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 r ty

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

- Killed

Application of the three Rs

1. Replacement

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.

(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 studies is that they cannot replicate the 3D structure of tissues or model the interactions between different tissues. Therefore, work is most informative when used in combination with whole animal (or) 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?

3. Refinement

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?

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.

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

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.

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?

: 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.

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 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

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.

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.