Home Office

NON-TECHNICAL SUMMARY

Can we modify the severity of inherited cardiac conditions? A study on novel treatments and environmental factors that modify the severity of inherited cardiac conditions

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

Inherited cardiac conditions, diet, pollution, systemic inflammation, low potassium levels

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

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?

Inherited cardiac conditions (also known as genetic cardiac conditions) are caused by genetic defects that cause abnormalities of electrical activity and/or pumping function of the heart. The overall aim of this licence is to get a better understanding of the mechanisms responsible for the onset genetic cardiac conditions and to develop treatments for these conditions.

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?

Genetic cardiac conditions tend to affect children, adolescents and young adults and can cause significant symptoms. In a subset of patients, inherited cardiac conditions cause cardiac arrest that without prompt resuscitation results in sudden death. Inherited cardiac conditions are the leading cause of sudden death in adolescents and young adults. Over the last decade these conditions have rose to prominence following the cardiac arrests of high-profile footballers on the football pitch. Their cardiac arrests were all caused by genetic cardiac conditions. Currently, there are no treatments that are specific for these conditions. In the majority of cases, we can treat patients to reduce the symptoms, but we are not able to give any treatments to specifically correct the abnormalities responsible for these conditions. In many cases patients need to have a cardiac defibrillator implanted to be protected from sudden death. Within families, there is significant variability in the severity of these conditions. Some family members are badly affected while others have no signs or symptoms of the condition despite carrying the same genetic defects responsible for the condition. There is a desperate need to identify novel treatments specific for these conditions. In addition, there is an urgent need to understand whether factors such as diet, low potassium levels in the blood, inflammation, and exposure to environmental pollution can modify the severity of these conditions. Identification and removal of factors that modify the severity of these conditions could help in their treatment.

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

This project is likely to generate several important outputs that will impact in the management of patients with genetic cardiac conditions, further advance our understanding of the mechanisms responsible for the onset of these conditions and help us understand the factors responsible for the variable occurrence (penetrance) of these conditions.

These will be:

Novel treatments of genetic cardiac conditions re-tasking existing drugs already used in patients for other conditions and novel drugs therapies.

A greater understanding of how genetic cardiac conditions cause heart failure through identifying the mechanisms by which abnormalities in electrical activity and/or pumping function of the heart cause heart failure.

Identify factors that influence penetrance and severity of inherited cardiac conditions. These will include diet, levels of potassium in the blood, levels of inflammation in the body and exposure to pollution.

Our principal output will be via publications in high quality journals, which will inform the work of other scientists around the world

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

Short term beneficiaries

Research scientists could benefit directly. We will report our findings in peer reviewed publications and presentations at national and international meetings. The study will explore novel therapeutic options for genetic arrhythmia syndrome and directly explore the mechanisms of arrhythmias in lamin A/C cardiomyopathy and mitochondrial dysfunction in hypertrophic cardiomyopathy. These studies in addition to potentially improving the treatment of these conditions will also provide a more detailed understanding of the mechanisms that are responsible for the onset of these conditions. The studies on factors that modify penetrance and severity of these conditions have the potential to provide novel answers to an issue that is the subject of intense debate among scientists investigating genetic cardiac conditions. Most of the current research is focusing on genetic factors and only a few studies have started investigating non-genetically acquired factors (such as diet, inflammation, and exposure to pollution). Identification of factors that modify penetrance and severity of inherited cardiac conditions is also likely to provide further mechanistic understanding of these conditions.

Medium long-term beneficiaries

Clinicians and patients could benefit directly because any positive findings could be readily translated into the clinic and have the potential of substantially improving the treatment and risk prediction of these patients. In addition, identification of factors that modify the penetrance and severity of inherited cardiac conditions could lead to the introduction of non-pharmacological interventions to prevent the onset of these conditions.

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

Our current mouse models of genetic cardiac conditions 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 will maximise access to our outputs by publishing in open access journals. Data sets, including those with negative outcomes, will be made available to other researchers via the institution's data repository and other public databases.

We will share good practice in terms of surgical techniques, disease model development and in vivo analyses. We already collaborate with numerous 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.

Species and numbers of animals expected to be used

- Mice: 10000

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.

The project will use mice, predominantly with genetic modifications which are similar to genetic defects that in human induce genetic cardiac conditions. We can therefore study the impact of these genetic modifications in the whole animal, allowing us to model the genetic cardiac conditions that occurs in patients with cardiovascular disease.

The mouse is a highly relevant animal model for understanding disease processes in the cardiovascular system. In comparison to humans the mouse heart has the same gross and cellular structure; the pressure and volume characteristics closely resemble the human situation; and the vascular system is similar in terms of structure, function and its response to changes in blood pressure.

In order to generate mice for use in experiments, we will have to maintain breeding colonies of genetically modified mice and will use adult mice in subsequent experiments.

Overall, the mouse is an excellent model to characterize the mechanisms responsible for the onset of inherited cardiac conditions, and hence develop treatment strategies which are needed to make progress in the field.

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

Typically, mice will be bred to carry a genetic alteration followed by subsequent study of their cardiovascular function. These genetic alterations are similar to the genetic defect that cause genetic cardiac conditions and cause in the mice electrical and structural alterations which are very similar to the one detected in humans. We will study two main groups of genetic cardiac conditions:

1) Genetic arrhythmia syndromes that are characterized by abnormalities of the electrical properties of the heart that can cause irregular heart rhythms (arrhythmias)

2) Genetic condition of the cardiac muscle (cardiomyopathies) that are characterized by abnormalities in the structure and function of the heart that can cause heart failure.

A series of methods similar to those used to assess human heart and vessel (cardiovascular) function in the clinic eg blood pressure measurement, an electrocardiogram (ECG) and cardiac ultrasound will be used to analyse cardiovascular structure and function in mouse lines carrying genetic alterations. These analyses may be carried out at a single time point, or at multiple time points to analyse changes over time.

Some animals will have small wireless devices implanted to continuously monitor their heart rhythms and determine whether they develop irregular heart rhythms. These will be implanted under general anaesthesia into their abdomen.

In some animals, when it is not possible to deliver drugs mixed with food or water, we will implant minipumps to continuously deliver drugs for 2-3 weeks. These minipumps will be implanted under general anaesthesia in their abdomen or under the skin.

In some of these experiments, we will assess the impact of an intervention. In some experiments, the mice will receive a potential therapeutic drug by injection or mixed into their food or though minipumps to asses whether the drug prevents arrhythmias or prevents the structural and functional abnormalities produced by the cardiomyopathy. In other experiments, we may feed mice modified diets with high fat and sugars or with high content of fish oils to determine whether these diets modify the severity of arrhythmias or the structural abnormalities that the mutations cause. In other set of experiments mice will be infected with influenza virus or similar virus and will have injections to prolong the infection and the inflammation associated with it to determine whether prolonged infection and inflammation modify the severity of arrhythmias or the structural abnormalities that the mutations cause. In other experiments, we will expose the mice to pollutants to determine whether exposure to pollution modifies the severity of arrhythmias or the structural abnormalities that the mutations cause. Finally some mice will be fed a diet without potassium and will be given 'water' tablets to reduce the level of potassium in the blood and induce arrhythmias. These experiments will enable us to determine how low potassium causes arrhythmias and test novel treatments that in single cells have been shown to prevent arrhythmias induced by low potassium levels.

To perform genetic testing of the mice with genetic modification, we will have to extract DNA from animal tissues. The tissue will be obtained with one of the following methods: 1) small ear clipping 2) blood sample, 3) hair sample 4) mouth swab.

At the end of each study described above, tissue will be collected after humane killing of mice for cellular, molecular, histological and in vitro analyses.

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

The genetic modifications that cause genetic arrhythmia syndromes do not affect the general wellbeing of the animals. They predispose the animals to have irregular heart rhythms when the animals are exposed to environmental stressors that cause physiological stress. These arrhythmias are well tolerated by the mice.

The genetic alterations that cause genetic cardiomyopathy can lead to the development of signs of heart failure. Genetic cardiomyopathy mice will represent 30-40 % of the mice utilised. The signs of heart failure include: respiratory difficulty, lethargy, cold tail, decreased interaction with other mice and lack of interest in food and water. Animals showing signs of heart failure will be promptly humanely killed.

Animals tend to tolerate arrhythmias well and the induction of arrhythmias should not be associated with significant adverse effects.

We expect the majority of animals to make a full recovery from surgical interventions conducted under anaesthesia. Post-operative pain will be prevented by administering analgesics. Some animals may not recover from anaesthesia as a result of surgical complications, and as such will feel no pain or distress.

High calorie diet (with high fat and sugars content) can result in obesity which can be associated with excessive grooming of fur and increased risk of infections.

Exposure to prolonged infection and inflammation causes signs of ill health that are typically characterized by weight loss (up to 15%) decreased food intake, decreased interaction with peers and other behavioural changes.

Similarly exposure to environmental pollutant such as phenanthrene and other derivatives of fossil fuels combustion can cause the same signs of ill health described above

Finally low potassium levels in the blood can be associated with dehydration and weight loss (up to 15%).

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 as expected to be mild for the genetic arrhythmia syndromes mice and moderate for the genetic cardiomyopathy mice.

Where animals undergo a surgical intervention with recovery from anaesthesia, we expect the majority to be within the moderate severity category.

We expect that modifications of diet should no cause significant adverse events and should be within the mild category .

We expects that chronic exposure to pollution, induction of sustained infection/inflammation and induction of hypokalaemia will be within the moderate severity boundary.

In summary we expect 10-15% mild severity (genetic arrhythmia models who have diet modification or treatment with no surgery) 85-90% moderate.

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

- Killed
- Used in other projects

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?

Non animal models of genetic arrhythmias syndromes and genetic cardiomyopathies (the two groups of conditions we want to study) provide some important and valuable mechanistic information however they have significant limitations that make the use of animal models essential

In the case of genetic arrhythmia syndromes non animal model can provide information regarding what happens at the level of the single cells but offer very limited information on the interactions between cells that result in the generation of the irregular heart rhythms (arrhythmias) These can only be studied in the intact hearts. Similarly the assessment of novel treatments can only be performed in the intact heart.

Genetic cardiomyopathies are associated with complex structural and functional alterations of the heart. It is impossible to study and characterize these alterations in single cells. Therefore the use of animal models and intact hearts is imperative in the study of genetic cardiomyopathies.

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

We have used isolated and cultured cells; heart muscle cells (cardiomyocytes) derived from stem cells generated from human tissues have been used to perform many of the experiments that underpin this project and we will continue to do so where appropriate. These studies mean that we are better informed to design our animal-based experiments.

Zebrafish and drosophila (fruit flies) are widely used in cardiovascular research. They are mainly used for identification of novel genes involved in cardiovascular function. However they provide limited mechanistic information and are not ideal for testing of novel treatments because the structure of the heart and the cardiovascular physiology are substantially different compared to humans.

Why were they not suitable?

Isolated cells are useful for providing proof of principle and for testing drugs (especially to determine tissue and circulating therapeutic levels) or understanding at the molecular level how signalling processes occur.

There has been a recent increase in the use of stem cells as an experimental model. There is a lack of human cardiovascular tissue available for research, which has led to the development of techniques to induce heart muscle cells (cardiomyocytes) from human stem cells. These cells have many of the characteristics of human cardiomyocytes and thus we are using them as a model system in which to characterise the effect of gene modification on hypertrophy and to understand the signalling pathways involved.

These in vitro systems can complement and enhance our research involving animals but will not act as a replacement for experiments, which require the understanding of gene function within the context of the whole organ and the whole body, especially in disease states

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?

For each step of the project we have calculated the number of animals required to complete each experiment. This calculation is based on our own experience of similar experiments and/or from experiments reported in the scientific literature. We will use the minimum number of animals required to determine whether there is a difference between experimental groups.

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

In many cases we will use the animals as their own control.

Animals utilised for in vivo experiments will also be utilised for in vitro experiments.

For in vitro experiments we will utilise each animal for more than one experiment.

We will utilise the PREPARE guidelines for planning and conducting high-quality research and testing on animals. We will utilise the NC3R's Experimental Design Assistant to design our experiments so that we are able to gain the most 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?

The majority of animals used in this project will come from our own breeding colonies of genetically altered mice. In maintaining these 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 constantly monitor whether to continue with a particular objective or not. Thus, if an experimental outcome indicates that there is no value in continuing, that aspect of the work will cease.

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.

We are using two main mouse models of genetic cardiac conditions: Models of genetic arrhythmia syndromes and models of genetic cardiomyopathies.

Models of genetic arrhythmia syndromes: During our previous licence we have been able to establish that mice tolerates arrhythmias well. Arrhythmias do not tend to be associated with signs of distress.

The models of genetic cardiomyopathy develop significant cardiac structure and function alterations that can lead to sign and symptoms of heart failure. In some models it will be inevitable to observe some signs of heart failure because they are closely associated with the structural and functional alterations. We will perform regular assessment of cardiac structure and function in order to prevent or minimise the onset o heart failure.

All surgical techniques are carried out under general anaesthetic to ensure that the animal does not feel any pain, and any post-surgical pain is treated with the use of analgesics. In the post-surgical period animals will be closely monitored to detect signs that suggest pain is not well controlled. These signs include piloerection of fur, hunched posture, decreased activity and decreased interaction with peers. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates, will be humanely killed. We have found optimal ages and weights for particular surgical procedures and we keep within these parameters.

Physiological analyses (eg ultrasound, imaging, ECG) are performed under general anaesthetic, in the majority of cases the mouse is under terminal anaesthetic from which it does not recover. .

Any stress caused by administration of pharmacological agents is momentary as the injection is given. Where applicable we will aim to administer drugs mixed in diet or drinking water. If necessary mini-osmotic pumps will be used to administer pharmacological agents. Although their use initially involves minor surgery, the technique in our experience leads to highly reproducible and consistent results requiring fewer animals per experimental group.

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

While non-mammalian models are available, such as drosophila and zebrafish, there are key differences in their cardiovascular physiology compared to humans (they do not possess a four chambered heart and have a different circulatory system). Whilst we do not use these model organisms directly in our own work we keep abreast of current findings using these models through discussions with colleagues and through the published scientific literature. We need to utilise adult animals because the full phenotype of genetic arrhythmias syndromes and genetic cardiomyopathies is only present in the fully developed adult heart.

Some procedures are performed under terminal anaesthesia to remove any form of animal distress.

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

Over the course of the previous licence we have made a number of refinements to minimise harm to the animals used.

Refinements to anaesthesia and surgical approaches

We will adopt surgical techniques and processes that fully incorporate current best practice .

Monitoring

Detailed study plans are drawn up for each experiment and named persons consulted. This allows us to readily monitor and question the benefit of each mouse added to the study.

Regular appraisal of surgical outcomes. Continually refine the monitoring documentation to aid in assessing mouse welfare

Telemetry implants

We will utilise wireless telemetry implants so that there will be no need to tether the devices and there will be no wound exposure.

Minipumps implantation

We will aim to deliver most of the drugs mixed with food. When this is not possible, we will utilise minipumps that will be implanted either under the skin or in the abdomen. These will be implanted by fully trained staff utilising current best practice.

Arrhythmia induction studies

The type or arrhythmias we intend to study occur mainly when the heart is stimulated by adrenaline and caffeine. Over the last licence we observed that it was very difficult to cause arrhythmias with adrenaline in animals under general anaesthesia and these studies had to be in conscious animals. In addition we observed that arrhythmias in conscious animals are not associated with significant signs of distress and the animals recover fully from arrhythmias within 1-2 hours

On the basis of these observations, we concluded that we can perform two arrhythmia challenges in each animal. This will allow us to perform one arrhythmia challenge while the animal is on treatment

and another arrhythmia challenge while the animal is not on treatment. This will enable us to use each animal as its own control. This will produce more solid data and will reduce the number of animals needed to complete the study

Animal housing

All cage enrichment will have adequate size entrance holes to avoid risk of skin catching/rubbing following implantation of the telemetry device/osmotic pumps and mice will be allowed to recover for at least 7 days after implantation of telemetry devices.

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

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/wp-content/uploads/2018/05/Aseptic-Surgery.pdf).

The administration of substances will be performed according to the guidance provided by the joint working group for administration of substances. (Morton DB et al Refining procedures for the administration of substances Lab Anim. 2001 Jan;35(1):1-41.)

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 our own and other institutions, regular updates provided by the establishment 3Rs Manager and through NC3Rs webpages newsletters.