Home Office

NON-TECHNICAL SUMMARY

Understanding the mechanisms and pathophysiology of heart failure and atrial fibrillation

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

heart failure, myocardial infarction, atrial fibrillation, therapy, comorbidities

Animal types	Life stages
Sheep	adult, aged, juvenile, neonate
Mice	adult

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?

Diseases of the heart including heart failure, myocardial infarction and atrial fibrillation are major causes of premature death and ill health. Many people with these diseases are elderly and also have a variety of other conditions including high blood pressure and obesity which are thought to contribute to disease onset and progression. However, the underlying mechanisms causing these associations are poorly defined. This project will firstly investigate how these various factors act to cause these cardiovascular diseases and, secondly, investigate how novel treatments might work in these diseases.

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?

- Diseases of the heart lead to ill health and premature mortality.
- There are no fully effective treatments for these diseases with five-year survival for key heart disease in Western society being worse than that of the commonest cancers e.g breast and prostate cancer.
- A key factor for the lack of progress in finding effective treatments and preventative measures for some of these cardiac diseases remains a lack of understanding of the underlying mechanisms responsible for their development.
- We know that ageing, high blood pressure and being overweight are associated with the development of many cardiovascular diseases but the mechanisms by which these cofactors act to increase disease prevalence are poorly understood.

To address these issues we will investigate key mechanisms that cause the development of heart disease and why heart disease leads to an increased risk of death. We will study the impact of heart disease on the function of the heart at both the whole animal and isolated cardiac muscle cell levels.

From these studies we will gain a better understanding of the mechanisms that lead to various cardiac diseases (the pathophysiology of disease) and from these findings we expect to reveal novel therapeutic targets.

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

The major output anticipated from the programme of work is an increase in our understanding of the pathophysiology (disease mechanisms) of key cardiac diseases and the role of significant cofactors (ageing, obesity and high blood pressure) in the development of heart disease.

We will also build on our recent findings, by furthering mechanistic understanding, where we reported that a class of drugs normally used to treat erectile dysfunction were highly effective at treating heart failure and reversing some of the changes that occur in the diseased heart.

From the increased understanding of the pathophysiology of heart disease and the role of key cofactors in the development and progression of these diseases, we anticipate that we may be able to develop more effective therapies for heart disease.

We will generate 'large' datasets from experiments where changes in protein abundance and gene expression are investigated

From these studies we anticipate identification of new and more effective therapies for heart disease.

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

The major beneficiaries of these outputs in the immediate term (lifetime of project) will be within academia and the pharmaceutical industry.

The increased mechanistic understanding we generate will drive further investigational studies by the wider scientific community and pharmaceutical industry. We anticipate that these 'secondary' studies will commence once our initial findings become publicly available via publications and conference presentations (3 - 7 years).

We would reasonably expect that a successful outcome of our pharmacological interventions could, within a 5 - 10 year period lead to in-human trials. Should these preliminary in-human studies show benefit, the next expectation would be for large scale clinical trials and ultimate adoption of the therapies into standard clinical practice (beyond 10 years).

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

We will maximise accessibility of outputs via open access publishing and depositing large datasets in public databases. This will allow other individuals and groups to use the available information and datasets with minimal delay and thus to greatest effect.

Any software for analytical work that is developed for this programme of work will be made available either through the journal associated with the data or open access sites such as GitHub upon publication

We will also communicate work (subject to intellectual property considerations) as widely as possible via scientific meetings and interactions with commercial partners via workshops and in response to calls for potential collaborations.

We also host a series of public (schools and adults) and patient engagement events within the host institution and contribute to these routinely to maximise the public understanding of our work. We will

look to maximise the reach of this work by establishing an online web resource for patients and members of the public as well as for sharing our scientific discoveries with the wider academic and industrial communities.

We are committed to sharing best practice via open access publications. In the event that studies are unsuccessful our intention is that this information is also made available through appropriate channels including appropriate publications and open access repositories.

Species and numbers of animals expected to be used

- Sheep: 1430
- Mice: 1000

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 principal animal model used is the sheep with mice used in some experiments. The sheep is a highly translationally relevant animal model for understanding disease processes in the cardiovascular system. The sheep heart is more similar to the human heart than is the case for species such as the rat, mouse or zebrafish. These similarities include structure, size, heart rate, response to various stimuli and stresses and the way in which the parts of the heart are supplied with blood. An important consideration for our studies is that we are able to employ standard clinical practices and devices to best reproduce what would be undertaken in patients. Due to availability arising from standard farm animal practices and potential welfare issues centred around single versus group housing, studies using sheep will only be performed using female animals.

Our experiments will mainly be conducted using fully grown adult animals. In a few experiments we will use aged animals and a small number of younger animals. The use of fully grown adult animals means that we are reducing confounding factors such as growth which is a neglected consideration in many other studies. In experiments where we will use aged animals, this is because in some experiments we are studying the role of cofactors in disease and age is a one such key factor. Similarly, in some experiments we will use young animals, this is because in the young animal heart there are still processes occurring such as changes in cellular structure which we may wish to validate and target as a therapeutic approach in the disease model and therefore the young animal model may help inform these choices.

Where mice are used, both genders will be studied. The reason for using the mouse in some experiments is that we will be able to take advantage of genetic modifications. This will allow us to understand the role of specific genes and their protein products where they have either been knocked out or over expressed. In all cases, we will use adult mice.

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

The typical experiment where animals have a surgical intervention will involve a number of non-invasive assessments of heart function in conscious gently restrained animals where we will measure their electrocardiogram (ECG), blood pressure and heart function by echocardiography (ultrasound measurements of heart function). We may also take blood samples during these procedures either by direct insertion of a needle into a vein or from a catheter that has been placed in the vein.

The majority of animals will then undergo a surgical procedure where we implant a cardiac pacemaker and pacing leads into the chambers of the heart in the same way that is used for patients who have a pacemaker inserted. In some of these animals this will be combined with either using an angioplasty balloon placed in one of the arteries in the heart to induce a myocardial infarction (heart attack). These procedures may also be combined with measurements of the electrical activity of the heart either using the implanted cardiac pacemaker or by leads that are temporarily inserted into the chambers of the heart using the same vessels into which the cardiac pacing leads are inserted or additional devices. Typically only one device approximately the size of a small box of matches will be implanted however, in a small number of animals a maximum of four devices (normally less than half the size of a box of matches) may be implanted to remotely monitor cardiac function (e.g. heart rate or blood pressure), activity or, to stimulate the vagus nerve in the neck. We will also take several blood samples during the anaesthesia period.

In a small number of animals where a vagal nerve stimulator is used, when this is activated we expect it will lead to a periodic cough and change in vocalisation when the nerve is actively being stimulated (a few seconds every few minutes). These changes will resolve spontaneously over the course of a few days and have no long-lasting effect.

In studies of heart failure we will, following recovery from surgery and a period of monitoring the animal to ensure full recovery from the surgical procedure, activate the cardiac pacemakers to cause the heart to beat faster and induce heart failure. Some of these animals will receive treatments (typically a tablet given orally every day) and some will also have a small blood sample taken weekly.

We will monitor the animals for signs of heart failure and once this becomes evident they will be terminally anaesthetised and a final set of measurements of the electrical activity of the heart made before the animal is killed and tissues harvested for a series of *in vitro* experiments.

The maximum duration over which any animal will be monitored following its first surgical intervention will be six months.

However, not all animals (approximately two-thirds) will undergo a surgical procedure. Here, animals will only be give a single injection of heparin to prevent blood clotting a few minutes before they are killed for tissue harvest for *in vitro* experiments.

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

We expect animals to make a full recovery from surgical interventions with recovery from anaesthesia and do not anticipate anything more than transient and mild discomfort from procedures performed on conscious animals.

In those experiments where we are studying the mechanisms that are altered in heart failure we do expect animals to develop signs of heart failure which can include increased breathing effort, coughing or tiredness. We monitor animals carefully for the development of these signs and they are humanely killed (as the end of the experiment) once these signs become evident and impact on normal behaviour.

In some experiments we may expect animals to develop abnormal heart rhythms in the main chambers (ventricles) of the heart; the large majority of these will be experienced in anaesthetised animals. However, there is a small possibility that these abnormal heart rhythms may develop spontaneously in conscious animals. If these are significant, we might expect the animal to rapidly lose consciousness and as such do not expect them to cause pain.

Beyond this expected impacts, there is the potential that a small number of animals could develop any of a range of adverse effects as a result of the procedures that have been performed. These could (they are not routinely expected) include; loss of blood during surgery or from a dislodged cannula, sudden death due to abnormal cardiac rhythm disturbances or adverse reactions to administration of investigational compounds. Animals are carefully monitored by experienced care staff and investigators such that should any of these unexpected adverse effects be experienced by the animal then it is for the shortest duration possible.

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)?

Where animals undergo a surgical intervention with recovery from anaesthesia we expect all of those animals to be within the moderate severity category. Conversely, for many animals the only intervention they will experience will be a single injection of heparin to prevent blood clotting a few minutes before they are humanely killed for subsequent *in vitro* experiments on the harvested tissues. In these animals there will be no more than minor transient discomfort and the overall severity limit will be mild.

Overall we anticipate approximately 70% of animals will therefore undergo mild severity experiments and the remaining 30% moderate severity experiments.

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?

We need to use whole living organisms because, whilst heart failure and atrial fibrillation are conditions that originate in the heart they are also influenced by the way that other parts of the body such as the kidneys, lungs and brain behave. Critically, the response of these other parts of the body can then also modify the way the heart behaves and therefore the overall progression of the disease. This cross-talk between the heart and the other parts of the body is complicated and not easy to predict as well as also changing quite rapidly. It is not yet possible to predict the outcomes of these interactions using either computer models or cells grown in a dish.

Additionally, some forms of heart failure are also more likely to occur if you have other underlying conditions such as being elderly, having high blood pressure or being overweight. Again these complex interactions and dependencies cannot yet be faithfully reproduced using non-animal alternatives such as computer models or cells maintained in culture dish conditions.

Our first major aim is to understand the mechanisms which cause the heart to stop functioning properly in diseases such as heart failure, atrial fibrillation or after a heart attack. Our second major aim is to also understand how factors such as age, high blood pressure or being over-weight lead to the onset of heart failure. In each of these aims, we hypothesise that changes in the way that the cells of the heart regulate their calcium levels is a major factor behind disease progression. Based on the findings of these initial studies and from some of our previous work we will also investigate the effectiveness of some new treatments for heart disease in these studies. Again, understanding how these new treatments work requires us to study them using approaches that are both reliable and reproducible where all the complex relationships between the heart and other tissues in the body are functioning.

Given these considerations, we need to undertake our experiments in appropriate animal models that allow us to address our key aims, advance our understanding of heart disease and to identify and test new treatments.

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

There are several possible non-animal alternatives that we have considered. These include:

- human tissues obtained from either deceased people or those undergoing heart transplants
- cells grown in the laboratory
- computer models

Although there are significant limitations with each of these that prevent us from using them to fully replace the use of animal models, it should be noted that we will use some of these approaches to address some very specific questions where it is not necessary to use an animal and thus we strive to replace the use of animals with these methods as much as is practicably possible.

Why were they not suitable?

In each case there are major limitations to using these non-animal alternatives and these are considered below:

Human tissues

These would mainly be available from hearts removed from patients undergoing heart transplant operations or donor hearts that were not considered suitable for use in the transplant programme. The main issues with using human tissues are:

- Lack of availability - There is a major need for more donor hearts to match the number of patients on the transplant list. This means that, particularly healthy human hearts, are very rare and secondly there is a limited number of diseased hearts for use. If we had to rely on these then we would be very unlikely to be able to complete more than a small fraction of our proposed studies.
- Variability - A major concern in using human hearts is that they are being taken from a very diverse population. For example, important factors such as age, obesity, blood pressure and medical treatments they are taking are highly varied and thus difficult to control for in our experiments. This is a significant concern which both limits our ability to draw firm conclusions and our ability to perform reproducible and reliable experiments that others can follow and work from.

Cells grown in the laboratory - There are several types of cells that could be used including those that were originally obtained from animal hearts or stem cells that have been treated to behave like heart cells. In each case there are number of major limitations to using these cells to answer our questions and prevent us from achieving our aims. The major limitations are:

- Cells grown in a dish are normally of a single type, for example HL-1 cells. This does not even remotely reflect the situation in the heart where lots of different cell types co-exist and interact with one another.
- Cells grown in a dish, even stem cells that have been treated to behave like heart cells, lack the structure of a normal heart cell. This is important because many key processes in the cells of the heart occur precisely because of the structure of the cell. If this structure is not reflected by the cells in a dish then it is unlikely that the response of the cells to a treatment will be the same as would occur in the heart.

Computer models - Whilst these are improving all the time:

- They still lack the ability to accurately reproduce what is happening in the heart.
- Computer models cannot reproduce the complex interactions that occur in disease between the heart and other organs
- Given these interactions between the different cells in the heart and the heart and other tissues, computer models lack the ability to accurately predict how new drugs may work in the intact animal (or human)

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?

At each stage of the programme of work we will estimate the number of animals that are required to complete the set of experiments before the series of experiments are started.

To calculate these numbers we use information from our own earlier experiments or those that have been published in the scientific literature or a combination of both of these to determine how variable this information tends to be. From this variability we can use statistical tests to determine how many samples are needed to detect differences between groups of animals.

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

We have, and our intention is to continue to, consulted with an external statistical advisor who is independent of this programme of work in order to minimise the numbers of animals that are required to address our experimental aims. We also utilise this advice in parallel with information that we have obtained as part of our consideration of key aspects of reproducibility in research as part of our regular laboratory meetings between the various groups that make use of animals in this programme of work. Tools such as the NC3Rs experimental design assistant are a useful adjunct to these processes and help guide considerations around the allocation of animals to groups.

All of the principal investigators leading their own groups which utilise the animal models in this programme of work are also members of different journal editorial boards and regular reviewers for major UK and international scientific funding agencies. As part of these roles, we are always asked to consider experimental design which reinforces our own thinking when planning our experiments.

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

For the work involving sheep there is no need to consider efficient in house breeding as animals are purchased from external suppliers as and when they are needed to complete our experimental protocols. Where mice are used, we will aim to purchase animals rather than breed them specifically. However, where we need to breed animals we will maintain colonies as efficiently as possible and, where possible, use littermates as controls rather than separately bred animals.

In the present programme of work, we do not envisage that we will routinely be performing pilot studies. However, if at any point we do use pilot studies, these will also be considered with a view as to the most appropriate way in which they can also be used to contribute to the final experimental data in order to both inform the number of animals required and reduce animal usage.

The programme of work supports a number of principal investigators and their research groups. Importantly, we share a combined laboratory meeting programme and plan our experiments collectively. This enables us to ensure that when experimental animals are available, they are used by the full range of groups and we do not need to repeat a particular experiment twice for different laboratories.

Where relevant we will also use cell culture experiments or computer models to complement or even replace animal-based research. These are important considerations when we plan our experiments and will be used to, for example, validate test substances or test potential pathways prior to investigating their effect in the intact animal or disease model

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 principal animal model is the sheep which has been chosen because its cardiac function and response to interventions very closely resembles that in man.

We will use a number of models which closely resemble common cardiac diseases of man (heart failure, atrial fibrillation, myocardial infarction). All of our approaches are designed to be as minimally invasive as is possible (which would generally not be achievable in smaller species) and accurately reflect the same procedures that would be performed in patients requiring only a small skin incision to enable pacemakers to be implanted.

All personnel involved in conducting animal experiments are fully trained in the theory and technical aspects of procedures and only take responsibility for completing these procedures as lead operator once they have worked closely with and been observed by experienced operators.

We also work closely with the named personnel and animal care staff to ensure that effective post-operative monitoring is in place and any remedial actions are effectively applied with minimal delays.

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

To deliver the most translationally relevant data from our studies and elucidate potential new treatments that are most likely to be applicable to man requires that we use appropriate animal models. To achieve this a key consideration is that potential confounding factors such as growth are removed from our experimental procedures. Another important consideration with immature animals is that their hearts are not fully developed and are structurally different to that in adults. As such, key signalling pathways which are heavily influenced by cellular architecture do not function the same way in immature animals as in adults. Thus, the translational relevance of using immature forms is a significant limitation in most of our experiments. Given these considerations, for the vast majority of our experiments, we therefore

use adult animals that are fully grown. Similarly, one of our experimental objectives is to understand how certain risk factors for heart failure interact; this requires that we use a model where these key factors such as ageing can be studied reliably. These considerations apply to the vast majority of our experiments; however, in some cases the lack of (e.g.) cellular ultrastructural development in immature animals is a useful tool with which we can address some very specific experimental aims.

Given these important considerations, and our aim to use minimally invasive surgical procedures requiring only a small skin incision, our preferred animal model is the sheep. Rodent models, immature life stages or (e.g.) zebrafish models are unable to address these key considerations and are in many respects fundamentally different to the human heart such that they are not appropriate to address our aims and objectives.

In many of our experiments we are using a disease model. In each case and in humans, the diseases that we are replicating takes a considerable time to develop following the initiating event. This therefore precludes us from completing all aspects of our experiments in terminally anaesthetised animals. However, we do strive to use terminal procedures where these are appropriate in order to minimise potential discomfort. Similarly, when investigating the potential value of a new treatment for disease, it is often necessary to apply this treatment over a prolonged period of many weeks to determine its effectiveness. This is therefore not achievable in terminally anaesthetised animals.

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

Our procedures have been refined considerably through a number of years of experience. We already seek to employ as minimally invasive procedures as possible to achieve our scientific objectives. However, we also strive to incorporate new methodologies and refinements if they become available and improve outcomes for the animals.

All surgical procedures require our team to ensure animals receive appropriate post operative care and pain management and we discuss these routinely with the named officers within our animal facility.

In some protocols we do expect animals to eventually develop some clinical signs of disease. In these animals we have an enhanced monitoring programme in place to ensure welfare harm to the animal is minimised as much as is practicably possible.

Our experimental animals become habituated to the investigators presence and in some experiments this allows us to train them to come to the side of the pen for food and completion of some aspects of our studies where remote monitoring is a feature. This means that we do not always need to restrain animals to achieve the experimental data that is being sought.

Animals are housed in social groups apart from the immediate post-operative recovery period (24 - 48 hours) when they are individually housed but in pens adjacent to and in sight of their original cohort so as to minimise any potential for separation effects.

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

There are no specific guidelines covering the animal models that we will employ. However, there are general guidelines covering aspects of our experiments such as blood sampling volumes; these are used to inform our standard operating procedures.

In addition, we regularly receive updates and guidelines through our local Animal Welfare and Ethical Review Board and we monitor websites and ensuing publications that promote best practice and refinements in methodologies including nc3rs.org.uk, felasa.eu, lasa.co.uk.

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

Our animal care facility, NC3Rs manager and ethical review board is responsible for delivering regular updates on 3Rs developments to users. We also monitor outputs published in the scientific literature and appropriate learned society websites.

When a 3Rs advancement becomes available we will consider if these can be adopted into our standard procedures as appropriate; for example, tube handling of mice, and employ them in our course of work as a matter of routine.

The project licence holder and associated investigators also attend a number of committee and scientific meetings each year where animal welfare and experimental practices are a key discussion topic. The members of the research team also attend a number of scientific conferences where animal welfare and refinements are frequent subject matter.