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NON-TECHNICAL SUMMARY

Vascular calcification in kidney dysfunction

Project duration

5 years 0 months

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

Key words

vascular calcification, chronic kidney disease, calcium

Animal types	Life stages
Mice	pregnant, adult, juvenile, neonate, embryo

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

To understand the mechanisms that lead to the hardening of blood vessels in patients with chronic kidney disease (CKD).

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?

Patients with CKD, diabetes and atherosclerosis have an increased risk of bone-like calcium deposits forming in their blood vessels. This process, known as vascular calcification, causes the blood vessels to become stiffer. Consequently they cannot stretch as easily as normal blood vessels, resulting in an increase in blood pressure which in turn places more stress on the heart. As a result, patients are more likely to die prematurely.

Currently there is no effective treatment for this condition. Therefore there is a clinical need to understand the underlying disease process in more detail so that potential drug targets can be identified.

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

The project has two arms, approaching the theme of vascular calcification from two different angles.

In the first part of the study we will use a genetically altered mouse strain that we have shown is more prone to developing vascular calcification than normal mice. One aim of the current study is to establish whether a drug that blocks the pathway activated as a result of the genetic modification is effective in inhibiting vascular calcification. As patients with CKD are known to show similarly increased activity in this pathway, by demonstrating the effectiveness of the drug our study would provide strong evidence for a new therapy.

In the second part of the study we will use a different genetically altered mouse strain to explore the relationship between calcium and phosphate in the development of vascular calcification. We have shown that the molecular sensor which detects the amount of calcium in the blood, and regulates the secretion of the hormone that controls the release of calcium from bones, can also detect phosphate. We have altered the phosphate sensing part of the molecule and predict that this will protect mice with CKD from developing vascular calcification. If that is the case this would point to towards a highly specific drug target that could not only reduce vascular calcification but may also be useful in the treatment of the bone and mineral disorder commonly found in patients with CKD.

Who or what will benefit from these outputs, and how?

In the short term, the project will help us to better understand the processes that lead to the formation of mineral deposits in blood vessels. This knowledge will help other researchers who are working in this field and more widely those who are seeking to understand blood vessel function and the control of calcium balance in the body.

In the longer term, the project may lead to the development of new treatments for patients suffering from vascular calcification. Both arms of the project could lead to the development of new drugs or the repurposing of existing drugs for the treatment of vascular calcification.

How will you look to maximise the outputs of this work?

The genetically altered mice strains that we have generated are likely to be of interest to other researchers, so we would be open to collaboration and the sharing of materials to maximise the impact of our work.

We already collaborate with other research groups, both at our own institution and elsewhere, so we envisage that the outputs of the current work will feed into further refinement of techniques and research partnerships. For example, we have collaborated with colleagues with expertise in imaging to develop a method which allows us to make two separate sets of measurements in the same sample. Previously, two tissue samples from two separate mice would have been necessary to achieve the same outcome.

We will make data sets, including those with negative outcomes, available to other researchers via the institution's data repository.

Species and numbers of animals expected to be used

- Mice: 1400

Predicted harms

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

Explain why you are using these types of animals and your choice of life stages.

We will use mice that have been genetically modified in one of two ways. One strain lacks the ability to make a target molecule of interest, making them more prone to developing vascular calcification than normal mice. In this way we can study the impact of the loss of this molecule on the calcification process and its related impacts on other parts of the body, such as the kidneys. The other strain of mouse has received a modification to the molecular sensor which detects calcium in the blood. We predict that a certain part of this sensor will also detect phosphate, and as such that it plays a role in the bone and mineral disorder that accompanies CKD in patients. We have changed the structure of this molecular sensor so that it no longer responds to phosphate, and in doing so we anticipate that these mice will be less prone to developing vascular calcification than normal mice.

We are using these mice because they have highly specific changes in the function of the molecules of interest. This means that we can study the impact of this altered function in the whole animal, thereby allowing us to model the complex disease process that occurs in patients with CKD and vascular calcification.

In order to generate mice for use in experiments, we will have to maintain breeding colonies of both strains of genetically modified mice. We will only use adult mice in the subsequent experiments, in order to model the form of vascular calcification that occurs in adult humans.

Typically, what will be done to an animal used in your project?

Mice will undergo a two-step surgical procedure to remove 5/6th of their kidney mass in order to induce CKD.

In the first part of this process, 2/3rd of one kidney are removed under anaesthesia. This involves making a small incision into the abdomen just below the ribs to access one of the kidneys. It does not matter which kidney is chosen, as they are identical in structure and function; however we typically start with the left kidney as it is easier to access. The upper and lower parts of the kidney are removed, tissue glue is applied to stop any bleeding and then the wound is closed and the mouse is allowed to recover. Analgesics are administered to prevent the mouse from experiencing pain.

7-14 days later a similar procedure is undertaken, again under anaesthesia. This time the whole of the second kidney is removed. The wound is closed and analgesics are administered to prevent the mouse from experiencing pain.

At least 7 days after the second surgical procedure has been completed, the mouse's diet is switched from normal chow to one that contains a higher level of phosphate. This is done to speed up the development of vascular calcification. The mouse will be maintained on this high phosphate diet for up to 16 weeks, but typically 8 weeks, before being killed and tissues collected for further analysis.

In some experiments, a small device will be implanted under the skin on the animal's back, between its shoulder blades, in order to deliver a drug. These tiny pumps are able to deliver a drug at a constant rate for up to 4 weeks: where a longer period of drug delivery is required the pump may have to be removed under anaesthesia and a replacement implanted. This would only happen on one occasion extending the total period of drug delivery to 8 weeks.

In other experiments a drug may be administered orally by inserting a tube briefly down the mouse's throat and into its stomach: this is called gavage. This approach will be used rather than dissolving the drug in drinking water and allowing the animal to self-dose as it is important to ensure that each mouse receives the correct dose. It is also helpful when drugs are not soluble in water, or are unstable in aqueous solutions over several hours, or are very expensive which prohibits making up large volumes. This method of drug delivery would be used once daily for up to 4 weeks.

Small blood samples will be taken every 7-14 days for up to 8 weeks by pricking the mouse's tail with a sharp needle. A small drop of blood will be collected in order to assess markers of kidney function.

What are the expected impacts and/or adverse effects for the animals during your project?

The genetic modifications do not cause any overt effects, so neither strain of genetically altered mouse is expected to suffer from any adverse effects prior to entering an experimental procedure.

Surgical removal of 5/6 of the kidney may cause acute kidney injury, a condition in which the animal's remaining kidney mass is not able to function adequately to remove waste products from the blood. The risk of acute kidney injury is minimised by performing the surgical procedure to remove the kidney tissue in two stages with at least 7 days recovery in between each step.

After the first surgical procedure the animal is unlikely to suffer any adverse effects and post-operative pain can be prevented by administering analgesics.

If acute kidney injury does occur, it is likely to do so in the period 10-24 hours after the second surgical step. The signs of acute kidney injury include lethargy and a lack of interest in food or water, fur standing on end and a pinched facial expression. Administration of fluids and analgesics by injection may help to alleviate these symptoms; however if there is no improvement the mouse will be killed to prevent further suffering.

The surgical procedure is designed to induce CKD in the mouse; however the animal will not experience the clinical consequences of this condition as the experiment will end before this stage is reached.

Once the mouse has recovered from surgery it will be fed a diet containing elevated levels of phosphate: this is designed to speed up the formation of calcium deposits in the blood vessels. The diet itself is palatable to mice, so they will eat it readily; however some animals can lose weight rapidly over 24-48 hours. Placing the animals temporarily in a warming cabinet can minimise this loss of weight, which will be detected by regular weighing.

Feeding a high phosphate diet in combination with removal of 5/6th of the kidney tissue will result in vascular calcification. Although this has adverse consequences for patients, which is the rationale for studying the condition in this project, the mice will be killed and tissue collected for further analysis long before they experience the clinical consequences of this condition.

The drugs that we plan to administer are not reported to cause any adverse effects at the intended doses and duration of treatment.

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 animal type)?

The expected severity level associated with the breeding and maintenance of the genetically altered mice is mild.

Some of those mice will then undergo the surgical procedure to induce CKD and subsequent feeding of a high phosphate diet, which has an expected severity limit of moderate.

Therefore approximately 70% of mice will experience mild levels of suffering and 30% experience moderate levels of suffering.

What will happen to animals at the end of this project?

- Killed

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?

CKD leads to complex changes in physiological function, both within the diseased kidney and in the body as a whole. Many of these changes are not understood fully and so cannot be reproduced using isolated cells or computer modelling. However it is precisely these systemic changes that result in vascular calcification. Consequently, it is necessary to study the changes that occur in blood vessels in the intact animal in order to understand what is happening as CKD progresses. This leaves us with no viable alternative to the use of animals for the proposed project.

Which non-animal alternatives did you consider for use in this project?

We have used isolated cells to perform many of the experiments that underpin this project and we will continue to do so where appropriate.

Why were they not suitable?

Isolated cells are useful for providing proof of principle and for testing drugs or understanding at the molecular level how signalling processes occur. Where they are less useful is in trying to understand the more complex interactions that occur in the organ or the whole body, especially in disease states. CKD is a complex disease which can alter the way that the body regulates calcium, ultimately leading to vascular calcification. This process cannot be modelled accurately using cell culture.

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 majority of animals used in this project will be required to generate and maintain breeding colonies of the two strains of genetically altered mice. Hence these numbers are based on the prior experience of both our group and that of numerous other researchers at our establishment and elsewhere.

For the remaining animals, we are able to base our estimates of the number of mice that will be required on our previous experience with the main experimental model.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We have designed our experiments using the NC3R's Experimental Design Assistant so that we are able to gain the most possible information from each individual animal.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In maintaining our genetically altered mice colonies we will employ efficient breeding strategies to ensure that the number of excess animals is kept to a minimum. Colony size will be reviewed regularly to ensure that breeding matches the anticipated demand for experimental animals.

We have designed the experimental plan in such a way that there are a number of points at which a decision is made whether to continue with a particular objective(s) or not. Thus if an experimental outcome indicates that there is no value in continuing, that aspect of the work will cease thereby preventing the unnecessary use of animals.

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.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The purpose of the animal model is to induce vascular calcification in mice in a way that mimics disease progression in humans with CKD. To do so, first we need to induce CKD. This can be achieved in a number of ways in the mouse, including exposure to radiation, obstructing the outflow of urine from one of the kidneys and administration of a number of drugs. However none of these methods is suitable for our purpose as they produce pathologies that differ from the type of disease typically seen in patients with vascular calcification.

Therefore, we will employ a surgical procedure to remove 5/6th of the kidney mass as the means to induce CKD. This involves removing kidney tissue in two stages and allowing the animals to recover between procedures. Thereafter, mice begin to develop CKD over a period of several months. As in humans, there are no overt clinical signs or pain associated with disease progression until the final stages. However, mice will not experience pain or distress due to CKD as the experiment will finish before they have reached this point.

The second part of the process involves feeding the mice a diet containing higher than normal levels of phosphate. We know from a pilot study conducted in earlier experiments how much phosphate the mice will tolerate (1.5%) and how long we need to feed the diet in order to induce the formation of calcium

deposits in their blood vessels (8 weeks), so we can have confidence that this model will work in our hands while at the same time causing the least distress to the animals. Vascular calcification associated with CKD will lead to an increase in the animals' blood pressure, but as in humans, there are no overt signs that hypertension causes pain or distress.

Why can’t you use animals that are less sentient?

We are not aware of any non-mammalian animal models of vascular calcification that mimic the pathology seen in humans as they age. There is a zebrafish model of generalized arterial calcification of infancy (GACI); however as its name implies, this is a disease that manifests either before birth or early in childhood as it is a rare genetic condition.

All published studies in this area, including our own, have either used isolated cells or rodents. The former are a valuable tool with which to study molecular pathways in specific types of cells. The latter are most frequently used in vivo because of the need to model a complex, multifaceted disease process that involves the kidneys, blood vessels and hormones that regulate calcium.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

During the course of earlier work authorised by a previous licence (P217A25EF) we have optimised the dietary conditions necessary to produce the calcification of blood vessels, minimising the time required to reach the scientific endpoint of the experiment while causing the least distress to the animals. Furthermore, we have refined our surgical procedure and post-operative monitoring regimen to ensure that any animal at risk of acute renal injury can be identified and killed humanely to prevent unnecessary suffering.

We will continue to review our procedures on an ongoing basis and make refinements where ever possible to minimise the welfare costs incurred.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All procedures will be undertaken in accordance with institutional standard operating procedures (SOPs) and guidelines.

The approach to surgical procedures will be further informed by the Laboratory Animal Science Association's (LASA) Guiding Principles for Preparing for and Undertaking Aseptic Surgery (https://www.lasa.co.uk/PDF/LASA_Guiding_Principles_Aseptic_Surgery_2010.2.pdf).

Blood sampling will conform to the LASA Good Practice Guidelines for Collection of Blood Samples (http://www.verutech.com/pdf/lasa_blood_sampling.pdf).

When publishing the outcome of our work we will adhere to the ARRIVE guidelines (https://arriveguidelines.org/).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We seek to stay informed of 3Rs advances through innovations published in the literature, discussions with colleagues at other institutions, regular updates provided by named individuals at our own institution and the local NC3Rs Regional Programme Manager and through subscription to the NC3Rs newsletter.