Home Office

NON-TECHNICAL SUMMARY

Ischaemia Reperfusion Injury and Organ Transplantation

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

No answer provided

Animal types	Life stages
Pigs	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?

The aim of this project is to characterise the mechanisms and consequences of ischaemia reperfusion injury in solid organ transplantation and to develop new therapeutic strategies using targeted drugs or cells.

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?

Organ transplantation is a life-saving treatment for end-stage organ failure, but there is a growing disparity between the number of patients in need of an organ transplant and the number of suitable donor organs available. In January 2020, there were 6183 people in the UK currently waiting for an organ transplant, an increase in more than 1% on the previous year. Conversely, the total number of patients whose lives were saved or improved by an organ transplant fell by 2% in the same year. The total number of transplants performed also fell, ranging from a 3% decline for pancreas transplants to 20% for lung or heart-lung transplants. This is in spite of a 2% increase in the referral rate of potential donors, as well as a 1% increase in the overall consent rate for organ donation.

Between March 2018 and 2019, 400 patients died while on the active waiting list. A further 777 were removed from the active waiting list, mostly as a result of their deteriorating health and subsequent ineligibility for transplant, and many of these patients would have died shortly afterwards.

When an organ is donated for transplantation, it can spend several hours outside the body, without any blood or oxygen supply, before it is transplanted into the recipient. This interruption and restoration of oxygenated blood to the organ at the time of transplantation is known as ischaemia-reperfusion (IR) injury. This results in damage and death to cells, and ultimately can result in the organ being unsuitable for transplantation.

This project aims to improve our understanding of the cellular mechanisms that underlie IR injury and test the safety and efficacy of promising novel therapeutic agents. New treatments that reduce cellular injury and organ dysfunction will help overcome the national and international organ shortage by increasing the availability of organs suitable for transplantation. Improved rates of organ transplantation would have an enormous societal impact by shortening waiting lists, improving the quality of life of patients, and reducing complications and death from organ failure.

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

This project will generate academic outputs, new treatment products, and the basis of clinical studies which will have health and economic implications.

So far, our understanding of how cells and organs sustain injury during transplantation is from results of experiments in cells grown in special dishes in the laboratory, and in mice. We anticipate that project will not only confirm that what we know is also true in pigs, which are much more like humans in terms of size and complexity but will also extend to discoveries in the precise steps underlying IR injury. If our hypotheses are correct, the findings will enable us to design and test targeted treatments, and perform experiments to measure whether injury levels can be reduced this way.

The results of these experiments will be published in peer-reviewed journals and presented at national and international conferences. The treatments that come out of the project may be new intellectual property. From our experiments, we hope to learn what the best ways to deliver the treatments are, and how often and how much of the treatments need to be given. Altogether, these will generate a blueprint for clinical studies, which will bring successful new treatments to patients.

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

Throughout the project, researchers both inside and outside the field of transplantation will benefit from the above outputs. IR injury occurs in many human diseases, including myocardial infarction (heart attack) and stroke. The processes that occur in mitochondria, cells and organs are applicable to all of these diseases. Because the cellular processes underlying injury in transplantation, heart attacks and strokes are similar, we anticipate that our findings, including new drug treatments developed for use in transplantation, could potentially also be of benefit in treating heart attacks and strokes. Importantly, our raw data, publications and presentations will disseminate new knowledge to other researchers, who can then build on it.

The surgical and scientific techniques used in this project are very specialised, and therefore limited to a few experts who are able to carry them out. As this project proceeds, more researchers will be trained in these specialist skills. They will go on to apply them in collaborative or new studies in the future, ensuring the dissemination of good experimental practice and model refinement.

In the longer term, future clinical trials of the new treatments will be based on the results of this project. These trials will ultimately deliver safe and effective new treatments to patients with end-stage organ failure and patients undergoing organ transplantation. The new treatments may also be applied to diseases such as myocardial infarction and stroke, so a wider range of patients may benefit.

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

This project represents an extensive multi-disciplinary collaboration involving multiple institutions. The knowledge created will be disseminated through presentations at national and international meetings and publications, which will include both successful and unsuccessful strategies investigated in the study.

Species and numbers of animals expected to be used

- Pigs: 100

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.

Pigs that weigh approximately 40–70kg have been chosen as they closely approximate the anatomy, size, physiology, and function of human organs. Techniques for organ retrieval, supplying blood to the organ outside the body (ex vivo perfusion), and transplantation are transferrable between pigs and humans, allowing us to closely model how organs are affected when their blood supply is interrupted (ischaemia) and when the blood supply is restored (reperfusion). This project will allow us to confirm that our mechanistic findings from mice are translatable to larger animals. Once the large animal (pig) model has been developed it will be used to test the safety and efficacy of our new treatments prior to the treatments being using in humans.

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

We will use commercially-sourced adult pigs for all of our experiments. When possible, animals will be trained to eat the substrate in which a sedative is administered before induction of terminal anaesthesia. Animals will undergo general anaesthesia, they may then be treated with therapeutic agents or cells, and finally multiple organs will be retrieved for the study of ischaemia reperfusion injury. The animals used in this project will be humanely killed at the end of surgery whilst still under anaesthetic (non-recovery). This means that any given animal will undergo anaesthesia and surgery once, and will not experience any pain, distress or suffering as a result of any procedure.

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

Animals will receive a general anaesthetic from the beginning of the experiment which will last to the end of the experiment after which they will be killed by the administration of an overdose of anaesthetic. The procedures do not involve reawakening the animal; therefore, no adverse effects are anticipated.

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)?

Pigs: 100% Non-Recovery. All animals will undergo non-recovery procedures, as described above.

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?

Ischaemia reperfusion injury in solid organ transplantation is a complex biological process involving crosstalk between cells, organs, the immune system and more. There is a gap in applying the knowledge we have generated using basic experimental models of ischaemia reperfusion injury to clinical transplant practices. We have used these basic experimental models to identify new therapies, but before these can be offered to patients, it is essential that we carry out clinical trials to ensure that these new therapies are both effective and safe. This project will provide us with a more complete understanding of the underlying pathological processes, help us shortlist the most promising new therapies, and inform the design of clinical trials in humans.

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

This project has been preceded and informed by experiments using cells grown in special dishes in the laboratory (*in vitro*) and donated human organs that have been declined for clinical transplantation. Non-animal alternatives have, therefore, been extensively used as far as possible, in order to replace animal experiments.

Why were they not suitable?

Use of cells does not enable the complexities of organ injury during transplantation to be assessed fully. While we have and continue to make extensive use of human organs, these are very rare and access to them is very unpredictable. Moreover, because some of the human organs are of less-than-ideal quality (which is why they were not used for transplantation), they are not always able to be used to generate robust and reproducible data.

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 number of animals per group have been estimated based on the preliminary data obtained from previous experiments, which we have used to estimate the variance in the data and the anticipated effect size in the treatment groups. We estimate a group size of 5 per group, with a total of 20 experimental/control groups, requiring a total number of approximately 100 animals. This is likely to be an overestimate, as in our experimental design, group sizes of 4 per group are anticipated to generate biologically and scientifically significant data. The overestimate is included to account for cases when it is not possible to generate data for technical reasons.

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

We have designed the experiments to maximise the number of organs and tissues that can be obtained for experimental use from every animal. For example, the heart, liver and kidneys, or biopsies from these organs, can be obtained from the same animal, enabling us to study mechanisms of injury (and response to therapy) to multiple organs in the same animal. In experiments using kidneys, we plan to use one kidney from each animal as a control and the other kidney as the experimental organ, thus reducing inter-animal variability and increasing the reproducibly of the data. By obtaining tissues under terminal anaesthesia, we will be able to ensure that the blood pressure and heart rate of the animals are consistent. This will reduce inter-animal variability, a key source of biological noise which can reduce the ability to generate biologically and statistically significant data.

We will continue to use the NC3Rs Experimental design Assistant (https://www.nc3rs.org.uk/experimental-design-assistant-eda) and the PREPARE guidelines (https://norecopa.no/prepare) for our ongoing experimental designs.

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

The proposed project builds on a wealth of pilot data, generated from small animal (mouse) models and use of human organs. We will therefore only pursue hypotheses and progress the use of therapeutic agents that have been shown to demonstrate promise in pilot studies. Moreover, we will actively share tissues from the animals with other researchers in order to reduce the number of animals used.

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 animals will be acclimatised to their new accommodation before the experiments, according to the guidance of the Named Veterinary Surgeon at our establishment. Animals will receive a general anaesthetic from the beginning of the experiment which will last to the end of the experiment after which they will be killed by the administration of an overdose of anaesthetic. The animals will therefore not endure pain or suffering.

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

Pigs are chosen for the proposed experiments because the size and anatomy of the organs closely resembles those of humans. The data generated from these experiments will therefore be directly applicable to humans and prime the design and conduct of human clinical trials. The research is using the least sentient model because all animals will be terminally anaesthetised in the experiments.

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

The Named Animal Care and Welfare Officer (NACWO) and animal technicians will be involved in sourcing animals, providing suitable acclimatisation and handling and environmental enrichment, all of which will improve animal experience. As housing and handling of the pigs, prior to the experiments, can contribute to contingent harm, we will monitor the pigs for any evidence of stress or adverse effects. If necessary, we will seek guidance and input from the Named Veterinary Surgeon (NVS) and explore measures to reduce such stress. For example, the acclimatisation period will be extended, or additional environmental enrichment will be provided as appropriate. Under the guidance of the NVS, palatable sedatives will be administered to reduce stress prior to terminal anaesthesia if necessary. During anaesthesia, the animals will be continuously monitored (heart rate, blood pressure, oxygenation and temperature) to ensure the depth of anaesthesia is appropriate.

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

The PREPARE guidelines and NC3Rs Experimental Design Assistant (EDA) have been used to design this project. These have aided in the comprehensive consideration of logistical, legal and ethical, quality control and experimental design and statistical analysis issues. Home Office Advice Notes, Codes of Practice, and regulations for personal and project license holders will be adhered to. We use the Establishment's 3Rs search tool as well as the NC3Rs and other websites (e.g., www.thepigsite.com) websites to keep up to date with new techniques and refinements as they are published. We will also actively explore and adopt any experimental procedures and techniques reported by other groups that can help refine the conduct of the experiments.

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

I have been actively involved in the ethical review of other scientific projects at the establishment. I actively engage with and keep informed about advances and developments in the 3Rs, including publications, conferences, meetings and new guidelines.