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NON-TECHNICAL SUMMARY

Understanding skeletal disease and pathological soft tissue calcification

Project duration

Years **5**
Months **0**

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Vascular calcification, Bone formation, Therapy

Animal types	Life stages
Mice	neonate, juvenile, adult
Rats	adult, juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The aim of this project is to improve our understanding of the processes which cause skeletal and vascular disease. It will also investigate the complications which can arise in bone and blood vessels as a result of diseases in other tissues.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Maintaining bone mass is important for healthy ageing; however, the skeleton does not exist in isolation and its function is influenced by other tissues. Consequently, many common diseases (e.g. chronic kidney disease (CKD or kidney failure), diabetes) are associated with significant skeletal problems (e.g. bone loss). Many of these conditions are also characterised by unwanted and harmful soft tissue calcification (e.g. calcification of the blood vessels which is known as vascular calcification). The processes which lead to the development of these skeletal and vascular problems are not fully understood and lack effective treatments. Therefore it is important to improve our understanding of what causes these issues. Ultimately this knowledge may lead to the development of new drugs to treat these common problems.

What outputs do you think you will see at the end of this project?

The studies performed under this licence will increase understanding of the processes that lead to the development of skeletal problems and harmful calcification of the arteries (known as vascular calcification) in several common diseases (e.g. chronic kidney disease (CKD or kidney failure), diabetes). Ultimately this may lead to the identification or development of compounds that can be used to prevent or treat vascular calcification without exerting negative effects on the skeleton. Experimental outputs will therefore include:

1. Publications/conference presentations describing our research findings. This will provide important new information to other researchers in the field about the processes involved. It may also be interest to industrial partners.

2. Refinement of protocols to reduce animal use.

3. Identification of compounds that warrant further investigation as potential treatments for vascular calcification and/or skeletal problems associated with other diseases.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Many of the conditions which lead to skeletal problems and/or vascular calcification are much more common in older people. Given the ageing population, these problems are expected to increase in prevalence and so finding new therapeutics is essential. Vascular calcification, in particular, does not currently have any effective treatments. This means there is a need for research to understand the processes involved and to identify compounds of interest. Due to the time required for drug development processes, translating basic science findings to clinical benefit is likely to take many years.

In the shorter term, the benefits will primarily be for the broader scientific research community. Data generated will be published in a timely manner to ensure effective dissemination to the skeletal and vascular biology fields, as appropriate. Any refinements in protocols was also be shared to ensure that improvements in methodology can be more widely adopted.

How will you maximise the outputs of your work?

Findings will be presented at appropriate national and international conferences to ensure rapid dissemination of new knowledge, protocols and refinements to a broader scientific audience. Data will also be shared with new and established collaborators within the field to inform and refine future studies of a similar nature. Once individual projects are complete the results will be written up in a timely manner for publication in a peer-reviewed journal. To prevent unnecessary repetition of *in vivo* studies we will aim to publish all findings, both positive and negative.

Publication of research findings is also disseminated to a general audience via the institution website.

Species and numbers of animals expected to be used

- Mice: 3,600
- Rats: 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Protocols 1-3: Here, animals with genetic changes will be bred for experimental purposes. Animals will either be used to maintain breeding colonies, to isolate cells or tissues or transferred for use in protocols 4 or 5.

Protocol 4: Here, animals will be fed a modified diet which results in the development of chronic kidney disease (CKD) and the associated skeletal and vascular problems. Animals will be fed this special diet for the duration of the study (up to 12 weeks). During this time, animals will have regular blood tests and be given compounds which could prevent the unwanted consequences of the disease. At the end of the study, all animals will be euthanised and tissues collected for experimental analysis.

Protocol 5: Here, animals will be fed a modified diet which results in the development of the skeletal and vascular problems associated with ageing. Animals will be fed this special diet for the duration of the study (up to 12 weeks). During this time, animals will have regular blood tests and be given compounds which could prevent the unwanted consequences of ageing. At the end of the study, all animals will be euthanised and tissues collected for experimental analysis.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

Protocol 1: The genetic alterations in these animals are not expected to cause any significant adverse effects.

Protocol 2: The genetic alterations in these animals may lead to the development of disease and the associated symptoms. In most cases these effects will worsen with age.

Protocol 3: The genetic alterations in these animals and the feeding of a high fat diet are not expected to cause any significant adverse effects.

Protocol 4: Animals on the modified diet in this protocol will develop kidney failure and the associated adverse effects which include reduced food intake and weight loss. Since this model reliably mimics the human clinical symptoms of kidney failure adverse effects are likely in all animals. Symptoms are expected to appear within 1-2 weeks of study initiation and will gradually worsen over time; after onset, adverse effects will last until the study ends. Animals will be carefully monitored throughout the study and if symptoms are approaching the limit of severity they will be euthanised via schedule 1 methods and tissues collected for analysis.

Protocol 5: Animals on the modified diet in this protocol are not expected to experience any adverse effects with the exception of transient weight loss.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per species)?

Protocol 1: The expected severity of this protocol is mild. It is anticipated that all animals will experience this level or a sub-threshold severity.

Protocol 2: The expected severity of this protocol is moderate. It is anticipated that all animals will experience this level or a mild severity.

Protocol 3: The expected severity of this protocol is mild. It is anticipated that all animals will experience this level severity.

Protocol 4: The expected severity of this protocol is moderate. It is anticipated that all animals will experience this severity.

Protocol 5: The expected severity of this protocol is mild. It is anticipated that all animals will experience this severity.

What will happen to the animals at the end of the study?

- Killed

Application of the three Rs

1. Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Within the body, there are numerous interactions between the different tissues. As a result a disease in one particular organ/tissue can cause problems in another. This project will investigate how diseases such as chronic kidney disease (i.e. kidney failure) and diabetes cause skeletal problems and harmful calcification of the arteries (vascular calcification). To learn more about these conditions and to find effective therapeutic treatments requires a number of experimental approaches.

In vitro (i.e. in the lab) work using cells obtained from animals can provide important information about how things work. Particularly useful in improving our understanding are cells that are isolated from rodents that have had genes switched on or off. However, one limitation of *in vitro* studies is that they cannot replicate the 3D structure of tissues or model the interactions between different tissues. Therefore, *in vitro* work is most informative when used in combination with whole animal (or *in vivo*) studies. This project will use both methods to address our research objectives.

What was your strategy for searching for non-animal alternatives?

Where possible we use human vascular cells for lab experiments but human bone cells are very difficult to obtain. Therefore bone cells for study need to be isolated from rodents. At present there are no effective non-animal models that allow the skeletal problems or vascular calcification associated with disease to be studied in the same system.

Why were they not suitable?

Non-animal systems that can reproduce the interactions between bone, blood vessels and other tissues do not exist. Therefore, at present there are no alternatives to whole animal models.

2. Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The estimated numbers are based on over 10 years of animal work. Extensive experience in bone and vascular research has provided a detailed understanding about the number of animals required for the different experimental approaches employed. It also takes into account typical inheritance patterns when breeding genetically altered animals and intragroup variability for whole animal *in vivo* work.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

The techniques employed in our research are continually being refined to maximise the experimental outputs from the animals used. These changes have been used to inform the experimental design in this project licence. For example, recent refinement of isolation methods means that four distinct cell types can now be obtained from a single animal; this represents a significant reduction in the number of animals needed for *in vitro* work. In addition, improvements in the methods used for *in vivo* studies has reduced the degree of variability between animals. This means that overall the number of animals needed in each experimental group can be reduced.

What other measures apart from good experimental design will you use to minimise numbers?

As mentioned above we have designed our protocols so that four distinct cell types can be isolated from a single animal or group of animals. This is particularly useful when isolating cells from genetically altered animals which may only be available in limited numbers. Furthermore, breeding programs will be designed to limit the generation of animal
s which cannot be used for experimental purposes.

Where necessary, pilot studies will be used to test poorly characterised compounds prior to commencing a full study. This will ensure that excess animals do not have to experience or be euthanised due to unforeseen adverse effects.

3. Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Protocols 1 and 2: This work will involve the breeding and maintaining of rodents (many with genetic changes) for research purposes. The majority of these animals are not expected to experience any significant pain or distress.

Protocol 3: This will involve feeding certain animals a high fat diet. This is not expected to have a significant impact on welfare.

Protocol 4: Chronic kidney disease and the associated skeletal and vascular problems can be induced in rodent models via the diet or by surgery. We have opted to use modifications in diet because this method is less invasive, shorter in duration and is more reproducible.

Protocol 5: This method uses a modified diet to allow the study of the vascular and skeletal problems that can develop as a consequence of ageing. This approach has been refined to minimise the adverse effects and reduces the need to use aged animals.

Animal suffering will be limited in all our studies by our strict monitoring of actual severity and severity limits. Our protocols are also designed not to produce excessive trauma or suffering. In all cases, animals will be euthanised if they approach the limit of severity.

Why can't you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

The conditions being investigated are most often associated with ageing and cannot be sufficiently reproduced in animals at an immature life stage. Furthermore, since bone loss and the development vascular calcification take a number of weeks to occur it is not possible to carry out these protocols on terminally anaesthetised animals.

What are you going to do to refine the procedures (for example increased monitoring, post-operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Protocols 1-3: All animals will be subjected to regular monitoring to ensure welfare is maintained. If an animal model is new or poorly characterised, levels of monitoring will be increased until the model is characterised.

Protocols 4 and 5: Animals purchased specifically for a study will have a 1-2 week acclimatisation period prior to work starting. Animals will undergo a health check twice a week unless part of a pilot study testing a poorly characterised compound. These animals will be subject to enhanced levels of monitoring. Options for using creatinine levels to refine protocol 4 will also be explored.

What published best practice guidance will be followed to ensure experiments are conducted in most refined way?

All whole animal *in vivo* studies will follow the ARRIVE guidelines. Administration of compounds will follow the LASA guidelines. Power calculations will be performed prior to every study to confirm that enough animals are included to ensure statistically relevant results.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

Advancing the 3Rs and ensuring animal welfare are central to our research ethos. Attendance at internal seminars and training courses aimed at promoting and improving best practice as well as external seminars and relevant conferences will ensure that the PPL holder and any PIL holders working under this licence are kept up to date with relevant new developments. Regular contact with international collaborators using similar whole animal models will ensure that any refinements developed in other research institutions can be quickly incorporated to the studies performed under this licence (subject to appropriate PPL amendments).

Explain the choice of species and the related life stages

Protocols 1-3: The ability to switch genes on or off in rodents (mice and rats) has yielded a lot of important information about how tissues work. Study of these animals provides an important research tool that helps to increase our understanding of how diseases develop. There are a number of diseases (e.g. chronic kidney disease (CKD or kidney failure), diabetes) which cause problems in the skeleton and also lead to the development of harmful calcification in the arteries (known as vascular calcification). In some cases, animals will be fed a high fat to mimic a western diet. Using these genetically altered animals in this project will help us to understand the processes which lead to the development of these unwanted effects. Furthermore, studying animals at different life stages provides important information on the impact of ageing on these processes.

Protocols 4 and 5: In order to determine whether potential treatments can prevent disease-induced vascular calcification and skeletal problems it is necessary to use animals with that disease (e.g. CKD). These experiments are performed on adult rodents as the diseases being modelling are typically associated with ageing.