biodegradability and persistent tissue accumulation. Therefore relatively few inorganic nanoparticles have progressed to clinical application. Further details are available in a review by Fadeel and Garcia-Bennett (2010) and in the references for the individual nanoparticles.

Ceramic nanomaterials

Ceramic nanoparticles made of materials such as silica, alumina and titania, have several advantages over polymeric nanoparticle systems in that they are easy to prepare and engineer to a desired shape, size and porosity, are biocompatible, have large surface to volume ratios, have high surface tailorability and are extremely inert. Additionally, they protect the adsorbed particles they carry against denaturation induced by extreme pH and temperature. However, titania nanoparticles appear to possess considerable in vivo toxicity (including causing distress and death in rats) and detailed toxicological studies of mesoporous silica nanoparticles are lacking (Fadeel and Garcia-Bennett, 2010).

Carbon nanomaterials

Carbon nanomaterials include fullerenes, carbon nanotubes and carbon nanohorns, and have been investigated as drug carriers. They also have potential for vaccine delivery as they amplify the immunological response (Pantarotto et al., 2003). However, single walled carbon nanotubes trigger oxidative stress and are cytotoxic in cultured cell lines (Fadeel and Garcia-Bennett, 2010) and toxicity and biodegradability are a significant concern in clinical application (Lamprecht, 2009; Surendiran et al., 2009).

Metallic nanomaterials

Various metals have been used to prepare nanoparticles. Gold, silver and copper are most commonly used, with gold nanoparticles being the most intensively studied (Ghosh et al., 2008; Mishra et al., 2009). The main applications of metal nanoparticles lie in biosensing/imaging and cancer thermotherapy, although they are also being explored for targeted drug delivery (Jain et al., 2007; Saha et al., 2007).

Metal nanoparticles can easily be synthesised with a range of sizes (1–150 nm), are stable and can be modified by conjugation with various functional groups (Jain et al., 2007). However, they have a range of toxic effects, including respiratory toxicity, disturbances to trace elements in tissues, inhibition of sodium–potassium–ATPase, and oxidative stress which, combined with their prolonged retention in tissues, is of significant concern (Shaw and Handy, 2011).

Magnetic nanoparticles

Magnetic nanoparticles are commonly composed of iron oxide due to its high in vivo degradability. They have been investigated for use as biosensors, for imaging and for drug delivery. In addition to performing targeted drug delivery by passive and active targeting, magnetic nanoparticles can be pulled out of suspension in the bloodstream and into localised disease sites by application of a high gradient magnetic field over that tissue (Mishima et al., 2007; Nishijima et al., 2007).

Despite having many characteristics that make them excellent agents for drug delivery and imaging, concerns over toxicity and the accumulation of metal-based particles are a significant barrier to the clinical application of magnetic nanoparticles (Veiseh et al., 2009) at the present time. Banerjee et al. (2010) provide a thorough review of the biomedical applications of magnetic nanoparticles.

Quantum dots

Quantum dots are semi-conductor nanoparticles that measure approximately 2–10 nm and fluoresce when stimulated by light. They are comprised of an inorganic core, an inorganic shell and an aqueous coating to which biomolecules can be conjugated. The size of the core determines the colour of light emitted. Biomedical applications of quantum dots are primarily focused on their use as imaging agents (Bentolila et al., 2009). Quantum dots can be targeted to various biomarkers providing a highly sensitive diagnostic and research tool (Halfpenny and Wright, 2010). However, clinical application of quantum dots is limited by their potential cytotoxicity and slow elimination (Iga et al., 2007; Hauck et al., 2009).

Conclusions

Whilst the hypothesised 'nano-bot' that can detect a disease and release a payload of pharmaceutical treatment is far from clinical reality, it is evident from the studies and results detailed in this review that the practical application of nanotechnology to veteri-

[9] See: www.who.int.

nary medicine is far from science-fiction. Rational scientific process underlies the mechanisms by which nanoparticles improve drug delivery, food supplementation, diagnostics and vaccines. Nanoparticle drug carriers are already enabling veterinarians to revisit challenging disease entities using a different approach and increasing their pharmaceutical armamentarium. The application of nanotechnology to veterinary medicine provides the opportunity for improved drug, vitamin, mineral and vaccine delivery by methods suitable for bulk application to wildlife and extensive production systems, and quick, sensitive on-site diagnostic tests for many veterinary diseases. This offers potential for improving herd management and productivity in food producing animals.

Although challenges remain in nanomedicine application, particularly relating to toxicity, nanocarrier residues and production costs, collaboration between various disciplines including human and veterinary medicine, engineering and materials science will expand existing knowledge to resolve these issues, increasing their applicability for veterinary use. Although not yet a dramatic revolution, nanoparticles are edging their way into the veterinary field, and are likely to continue to do so into the future.

Conflict of interest

Neither of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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