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Application of pharmaceutical drug delivery for biological control of the common brushtail possum in New Zealand: a review

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Abstract. The common brushtail possum (*Trichosurus vulpecula*) is the most significant vertebrate pest in New Zealand, being a major ecological threat to the indigenous biodiversity and an economic threat as a vector for bovine tuberculosis. Novel and effective strategies to reduce the population of *T. vulpecula* are needed urgently. Several biocontrol agents are currently being assessed and from research to date it is likely that the biocontrol agents will be peptide or protein molecules. It is not possible to administer such biocontrol agents alone because they would be degraded rapidly in the animal, especially if delivered orally. Technologies used in the pharmaceutical industry to design efficacious drug-delivery systems for humans and animals can be applied to the design of delivery systems for biocontrol agents used in wildlife management, although there are some unique challenges that must be overcome.

Introduction

Overabundant and introduced mammals continue to have unwanted impacts worldwide and there is considerable interest in the development of new and effective methods of control for pest wildlife. The aim of this review is to provide information for biologists about the technologies used in the formulation and delivery of pharmaceutical compounds that could have application in the biological control of pest wildlife.

In New Zealand, 54 species of mammal (including deer, ferrets, stoats, rabbits, rats and hedgehogs) have been deliberately introduced (Cowan and Tyndale-Biscoe 1997) and 25 species are currently managed owing to their pest status (Parkes and Murphy 2003). Although each of these species has had deleterious impacts on the natural flora and fauna, the common brushtail possum (*Trichosurus vulpecula*) is considered to be the major threat to New Zealand's biodiversity and, as such, will be the focus of this review.

The common brushtail possum, a marsupial native to Australia, was introduced into New Zealand in the 1850s with the aim of establishing a fur trade (Pracy 1974). A lack of predators and competitors, and a palatable native flora that lacks chemical defences against browsing herbivores, has enabled the possum population to reach an estimated 70 million with a distribution across more that 90% of New Zealand at densities up to 20 times greater than in their native Australia (Cowan 1990). The pest status of the brushtail possum in New Zealand has been established because possums defoliate native and introduced forests, consume pasture grasses and threatened fauna (e.g. kiwi chicks and land snails) (Cowan and Moeed 1987; Brown *et al*. 1993). The common brushtail possum is also considered to be the primary wildlife reservoir for bovine tuberculosis (Tb) in New Zealand (Coleman and Caley 2000).

The current lethal methods used to reduce the number of brushtail possums in New Zealand are shooting, trapping and poisoning. The humaneness of these methods is increasingly coming into question and, while effective on a local scale, they are not sufficient to reduce the national population over the long term (Cowan 1996). Consequently, much research effort has been invested into developing new methods of control that can be added to the existing arsenal. There are several advantages to using biological control programs rather than conventional control methods. The quantities of poisons used in the environment could be reduced and this would also reduce the risk of poisoning non-target species (Cowan 2000). In addition, after traditional control operations (trapping and poisoning), populations recover more rapidly than can be predicted by birth rates and dispersal alone (Clout and Efford 1984). Described as the 'vacuum effect', neighbouring animals will move into a new area where the local population density has been reduced by control operations (Efford *et al*. 2000; Ramsey 2005). Population numbers are able to grow rapidly because of the local abundance of resources. A strategy that impairs reproduction will have a more sustained effect of reducing the brushtail possum population over the long term because individuals remain in the population and so are not replaced by immigrants but they fail to reproduce and so do not contribute to population growth. It has also been predicted from modelling studies, that bovine Tb would die out in stable, low-density possum populations (Barlow 2000).

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The list of desirable characteristics to be incorporated into the design of an ideal biological control agent is daunting. Ideally, it should be:

- affordable,
- safe,
- not modify animal behaviour,
- target-specific,
- highly potent and induce a permanent effect,
- stable under widely differing environmental conditions.

Future control methods

For the New Zealand public the most acceptable method of eradication of the common brushtail possum is biological control, particularly fertility control (Fitzgerald *et al*. 2000). Biological control relies upon interrupting one or more key biological processes in the target species to cause either death or infertility. Key reproductive events identified in the common brushtail possum that could be targeted to impair fertility include ovarian function, hormone production, sperm maturation, fertilisation and lactation, although targeting the last is deemed unacceptable on ethical grounds (Cowan 1996). Target systems under investigation include both immune-based and non-immune-based (direct) fertility control. Both present formidable formulation challenges for their delivery, especially if via the oral route. The first challenge is to deliver the biocontrol agent to the animal in the field. Delivering biocontrol agents to the brushtail possum orally (in baits) is the most practical choice for delivery: the brushtail possum has a widespread distribution across New Zealand, including remote areas of inaccessible terrain, and baits can be distributed aerially to allow broadcast distribution. The brushtail possum is also a wild animal, which cannot be herded into a confined locality in order to administer a biocontrol agent at close range.

Immunocontraception has received a great deal of attention as a method of fertility control for several pest species, including foxes (*Vulpes vulpes*) and rabbits (*Oryctolagus cuniculus*) in Australia (Seamark 2001) and brushtail possums in New Zealand (Polkinghorne *et al*. 2005). With this technique, animals are immunised with key proteins involved with reproduction, such as egg coat (zona pellucida) proteins or sperm proteins. The body then recognises these self-proteins as foreign and mounts an immune response against them, thus inducing infertility. That is, they act as a vaccine. Immunocontraception has been used with success in some populations of feral animals injected with zona pellucida (ZP) antigens. For example, in wild horses (*Equus caballus*), more than 90% infertility that lasted for 1 year has been reported in mares given injections of a porcine ZP vaccine (Turner *et al*. 2002). However, when ZP proteins are delivered using recombinant virus vectors that express ZP proteins, no detectable immune response is reported in foxes (Reubel *et al*. 2005) and rabbits (Mackenzie *et al*. 2006). ZP proteins have been characterised for the common brushtail possum (Mate *et al*. 2003) and Duckworth *et al*. (1998) reported that in captive brushtail possums there was a 75% reduction in fertility compared with the control group when females were immunised with whole ZP by subcutaneous injection, although the duration of this infertility is unknown. Marsupial-specific epitopes of ZP have also been identified (Cui and Duckworth 2005) and are being investigated as a possum-specific vaccine.

There are several potential delivery issues with the immunocontraception strategy. The first of these is how to administer an immunologically effective dose of the agent to free-ranging feral animals in the field. The most successful studies to date have involved delivery via injection, co-administration of an adjuvant and sometimes more than one vaccination (Duckworth *et al*. 1998; Cooper and Herbert 2001). A system that relies upon injecting the animal is not feasible for free-ranging animals and thus alternative delivery systems will be required. Furthermore, the degree of genetic variation in the immune system between individual animals means that the response to vaccination is likely to vary widely, so a proportion of the population subjected to a standard dose of immunogen may remain fertile (Barlow 1997; Nettles 1997). For example, Deakin *et al*. (2005) found that the antibody response by brushtail possums to synthetic peptide hormones was highly variable and these authors questioned the feasibility of immunocontraception as a control method for this species. The variable immune response has ongoing consequences as only non-responding possums would produce offspring, which most likely would also be non-responders. It has been suggested that the proportion of the possum population that fails to respond to an immunocontraceptive agent would rapidly increase over time (Cooper and Herbert 2001).

In contrast, non-immune-based biocontrol agents (chemical sterilants or hormonal contraceptives) are alternative control agents that would act directly on target cells or tissues and not rely upon an immune response. Research in this area has been largely focused around luteinising hormone-releasing hormone (LHRH) because of its critical role in the regulation of reproductive function. An example of a hormonal sterilant under investigation is a cytotoxic antiviral protein isolated from the pokeweed plant (*Phytolacca decandra*). The protein (pokeweed antiviral protein, PAP) is conjugated to LHRH and then internalised into the gonadotroph cells of the pituitary. Here, PAP kills the cells that produce luteinising hormone (LH) and follicle-stimulating hormone (FSH) (Eckery *et al*. 2001). An alternative approach is to deliver highly potent analogues of LHRH that induce down-regulation of the LHRH receptors (D'Occhio *et al*. 2000) and thus block the secretion of LH and FSH. This method requires a controlled-release formulation that delivers the LHRH analogue over a prolonged period following a single administration. Direct-acting sterilants or contraceptives are not likely to be influenced by

genetic variation between animals, but they can involve special challenges in delivery of the bioactive compound. For example, those methods described above require the bioactive compound to be delivered to the pituitary gland.

Species specificity

In New Zealand, chemical control methods (both lethal and fertility control) may be based on exploiting physiological differences between eutherian mammals and the marsupial brushtail possum, to achieve some level of specificity within the biocontrol agent. It has been demonstrated that some aspects of gastrointestinal (GI) physiology in the brushtail possum are markedly different from those in eutherian mammals (Butt *et al*. 2002*a*). Such differences could potentially be utilised in the design of a species-specific bioactive compound, either by targeting specific differences to disrupt physiological function, or by utilising specific mechanisms for delivery and uptake of the biocontrol agent. For example, the mechanism that drives the secretion of fluid in the intestine of the common brushtail possum is markedly different from the mechanism identified in placental mammals. Butt *et al*. (2002*b*) have found that the secretion of fluids in the intestine of the brushtail possum is driven by bicarbonate ions, not by chloride ion transport as it is in eutherian mammals. Research is now focused on how these mechanisms function in the brushtail possum and on the identification of compounds that can irreversibly turn on fluid secretion in the gut. Hypersecretion in humans causes blood pressure to fall abruptly and the presentation of clinical signs of dehydration, such as lethargy, before coma leading to death. The effect of such a toxin is yet to be tested in the brushtail possum. The action of such a toxin would be much more rapid and have fewer clinical signs of distress than the poisons currently used in New Zealand, which include anticoagulants (e.g. brodifacoum), cyanide and the most commonly used compound, 1080 (Littin *et al*. 2002).

Target specificity can potentially be incorporated at the level of the bioactive compound, but also in the bait if the oral route is chosen for delivery. This is an important consideration to reduce the risk of affecting non-target species, primarily native birds in New Zealand. For example, colouring the baits green minimises the attraction of the bait to birds and a cinnamon-scented lure masks the taste of the poison in baits and also repels birds (see Spurr 2000). Acceptance of the bait is also important to the success of a control program to ensure that the animals do not become bait shy (O'Connor and Matthews 1999; Ogilvie *et al*. 2000). Carrot baits are considered more desirable than cereal baits because they are more palatable to possums and are also more robust in the field (Bowen *et al*. 1995), also an important consideration when there will be little control over storage conditions for distribution in remote areas. The infrastructure for producing and distributing poison baits is already well established and the acceptance of baits by the animals is high (Morgan and Hickling 2000), thus there is the potential to incorporate a biocontrol agent within oral baits.

Delivery systems

From research to date, it is highly likely that any biological control agents developed to reduce fertility in the common brushtail possum will be proteins or peptide molecules (Duckworth *et al*. 2001; Eckery *et al*. 1999). Whether the agent is an immunocontraceptive, a chemical sterilant or a toxicant, the challenge is to deliver these biological control agents safely and specifically to a free-ranging, feral animal that has a widespread distribution across most of the New Zealand landmass, including remote and inaccessible areas. Delivery systems can be broadly classified as disseminating (transmissible) or non-disseminating, based on their mode of distribution.

Disseminating delivery systems

The advantage of disseminating delivery systems is their ability to spread the bioactive compound unaided through the target population by means of a vector that has been administered to only a few animals within that population. An example of a potential disseminating system for possums under investigation is the naturally occurring, parasitic gut nematode *Parastrongyloides trichosuri*, which is brushtail possum–specific (Cowan *et al*. 2005). The nematode can rapidly infect large numbers of animals and this has been shown in a field trial in the Kahurangi National Park near Nelson in the South Island of New Zealand (Ralston *et al*. 2001). When introduced into a few possums within a parasite-naïve population, the nematode had spread across 400 ha in 52 weeks (over 5000 ha by Week 135) and was present at a high prevalence (Ralston *et al*. 2001). The assumption is that nematodes that have been genetically modified to contain a gene encoding for a biocontrol agent would be equally effective in infecting large populations of animals, thus rapidly disseminating the biocontrol agent.

Viruses engineered as vectors to carry genes for immunocontraceptives, or as pathogens, are also under investigation in New Zealand as potential disseminating delivery systems. Viruses identified from brushtail possums in New Zealand include adenovirus, coronavirus and herpes virus (Rice and Wilks 1996), a possum papillomavirus (Perrott *et al*. 2000) and a retrovirus (Baillie and Wilkins 2001). Possum adenovirus (PoAdV-1) has been selected as the most promising candidate as a vector because the genomes are easy to manipulate and the viruses are readily transmitted between individuals (Thomson *et al*. 2002). A single genotype of PoAdV-1 has been isolated and sequenced from individual possums from different localities in New Zealand (Thomson *et al*. 2002), although it has not been possible to replicate this virus in cell culture. The macropod herpes virus causes fatal infections in marsupials such as wallabies and kangaroos in Australia. The virus can cause mild infections in the brush-

tail possum (Zheng *et al*. 2004); however, the transmission from infected animals has not been demonstrated and so the usefulness of this delivery system remains to be established. Another virus infecting brushtail possums is the wobbly possum virus (Mackintosh *et al*. 1995), but as this invariably causes death it would not be suitable as a transmission agent.

With all genetically modified organisms and disseminating systems, there is considerable public concern about the inadvertent transfer of these organisms, and the genes they carry, particularly as it would be impossible to withdraw the organism once released. This is especially so in the case of the common brushtail possum, which, while a major pest in New Zealand, is a protected and valued endemic animal in Australia. The potential for inadvertent or illegal transfer to Australia is considered to be high, owing to the frequent exchange of people and goods between the two countries and the precedent of rabbit hemorrhagic disease being illegally brought from Australia to New Zealand (Cooke *et al*. 2004; Gilna *et al*. 2005). There is ongoing debate in the scientific community about what regulatory processes would need to be in place and the management strategies for the implementation of genetic technologies to control overabundant wildlife (Cooke *et al*. 2004).

Non-disseminating delivery systems

While having the obvious disadvantage that the biocontrol agent must be administered to each individual animal, non-disseminating delivery systems offer the advantage that the risks of uncontrolled spread are removed and that the biocontrol agent can be withdrawn if necessary. There is a wide range of potential non-disseminating delivery systems including genetically modified plants, such as carrots and potatoes, that express an immunocontraceptive vaccine within the genome of the transgenic plant (Smith *et al*. 1997). The plant material can then be fed directly to the animal or incorporated into baits. Carrots are already used as baits in poison operations for possums and this root plant material has the advantage that it is robust in the environment (Polkinghorne *et al*. 2005).

Another example of a non-disseminating system is bacterial ghosts, which are empty envelopes of cell membrane from dead Gram-negative bacterial cells that retain the surface antigens of the living cell (Mayr *et al*. 2005). Recombinant proteins and antigens can be loaded onto the inner or outer cell membranes, or within the periplasmic space or in the lumen of the cytoplasmic space of the bacterial ghosts (Mayr *et al*. 2005). The ghosts are taken up by the dendritic cells of the immune system of the target animal, processed and the recombinant proteins expressed within the host (Jalava *et al*. 2002). An advantage of bacterial ghosts as an antigen carrier is that the ghosts retain their immunostimulatory membrane structure. However, in studies to date, the immune response after oral administration of ghosts containing ZP antigen to the common brushtail possum has been low (Duckworth *et al*. 2001), which may be due to degradation of the ghosts in the gastrointestinal tract. Their stability following oral ingestion is an area of current investigation (J. A. Duckworth, personal communication).

Virus-like particles (VLPs) are another non-disseminating delivery system that targets the immune system. VLPs are composed of structural proteins that are able to self-assemble into hollow particulates and they have been investigated for application in delivery of DNA and therapeutic proteins to treat human diseases (Pattenden *et al*. 2005). VLPs are non-infectious, but able to stimulate the immune system because they mimic the structure of viruses (Jiang *et al*. 1999). VLPs from more than 30 viruses are being developed for use in both humans and animals (Noad and Roy 2003). Although there is evidence that these delivery systems are effective and safe, they are more costly than attenuated vaccines because higher doses of antigen are needed compared with attenuated vaccines (Noad and Roy 2003).

Routes of delivery

The preferred route for the administration of biologically active compounds, in terms of maintaining potency and maximising concentrations in the circulation, is via injection (either intravenous, subcutaneous or intramuscular). However, the distribution of pest species over large areas and the wild nature of pests means that injections are not practical. The nasal route offers some advantages for the delivery of peptide and protein biocontrol agents for several reasons: this route avoids first-pass metabolism in the liver; enzyme degradation is much lower than in the intestine; and the mucosal surface is highly vascular and has a large surface area (Illum 2002). However, it would be necessary to administer such a nasal formulation from a mechanical device designed to deliver a spray to animals in the field. A spray device would be technically very difficult to achieve and to maintain in the field. Furthermore, a study by McLeod *et al*. (1998) on the common brushtail possum reported low systemic uptake of a model peptide hormone, LHRH, following nasal administration.

Delivery using an oral bait is likely to be the easiest, safest and most convenient method for delivery in the field to widely dispersed wild animals such as the common brushtail possum (Rodger 1999). The oral route is used primarily to achieve a systemic effect following absorption of the bioactive compound from the GI tract. However, before compounds can be absorbed from the GI tract, the formulation must be able to withstand the acidic environment of the stomach, be emptied from the stomach within an appropriate time to limit degradation and reach the site of absorption.

Formulation strategies to enhance oral delivery of peptides

Peptides and proteins have poor intrinsic permeability across membranes owing to their hydrophilicity, electrical charge and structural conformation (Yang *et al*. 2002), which results

in a bioavailability (the fraction of an administered dose that reaches the systemic circulation) of typically 1–2%. In addition to the physical barrier of the epithelium lining the GI tract, there is also a chemical barrier, where compounds may be exposed to enzymatic degradation by the secretions of the GI tract (Mahato *et al*. 2003). These barriers to the oral delivery of proteins and peptides can, at least in part, be overcome by using formulation strategies that protect the bioactive compound and facilitate absorption across the intestinal mucosa.

Chemical modification

One technique that can be used to enhance the stability and uptake of peptides in the body is to attach a lipid portion to the peptide. Such chemical modification of the bioactive compound by lipidification results in a higher affinity with the phospholipid cell membrane, which enhances passive transport across the intestinal membrane (Toth *et al*. 1999). In addition, the stability of the peptide is improved by the attachment of a lipid group, because enzyme recognition of cleavage sites is interrupted by the presence of the lipid side chain (Toth *et al*. 1999). Lipidification of the polypeptide salmon calcitonin has been shown to increase its retention in the plasma of rats when injected subcutaneously (Wang *et al*. 2003) and thus increase the time available for uptake into target tissue(s). Similarly, after oral administration to rats, radio-labelled LHRH–lipid conjugates were absorbed into the systemic circulation and retained in plasma for up to 10 h (Flinn *et al*. 1996).

Penetration enhancers and enzyme inhibitors

Co-formulation with penetration enhancers (bile salts or detergents), which disrupt the tight junctions that bind epithelial cells to their neighbouring cells, can enhance paracellular absorption of bioactive compounds (Aungst 2000). The transcellular pathway can also be disrupted by penetration enhancers and lead to increased transport of peptides and proteins in the intestine. Examples of the types of penetration enhancers used include chelating agents that bind Ca^{2+} in the tight junctions (Noach *et al*. 1993) and surfactants and bile salts that disrupt the cell membrane integrity (Schep *et al*. 1997) (Table 1). McLeod *et al*. (2005) have demonstrated in the common brushtail possum that, when co-administered with sodium deoxycholate (SDA), the intestinal permeability of the reference compounds fluorescein and LHRH increased 33-fold and 63-fold respectively.

Enzyme inhibitors (Table 1) can also be used as excipients (those ingredients other than the bioactive compound included in a formulation) to reduce the degradation of bioactive peptides and proteins by hydrolysis in the GI tract. In the specific case of the common brushtail possum, soybean trypsin-chymotrypsin inhibitor, SDA, carbopol and bacitracin all significantly inhibited the degradation of LHRH and bovine serum albumin (BSA) *in vitro* in the presence of enzymes from the GI tract (Wen *et al*. 2002*a*).

Particulate delivery systems

A particulate delivery system that incorporates the bioactive compound is an attractive formulation option for protecting the bioactive compound from enzymatic degradation in the body and for facilitating its subsequent uptake at the target site. For example, liposomes are a versatile colloidal delivery system because both their size (20 nm to 2 μm) and composition (single or multiple bilayers) can be manipulated. In addition, they have both lipophilic and hydrophilic domains within their structure that can be utilised to carry a range of drugs and vaccines (Sihorkar and Vyas 2001). Liposomes have been successfully used in mice to deliver the protein insulin via the lungs, leading to a reduction in blood glucose levels (Huang and Wang 2006). Liposomes may have some potential to be used for the oral delivery of peptides and proteins, although *in vivo* results to date have been variable (Rogers and Anderson 1998) because liposomes are unstable in the lower GI tract (Faas *et al*. 2001).

Another type of particulate delivery system that can protect the bioactive compound *in vivo* is microparticles (microspheres or microcapsules). In the size range between

Table 1. Examples of permeation enhancers and enzyme inhibitors used to improve the oral delivery of peptides and proteins in mammals
From Mahato *et al*. (2003)

	Group of compounds	Example compound
Permeation enhancers	Chelating agents	Ethylene diamine tetra-acetic acid (EDTA)
	Surfactants	Sodium dodecyl sulfate (SDS)
	Bile salts	Sodium deoxycholate (SDA)
	Fatty acids	Lauric acid
	Mucoadhesive polymers	Chitosan
Enzyme inhibitors	Chelating agents	Ethylene diamine tetra-acetic acid (EDTA)
	Bile salts	Sodium deoxycholate (SDA)
	Mucoadhesive polymers	Polyacrylic acid
	Protease inhibitors	Aprotinin
	Peptidase inhibitors	Bacitracin

1 μm and 1 mm, microparticles are spherical drug carriers made of selected polymers that have low toxicity and high biocompatibility (Kissel and Koneberg 1996). The polymers used most extensively to prepare microparticles are poly(lactic acid) (Vila *et al*. 2002) and poly(lactic-co-glycolic acid) (PLGA) (Damgé *et al*. 1996). Recently there has been considerable research into the use of PLGA microparticles for the delivery of vaccine antigens and DNA because they are able to act as adjuvants and elicit a cytotoxic response *in vivo* (Jiang *et al*. 2005), an essential quality when the immune system is the target. Despite the advantages of microparticles, the stability of proteins once entrapped within them can be low (Schwendeman 2002).

Nanoparticles are a promising and versatile delivery system suitable for the delivery of a range of therapeutic agents ranging from small-molecular-weight drugs to macromolecules (proteins and peptides) and DNA (Panyam and Labhasetwar 2003; Tiyaboonchai *et al*. 2003). Nanoparticles are polymeric, spherical structures in the size range of 10–1000 nm and have the advantage that they are stable in biological fluids, can protect therapeutic agents from enzymatic degradation and can provide controlled drug-release profiles owing to their polymeric nature (Damgé *et al*. 1997; Moghimi *et al*. 2001). Depending on the method of nanoparticle preparation, therapeutic agents can be dissolved, encapsulated or adsorbed to the nanoparticle. There are two different types of nanoparticles recognised in the literature (Fig. 1): nanocapsules that contain the drug within an internal cavity surrounded by a polymer wall, and nanospheres that are solid matrix systems with the drug dispersed throughout the matrix (Soppimath *et al*. 2001). The core of the nanoparticle can be an oily compartment for the encapsulation of lipophilic drugs (Al Khouri Fallouh *et al*. 1986; Cournarie *et al*. 2004) or an aqueous core for the incorporation of hydrophilic drugs (Watnasirichaikul *et al*. 2002*a*).

Particulates in this submicron size range have been shown to move across the gut epithelium and enter the systemic circulation (Kreuter 1996; Aboubakar *et al*. 2000; Damgé *et al*. 2000), and they can be taken up by epithelial cells (Florence *et al*. 1995). For example, in the rat, uptake of nanoparticles from the intestine was up to 250 times greater for 100-nm-sized particles than for 1-μm particles (Desai *et al*. 1996). The particle size is a property that can be controlled during the preparation of nanoparticles (Damgé *et al*. 1996; Vila *et al*. 2004). Watnasirichaikul *et al*. (2002*b*) have demonstrated the effectiveness of poly(ethyl cyanoacrylate) nanoparticles containing insulin to reduce blood glucose levels in diabetic rats.

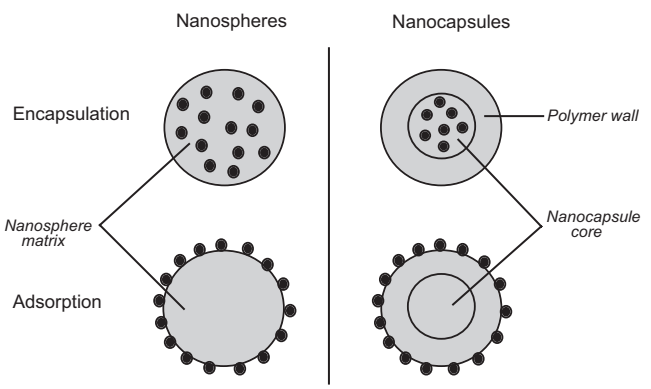

Fig. 1. Schematic diagram of the types of nanoparticles produced from the encapsulation and sorption methods of entrapment. ● = Bioactive molecule. Modified from Krauel *et al*. (2005).

Targeting the colon

There are advantages to delivering bioactive compounds to the colon rather than other regions of the gut, including a near-neutral pH, lower proteolytic activity than in upper regions of the GI tract and a long transit time increasing opportunities for uptake (Sinha and Kumria 2003). For the common brushtail possum, Wen *et al*. (2002*b*) have demonstrated that the proteolytic activity of enzymes from both the lumen and mucosa of the hindgut were 100–1000-fold less aggressive towards a model peptide (LHRH) and protein (BSA) than those in the small intestine. Several strategies have been suggested to achieve site-specific delivery of bioactives to the colon (Friend 2005). Many of these rely on the physiological differences between this region and the small intestine. For example, there is a gradual change in pH throughout the GI tract that could potentially be used to trigger the disintegration of polymer-coated systems. However, Ashford *et al*. (1994) have shown that there is great variability in the site of release when using pH as the trigger mechanism with polymethacrylate coated tablets *in vivo*.

Surrounding a particulate formulation with an enteric coating can protect the delivery system from the acidic environment in the stomach after oral ingestion. Pectin, ethylcellulose, guar gum and chitosan are examples of the polymers that have been used for colon-targeted delivery (Wakerly *et al*. 1996; Macleod *et al*. 1999; Sinha and Kumria 2001). Contained in the colon is an abundant and diverse microflora that functions to ferment fibre with hydrolytic enzymes (Watts and Illum 1997). The enzymes secreted by the microflora in the colon can also be utilised to degrade the polymer and thus to release the bioactive compound. Bacteria-mediated degradation is a promising area for targeted delivery because of the abrupt increase in microflora between the small intestine and the colon (Sinha and Kumria 2003).

The use of pro-drugs is another strategy that has been used to target the delivery of drugs to the colon. A pro-drug is a polymer–drug conjugate and is defined as an inactive derivative of a drug that requires enzymatic transformation to release the active drug (Reddy *et al*. 1999). To target the colon, the system relies on the enzymes produced by colon-specific bacteria. The most commonly used and most extensively studied pro-drugs are those with an azo-bond between

the active molecule and the inactive moiety (Sinha and Kumria 2003). An example is the pro-drug sulphasalazine containing the active drug mesalamine, used for the treatment of irritable bowl syndrome in humans (Friend 2005).

In order to target the colon as a site for drug release following oral administration, it is essential to have knowledge of gastrointestinal transit times and of the factors that influence transit through the GI tract. These have been extensively characterised in humans (Wilding *et al*. 2001), where total gastrointestinal transit times can be influenced by factors such as posture, physical activity and stress level of the individual (Coupe *et al*. 1992), and are determined largely by gastric emptying (Smart and Kellaway 1989). The gastrointestinal transit of oral formulations has been investigated in the brushtail possum (McDowell *et al*. 2005). To target the hindgut for oral delivery of protein and peptide biocontrol agents, a formulation would need to protect the bioactive compound and prevent release for ~12 h. Gastrointestinal transit was shown to be independent of body mass, sex and time of day that the animal received the dose (McDowell *et al*. 2005). This is valuable information when considering remote delivery of oral baits to brushtail possums in the field where it is not possible to control when the animal consumes the bait.

Delivery system considerations

A key issue when considering broad-scale distribution of a delivery system containing a biocontrol agent is the potential effect on non-target species. As discussed previously, specificity of the biocontrol agent for common brushtail possums in New Zealand is being investigated by targeting physiological characteristics that are unique to marsupials. The disseminating delivery system that has the most potential for specificity is the nematode vector, *Parastrongyloides trichosuri*. For the non-disseminating delivery systems (baits), the knowledge obtained through poisoning control operations could be adopted (Morgan and Hickling 2000).

Assessment of delivery systems in vivo

Any potential delivery system must be evaluated *in vivo* in the target species. Achieving appropriate physiological concentrations of the bioactive compound in the plasma of the animal is the first critical part of successfully achieving the desired response of a biological control agent (e.g. a reduction in fertility). The development of formulation strategies for oral delivery of peptides and proteins to be applied to a marsupial species, the common brushtail possum, is an especially difficult challenge because there are gaps in our knowledge of basic physiology of the brushtail possum and very little information on formulations for use with marsupials is available. McLeod *et al*. (2005) have investigated the pharmacokinetics following intestinal absorption of the model compounds fluorescein and LHRH in the common brushtail possum. These authors found that the plasma concentration of fluorescein increased from 0.3 ± 0.01 μg mL^{-1} to 7.8 ± 1.64 μg mL^{-1} with the addition of SDA as a permeation enhancer and LHRH rose from 0.1 ± 0.02 ng mL^{-1} to 6.3 ± 0.45 ng mL^{-1} with addition of the enhancer.

Until specific biocontrol compound(s) are identified, research on the development of pharmaceutical delivery systems for the common brushtail possum is being conducted using 'model' peptides and proteins. Once a potential bioactive candidate has been identified, it will be necessary to understand the physiochemical properties of the active compound (e.g. stability) in addition to the biological factors that influence absorption and degradation.

The effectiveness of biocontrol agents will also need to be evaluated in the target species at the population level. When used in the situation of managing pest species, population modelling can be used to predict the outcome of different control regimes, estimate the amount of control effort that needs to be applied and estimate the success of control operations on the target population (Barlow 2000). In New Zealand, population modelling has been used to predict the population dynamics of brushtail possums in response to harvesting for their fur (Clout and Barlow 1982). More recently there has been an emphasis on modelling *Mycobacterium bovis* infection, the cause of bovine Tb, and the role of the brushtail possum population in transmitting this disease (Caley and Ramsey 2001; Arthur *et al*. 2004). Through such modelling studies, useful information has been obtained about the population dynamics of the brushtail possum in different habitats in New Zealand, such as density, survival and distribution (Efford 2000).

Stability of formulations in the field

Preserving viability of the bioactive compound during manufacture, storage and administration is of vital importance. Hydrolysis and oxidation are the most common forms of chemical degradation for bioactive compounds (Florence and Attwood 1998), although the specific type of degradation pathway will depend on the chemical nature of the bioactive compound in question.

An aspect of delivery systems important to wildlife applications, not usually considered in the pharmaceutical setting, is the stability of formulations under extremes of environmental conditions, as would be encountered in the field. Formulations developed for field use for control of the common brushtail possum in New Zealand will be exposed to a range of temperatures including wide day/night fluctuations, extremes of humidity, freeze/thaw cycles, ultraviolet light and rainfall, and fungal and microbial contamination. Consequently, the delivery system or dosage form can undergo chemical, physical and microbial degradation. Thus, the stability of the dosage form under environmental conditions will need to be assessed and the formulation must incorporate features that will protect the bioactive compound without compromising potency or delivery to the targeted region of the gastrointestinal tract.

Conclusion

The development of biological control methods for vertebrate pest wildlife requires innovative approaches. For a biological control program to be successful, it must be underpinned by the ability to effectively administer the control agent(s) to the target population. Approaches used in the pharmaceutical industry can potentially be applied to the delivery of biocontrol agents to wildlife. To apply the techniques developed in the pharmaceutical industry to management of pest species, it is necessary to characterise relevant aspects of the biology and physiology of the target species and to develop *in vivo* methods to assess the effectiveness of delivery systems. Any future biological control strategy should be used in combination with traditional control methods in an integrated management system to achieve a sustainable reduction in the population.

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