Home Office

NON-TECHNICAL SUMMARY

Reducing tissue damage during heart attack or stroke

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

Heart attack, Stroke, Therapy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 understand how heart and brain tissue can be saved from damage due to the lack of oxygen and nutrients during a heart attack or stroke.

A retrospective assessment of these aims will be due by 7 July 2030

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

Heart attack and stroke are the leading cause of death worldwide and present an increased burden on health systems such as the NHS. These diseases occur when an artery supplying either the heart muscle or the brain respectively is blocked, disrupting the delivery of oxygen and nutrients as well as the removal of waste products. This inevitably leads to death of the tissue that is without blood supply, which can lead to both severe long-term disability and increased mortality in patients.

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

This project will develop and test novel protective drugs for heart attack and stroke, as well as the resulting organ damage of chronic heart failure or chronic neurological disability, such as the inability to move a limb, in order to pave the way towards the use of these compounds in patients. The information gathered from this project will allow us to see how these substances affect the heart or brain and what doses and treatment regimens are most likely to be effective. We will also examine whether lifestyle changes (such as diet and exercise) or pre-existing diseases alter their effectiveness.

Treatment of an acute heart attack and stroke has markedly improved over the last decades resulting in fewer deaths in hospital. However, once patients survive the acute event, they are prone to chronic changes of the organs, resulting in chronic heart failure or chronic neurological disability. The present project will therefore address both of these important endpoints: the acute damage to the heart or brain, and, maybe even more important, chronic changes leading to heart failure or disability.

We aim to publish in high-ranking journals in order to inform the scientific community. I also regularly take part in public engagement activities and appear in the popular press in order to inform the general public about the progress and new findings of our research.

Ultimately, the above results and achievements will pave the way towards the use of novel compounds in patients with heart attack, heart failure, and stroke.

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

Initially, our fellow scientists will benefit from our findings about the underlying mechanisms of health and disease in heart attack and stroke. We will publish in prestigious journals and are active members of many scientific societies with regular participation at meetings. This will enable fellow scientists to have a better understanding of the disease mechanisms and helps them to develop alternative therapies.

In the second line, we anticipate bringing at least one of our novel compounds towards use in patients with heart attack or stroke within the next 5 years. Drug development is a long and complicated process, but we are confident that this project will enable us to move forward to a first-in-man study on this timescale.

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

My group is very well connected, not only locally, but also on a national and international level. The quality of our animal work has made my group one of the leading groups in the country investigating tissue damage due to loss of oxygen during a heart attack or stroke. We regularly publish our results, including negative outcomes or unsuccessful approaches. All our publications will be published with immediate full open access, making sure that fellow scientists and the general public can benefit from the results without delay in in full.

My group and my collaborators are very active on various outreach channels, including twitter, popular press, radio, and websites. The general public has access to all our findings, and we widely share our knowledge. We have a large number of collaborators world-wide, which work together with us to maximise our findings and make translation towards patient care more likely.

Species and numbers of animals expected to be used

- Mice: 10550

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.

Since the effects on blood flow, blood pressure, and the elimination/distribution of compounds are essential parameters of drug therapy, isolated organ systems cannot be used. For example, over the years, we have successfully used an isolated heart system, called Langendorff, whereby the heart is mounted on a rig and being perfused by a solution. It is not possible to gain this information in cell models since the whole-body system needs to be intact due to the influences that administration, distribution, metabolism and excretion have on the availability and efficacy of the compound.

Furthermore, changes in exercise levels, diet or pre-existing diseases cannot be mimicked without animals.

Rodents are the smallest possible species where interventions, such as ligation of a coronary artery or occlusion of a brain artery in order to induce a heart attack or stroke respectively are possible.

In addition, rodents offer the possibility to use genetically-altered models in order to mirror patient conditions (such as pre-existing diseases) more closely.

We use animals of an adult and aged life stage since this best reflects the time of disease onset in patients.

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

Typically, either purpose-bred genetically-altered animals or genetically normal mice will be used. The breeding protocols follow standard breeding procedures.

In a subset of animals certain conditions such as exercise, administration of substances or alteration of diet will be applied in order to mirror more closely the conditions in humans. This includes pre-existing lifestyle (exercise or diet) or pre-existing medication. Substances will be administered in the most-suitable way, which includes into a vein, under the skin, or into the abdominal cavity. In addition, if the animals are on the voluntary wheel running protocol, they will be single housed. Rodents much like the additional wheel running, while the single housing will only be done as short as possible and no longer than 6 weeks.

Finally, animals will be subjected to surgical procedures to induce either a heart attack or a stroke. These procedures will be performed either under terminal anaesthesia and the animal will not be allowed to recover, or animals will be allowed to recover. After a certain recovery period, the animals might develop heart failure or neurological disability which mirrors very closely the conditions of patients surviving a heart attack or stroke following treatment.

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

Overall, most of the animals will only suffer mild discomfort and no lasting harm. Most of the interventions, such as exercise or dietary intervention, are not expected to have any discomfort and certainly no harm to the animals.

The surgical procedures will be performed solely under confirmed general anaesthesia and in case of the recovery protocol, pain medication will be given during the wound healing and recovery period from surgery. The development of heart failure or neurological disability itself is without any pain. However,

the animal might experience suffering due to the development of heart failure, such as fatigue and breathlessness, or in case of stroke, neurological disabilities, weight loss, or general lethargy.

Nevertheless, some animals may die during surgery due to complications in the procedure itself or the anaesthesia. These deaths will be rare (<5%) and will happen during full anaesthesia. In addition, very rarely (<3%) animals will die during the follow-up period post-surgery. These deaths are almost always due to fatal cardiac arrhythmias which are entirely pain-free and can be compared to "sudden cardiac death" in patients with heart conditions. Due to the sudden and unexpected nature of these deaths, there is no possibility to make any prevention beforehand.

We further aim to perform interventions, such as imaging with MRI, PET, or echocardiography, or administration of substances. Only rarely these interventions cause discomfort.

Taken together, the likely adverse effects will only very rarely exceed a moderate level at any point.

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)?

Non-recovery:	Mice	40%
Mild:	Mice	30%
Moderate:	Mice	25%
Severe:	Mice	5%

What will happen to animals used in this project?

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

A retrospective assessment of these predicted harms will be due by 7 July 2030

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

Information about the distribution of the drugs, effects on the heart and brain, or blood pressure and exercise tolerance cannot be answered using cell-based models.

The novel drugs which will be tested with this license have already undergone extensive testing in suitable non-regulated models in cell culture, isolated cells or isolated organ preparations. Therefore, we already know most of the mechanisms of action and have information about possible toxicity, dosing and the most effective treatment regimen. However, we need to confirm efficacy in animal models before we can translate these compounds to human use. Furthermore, these animal models have to mimic the situation of patients as close as possible. This can be done by an alteration of diet, exercise, application of pre-existing medication, and the use of pre-diseased genetically-altered models.

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

We routinely use cell lines for very important preliminary questions, such as drug transport into cells, metabolism, and basic toxicology.

Other applications, such as "organ-on-a-chip" are not suitable due to the complex nature of ischemic damage to the heart and brain and their complex structure. However, we will monitor scientific advances regularly and reconsider should suitable models become available.

Why were they not suitable?

Cell lines cannot mimic important conditions, such as exercise, and do not have crucial confounding elements, such as circulation or the blood brain barrier.

There have been decades of research trying to find a suitable cell-based model of ischaemia (lack of oxygen to tissue)/reperfusion (re-introduction of oxygen and blood flow to tissue), but so far none have been found.

A retrospective assessment of replacement will be due by 7 July 2030

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 is determined by more than 20-years experience with an identical methodological approach under a different license. Furthermore, we will use statistical calculations based on our previous data to calculate numbers for each individual experimental setting.

There is an increase in the overall number of animals used compared to previous licenses by my group. This is partly due to the increased number of genetically-modified mouse strains used to simulate better conditions happening in patients (such as the use of exercise and fasting) and the investigations of pre-existing conditions, such as obesity and diabetes.

Furhtermore, the activity in my group has increased overall with recent grant success and the promising move towards a new therapy in stroke.

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

I use the NC3R Experimental Design Assistant (EDA) in order to thoroughly design the studies.

In addition, I am on an EU committee which aims to standardise ischaemia/reperfusion experiments throughout Europe considering the 3Rs as well as highest scientific standards. Within this consortium, my group is the reference group for acute heart attack in mice in the UK.

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

Wherever possible, we try to assess as many parameters in a single animal as possible and reduce the numbers in one treatment group to an absolute minimum that the statistical analysis will allow us. This includes performing sophisticated imaging techniques which allow us to gain much additional information about the heart attack or stroke in a single animal.

We are part of a tissue and animal colony sharing system within our institution. Furthermore, we share certain knowledge, such as surgical procedures, word-wide within the scientific community.

A retrospective assessment of reduction will be due by 7 July 2030

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 present project will use either wild-type mice or genetically-altered mice. The genetically-altered strains are lacking important mechanistic elements which helps us to understand the mechanisms of how we can protect against heart attack or stroke. We are already breeding some of these genetically-altered lines under a different license and the animals do only show mild clinical signs.

The main aim of the license is to test protective effects in heart attack and stroke as well as during the following recovery period where the animal might develop heart failure or neurological disabilities. Both surgical models will be performed under adequate anaesthesia and pain medication during the immediate recovery. While long-term recovery is pain free, it might however cause moderate harm due to signs of heart failure (such as breathlessness and fatigue) or neurological disability (such as inability to move and climb, reduction in food intake). Since the experiments are aimed to find treatments to ameliorate these long-term effects, there are no models available with less suffering, The chosen models to examine long-term effects after heart attack or stroke are the ones with the least suffering for the data obtained. Many other models used in the literature are of increased harm.

The alteration of diet or exercise and administration of substances are very close to the patient situation and therefore ideally suited to test these important factors on their influence on the outcome of heart attack and stroke. There will not be any lasting harm and only mild discomfort for the animals due to these models.

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

Mice are the most useful species from which it is possible to obtain relevant and meaningful physiological and pathophysiological information. Especially non-invasive imaging such as MRI and PET cannot be performed in smaller species. Furthermore, mice allow genetic manipulations in order to more specifically study the underlying mechanisms.

The development of heart attack and stroke is dependent on many factors, such as oxygen and temperature, which makes fish or toads not suitable.

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

We closely monitor the animals throughout the experiments. This includes the use of sophisticated heart and brain function monitors, such as Ultrasound, ECG and blood flow monitor (doppler), as well as temperature control. If anaesthesia is applied, the efficacy is monitored closely throughout the procedure. Should any anaesthesia or surgical problem occur which would potentially harm the animal, the experiment will be terminated, and the animal humanely killed.

In protocols or procedures where anaesthesia is not used, animals will be monitored for any deviation from normal health and behaviour and should any signs appear animals will be killed immediately.

The animals are allowed to acclimatise to either the exercise protocol or the water supplementation and dietary changes. In protocols where treadmill running is used, animals have warm-up

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acclimatization prior to the exercise protocol.

Within the previous license, we gained much experience in pain management after the described surgical procedures. While the development of either heart failure or neurological disability itself is painless, we will make sure that adequate pain medication is given after surgery.

In addition, the animal will be kept in a warm environment for the immediate recovery and receive mashed food as long as necessary.

In a past license, we have used streptozotocin (STZ) to induce a diabetic phenotype in mice. This model is used by many investigators. However, we discovered that STZ has many side effects leading to severe suffering of the animals. Furthermore, due to the toxic potency of STZ, the obtained results have been of dubious quality. We therefore stopped the model. In the present license we now use a genetic model of diabetes, db/db mice. These animals are very obese and slowly develop a mild diabetic phenotype. The model is much closer to the human condition and, hence, the obtained results are much superior. Furthermore, the animals do not develop any significant phenotype apart from a diabetes-induced increase in urination and thirst.

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

We will follow the NC3Rs Experimental Design Assistant (EDA) see https://www.nc3rs.org.uk/experimental-design as well as the following guidelines:

for pilot studies (https://www.nc3rs.org.uk/conducting-pilot-study);

ARRIVE guidelines, https://www.nc3rs.org.uk/arrive-guidelines;

IMPROVE guidelines for stroke models: https://www.nc3rs.org.uk/news/improve-ing-animal-welfareexperimental-stroke-research;

published guidelines to assist with planning animal research and testing, such as the PREPARE guidelines: http://journals.sagepub.com/doi/full/10.1177/0023677217724823

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

I receive regular updates about news and advances in the 3Rs through our local information system. This includes information about relevant NC3R platforms and NIO communication.

A retrospective assessment of refinement will be due by 7 July 2030

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?