Developing and delivering oral contraceptives for Grey Squirrel

G. Massei 23 May 2017

Summary

This is a proposal to move the existing fertility control science to the point that a strategy for the British grey squirrel is available and a product is ready for manufacture.
The research, which would be expected to last 5 years, will involve using existing and proven US developed contraceptives and marrying them with leading edge British technology which would allow these contraceptives to be delivered orally. In parallel a species specific hopper, based on existing and proven designs for warfarin hoppers, will be tested to deliver oral contraceptives and form part of the overall end research product.
The research will be carried out by the National Wildlife Management Centre, which is part of the Animal & Plant Health Agency unit based outside York.

Background

Fertility control is widely advocated as a safe, effective and publicly supported alternative to lethal control. Single-dose injectable immunocontraceptives, based on vaccines that generate an immune response to proteins essential for reproduction, are increasingly used for wildlife management. For instance, a single dose of the injectable immunocontraceptive vaccine GonaCon, which targets the gonadotropin releasing hormone (GnRH), can induce infertility (in both sexes) for several years in many wildlife species.
In contexts where capture, injection and release are not feasible or economically viable, the availability of oral contraceptives would multiply the breadth of field applications, particularly as species-specific oral delivery systems already exist for some potential target species.

Defra-funded projects demonstrated that a novel GnRH-based compound, tested as an oral contraceptive vaccine, generated an immune response in laboratory rats and reduced fertility in some animals. This was the world's first ever demonstration that an animal's immune system can be triggered by oral administration of an immunocontraceptive vaccine. Building on these results, novel formulations are required to develop an oral vaccine that elicits a substantially greater and more persistent immune response. A promising approach is an encapsulation technology patented by the UK company Sporomex Ltd. and based on pollen grains and spore shells (SpECs, see technical Addendum).

Developing safe, effective and relatively long-lasting oral contraceptives is the first step for controlling overabundant wildlife populations. Another key step is assessing how these contraceptives can be delivered to a sufficient proportion of the target population so that fertility control can reduce significantly population size. As bait uptake by squirrels will affect the success of a campaign aimed at reducing population size through oral contraceptives, the study will also focus on conducting captivity and field trials to assess bait uptake by grey squirrels and to address the practical aspects of delivering these drugs to grey squirrels. In parallel, modelling will be used to examine the effects of fertility control and culling on population size and to compare the effort required by these methods to eradicate grey squirrels.

Specific objectives of this project are:

1. Identify formulations of SpECs for effective delivery of an orally active, microencapsulated immunocontraceptive vaccine in a model species;
2. Test formulations of putative oral contraceptive vaccines to induce infertility in a model species;

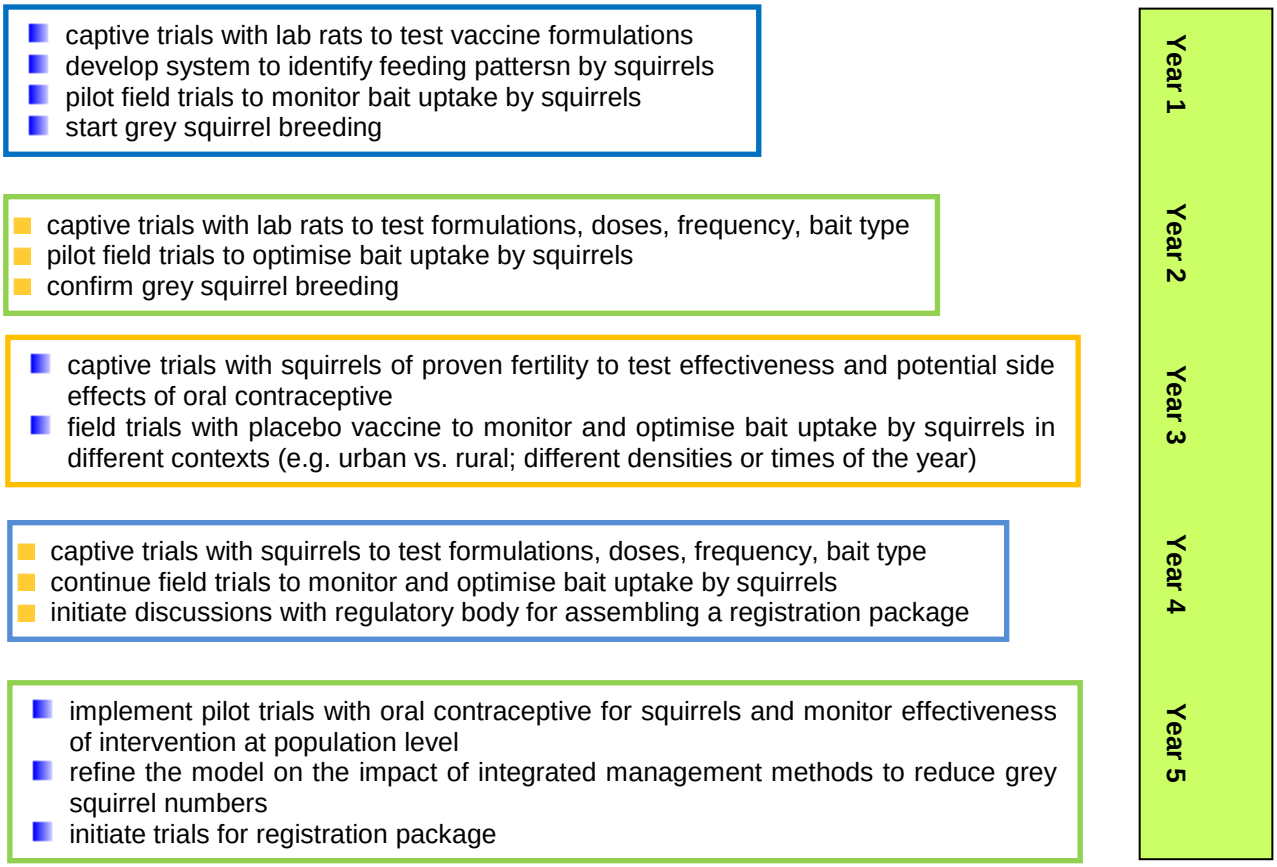
3. Test effectiveness, longevity and potential side effects of an oral contraceptive in a model species in captivity trials;
4. Set up a breeding colony of grey squirrels in captivity;
5. Test effectiveness, longevity and potential side effects of an oral contraceptive in grey squirrels in captivity trials;
6. Develop a monitoring system to record the number of squirrels' visits to bait stations;
7. Formulate a liquid or paste-based bait, test palatability and monitor potential spillage of this bait in captive and pilot field trials;
8. Test bait uptake in captive and field trials, with baits containing placebo contraceptives and bait markers, to quantify the proportion of the target population consuming baits and to ensure bait uptake by non-target species is minimum or nil
9. Model the impact of fertility control and culling on grey squirrel population size
10. Implement pilot trials to monitor the effect of fertility control on grey squirrels populations.

Cost and timing

The project will last 5 years, starting in April 2017 and will cost £ 1,000,000.

The majority of the funding will cover staff time; the remaining funding will cover consumables and contribution by collaborators such as Sporomex that will provide the SpECs formulation technology, by the US National Wildlife Research Center and by a French company that will provide the immunocontraceptive vaccine.

Overall 5 year workplan



PLEASE NOTE: the results obtained each year, and discussions with the customer, will guide the phases of the project in subsequent years.

Expected benefits of the 5-year study

- An effective, safe, long-lasting oral contraceptive for grey squirrels
- A system (feeder and bait) developed to optimise delivery of the oral contraceptive to free-living squirrels
- Proof of concept that microencapsulation with SpECs can be used to optimise the delivery of vaccines, with potential use for other vaccines such as the parapox vaccine for the red squirrel
- A model predicting the impact of fertility control on grey squirrel population size
- Proof of concept that oral contraception can be employed, alone or in conjunction with culling, to eradicate local populations of grey squirrels
- An oral contraceptive vaccine which can be considered for other wildlife mammal species, both in the UK and in Europe, particularly for wildlife for which species-specific feeders already exist, thus strengthening the business case for its registration.

Project Staffing

The Project Lead will be Dr Giovanna Massei who sits on the US-based Botstiber International Institute for Wildlife Fertility Control and on the European Group for Zoo Animal Contraception and has more than 12 years of experience of this area. Dr Massei is currently leading the R&D in fertility control for wildlife. She has been at APHA for 19 years and she is now the head of a team of 14 staff.

Collaborators include the National Wildlife Research Center (NWRC, USA), world leader on developing contraceptives for wildlife, Sporomex Limited (UK) which patented the SpECs technology and French partners that developed part of the vaccine conjugate in collaboration with the NWRC.

All organisations have agreed to collaborate with APHA on this study and have contributed technical expertise to the proposal.

Contribution to funding

A number of organisations have already contributed to the funding of this project. In addition, Defra committed funding, for at least two years to develop the model on the impact of fertility control on population size. Many other organisations have pledged funding for this project.
The funding has been coordinated by the UK Squirrel Accord.

Regular project updates, at least every 6 months, will be given to funders, by the Project Lead and by the Chair of the UKSA in layperson reports, newspaper articles and public presentations. The results of the project will be published in open-access, peer-reviewed journals, in popular science articles and presented to scientific conferences, workshops and webinars. The contribution of all funders will be formally acknowledged in presentations, reports and scientific articles.

Technical Addendum

Encapsulation technology is commonly employed in pharmaceutical preparations to achieve controlled drug release. Mucoadhesive polymers are sometimes used to produce microcapsules that maintain intimate contact with the mucosa of the gastrointestinal tract thereby achieving improved bioavailability and absorption (e.g. Wakil et al. 2010, Tomaro-Duchesneau et al. 2013, Ma et al. 2014). Amongst novel technologies for delivering pharmaceuticals, the use of sporopollenin shells has recently emerged for microencapsulation of a wide spectrum of drugs. This quickly expanding technology, patented by the UK company Sporomex Limited, is based on pollen grains and spore shells that are employed for microencapsulation of pharmaceuticals. The shell of pollen grains and

spores is composed of two layers, the inner layer (intine), made largely of cellulose, and the outer layer (exine), composed mainly of the chemically resistant polymer sporopollenin. Chemical or enzyme processes are used to remove the internal genetic material of pollen grains and spores to obtain the ssporopollenin exine capsules (SpECs). SpECs are elastic and porous, due to the presence of multidirectional nano-diameter sized channels through which SpECs can be filled with an active ingredient that is protected inside the shell until its later release (Barrier et al. 2010 and 2011, Diego-Taboada et al. 2014).

In particular, SpECs have many advantageous properties for microencapsulation such as:

- availability of a range of sizes (from 5 to 250 μm), depending on the species of plant used to produce the exines. SpECs of different sizes can be selected in relation to the type of drug to be delivered and to the route for drug administration: larger microcapsules contain more active ingredient, whilst smaller exines may be required to target specific areas of the body such as the lower gut;
- uniformity of size, morphology and topology within a plant species, which allows even uploading of drugs;
- proven resilience to both alkalis and acids, which means SpECs can resist digestion in the acidic stomach environment. The exines do not enter the stomach wall cells but can be trapped in the intestine microvilli and the active ingredient released by pressure and/or ingress of the intestinal fluids into the shells. A pH-sensitive coating/pore-blocking excipient can be used to provide protection of the drug through the stomach;
- chemical and structural stability as pollens and spores have evolved to survive in a wide variety of environmental conditions;
- UV (ultraviolet) shielding capability and antioxidant activity, which may enhance the shelf life of drugs;
- proven relative high mucoadhesion to intestinal tissues (compared to other bioadhesives such as chitosan), likely due to the "rugged" external structure of the outer walls of the SpECs. This contributes to the extended contact of the SpECs with the intestinal mucosa leading to an increased efficiency of delivery of pharmaceuticals;
- proven increased bioavailability of drugs when compared to the same drugs delivered without microencapsulation;
- proven controlled release of a spectrum of drugs;
- proven taste-masking;
- high loading, usually 1:1 (w/w, SpECs:drug);
- relative low cost as pollen grains and spores can be obtained in multi-ton quantities at a price that is commercially viable for many purposes. The extraction and filling processes use standard food processing machinery, thus keeping the cost of the process relatively contained;
- renewable supply, as SpECs can be obtained from plants such as club moss (*Lycopodium clavatum*), sunflower (*Helianthus anuus*) and green algae (*Chlorella vulgaris).*

The release of the active can be controlled by adding a coating on the shell, or by co-encapsulation with the active inside the shell (Diego-Taboada et al. 2014). For orally delivered drugs that can be destroyed by the acidic environment of the gut, a co-encapsulant (such as shellac or a proprietary product such as Eudragit ®) has been used to block up the pores in the shell or to provide a surrounding barrier. Eudragit ® can be especially useful for targeting the lower gut, as the drug receives protection until it reaches this point.

Examples of use of SpECs in double blind cross-over trials using human volunteers include 10-fold enhanced bioavailability of both vitamin D and of eicosapentaenoic acid from fish oil, compared to the non-encapsulated formulations respectively (Wakil et al. 2010, unpublished work). Also, SpECs-encapsulated ibuprofen and the peptide enfuvirtide coencapsulated with Eudragit, were shown, respectively, to be retained inside SpECs during transit through simulated gastric environment and released in simulated intestinal fluids (Diego-Taboada et al. 2013). In addition, SpECs have been successful for encapsulation of fats, polyunsaturated oils, vitamins, enzymes,

flavours, hormones and several pharmaceutical drugs of different polarities and molecular masses (Diego-Taboada et al 2014, Mackenzie et al. 2015, Sporomex in preparation). In these trials, no oxidation of the oils or denaturation of the enzymes was observed following their full recovery (Barrier et al. 2011). More recently, SpECs have proven successful for the delivery of *in vivo* vaccines: using ovalbumin (OVA) as a model antigen, SpECs of club moss formulated with OVA were fed to mice. Significantly higher anti-OVA serum IgG and fecal IgA antibodies were found in treated animals compared to antibodies induced by use of cholera toxin as a positive-control adjuvant. The antibody response was not affected by pre-neutralization of the stomach acid and persisted for up to 7 months. Examination by confocal microscopy has indicated that sporopollenin exines extracted from club moss could translocate into mouse intestinal wall, thus offering a putative mechanism for oral vaccination and the potential to stimulate mucosal immunity through antigen-processing by the gut-associated lymphoid tissues, and to maintain this response for at least a few months (Atwe et al. 2014).

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