Home Office

NON-TECHNICAL SUMMARY

The influence of metabolic disturbances on platelet function, thrombosis and vascular inflammation

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

No answer provided

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

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?

Blood platelets play critical roles in the prevention of blood loss, termed haemostasis, but also participate in pathological thrombosis, cancer and cardiometabolic diseases. This project aims to dissect the molecular mechanisms unpinning platelet driven disease processes.

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?

Blood platelets are cells that become sticky and clump together to form blood clots to stop us from bleeding after injury. However, in some people platelets also form blood clots inside intact blood vessels leading to heart attacks and strokes. The formation of these blood clots, called thrombi, account for large numbers of deaths in the UK. We know that people at risk of these diseases often have increased levels of fat and sugar in their blood, and that raised fat and sugar concentrations may cause platelets to form clots more readily in the blood stream. However, we do not know how these fats and sugars cause excessive blood clotting. If we can identify the proteins on platelets that respond to fats and sugars it will help doctors understand the causes of blood clots and to develop new medicines to prevent thrombotic disease. The only way we can really examine the importance of these proteins is to increase the amounts of fat or sugar in blood of mice and then determine how blocking or deleting them affects blood clotting. Therefore the work we propose is critically important to help doctors and the pharmaceutical industry to develop new strategies in preventing a major cause of death in the UK and worldwide.

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

Our key endeavours are academic excellence, unravelling the molecular mechanisms that drive platelet hyperactivity, which could be used to improve the lives of patients at risk of thrombotic diseases. For example, in the lifetime of our previous licence we published two high profile papers describing novel mechanisms of platelet activity and how in principle they could be targeted therapeutically for clinical benefit. Our primary outputs will in the form of academic publications describing these mechanisms. We hope that these publications act as platforms for further studies by our group, and others, to translate our findings to patients at risk of thrombotic disease.

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

Pathological thrombosis is a major component of the pathology that underlies cardiovascular diseases (CVD) and other chronic diseases. In the UK, CVD accounts for 28% of annual deaths is the greatest

cause of mortality, killing approximately 161 000 people in 2012 (www.heartstats.org - British Heart Foundation). Furthermore, these numbers do not take into account the thrombosis related deaths associated with diseases such as cancer and diabetes. Thus, thrombotic disease inflicts a significant health and financial burden on the UK.

The beneficiaries from these output will be varied and include the academic, clinical and pharma sectors. The outcomes we generate from our programme of work will provide new knowledge of platelet driven disease processes providing further opportunities to drive academic excellence. A second element of this will be the generation of new animal models, for example, the generation of platelet specific genetically modified mice that may be applicable to other areas of research including diabetes, cancer and immunity/infection. As is standard with good academic practice all models developed in our laboratory would be shared with the wider scientific community, both directly within our institute and wider afield. Should our work lead to refinements in laboratory practice for example as improved surgical, imaging or experimental procedures these would be made available for other researchers to utilise. Our work programme is focused on a key unmet clinical need. Further exploration of the mechanisms of platelet hyperactivity would allow clinical colleagues insight into the potential causes and management of disease. There are also potential advantages for the pharma industry, who remain highly interested in developing agents to control unwanted platelet activation.

Therefore, the project will continue to help us identify the specific roles of the proteins that regulate or inhibit platelet function and allow us to evaluate their potential as targets for the development of new antiplatelet drugs that could reduce/prevent heart attacks and strokes. While we have no guarantee that the proteins studied will in the future become useful therapeutic targets, it will add to the overall of knowledge of this area of research and help other researchers, both in the UK and internationally, who are focussed on solving problems associated with thrombosis.

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

We will publicise our findings to the wider scientific community, industry and the public through annual conference presentations, publications and through other media.

Our work is presented on a regular basis within our institute, through research seminars, and often disseminated through academic conferences. These are used to discuss the work in progress and provides a forum with which we can discuss experimental issues (including animal welfare and phenotypes) informally with other experts in order to refine techniques and to help develop best practice. The publication of our work is another major form of dissemination, where we report the findings in a more formal manner. In our recent work we used a similar approach to that outlined in this application to identify a new mechanism of thrombosis.

We aim to publish at least two high impact publications, which build on our previous work. Upon publishing our research results in scientific journals, we will prepare press releases in collaboration with the press offices of the University. This transparency of approach to sharing of data will maximise our outputs, ensure that our research activities are complementary rather than competitive and that the field moves forward as quickly as possible.

Species and numbers of animals expected to be used

- Mice: 3000

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.

Mice are the smallest mammal with a haemostatic system similar to that of humans and therefore is an excellent model for evaluating thrombosis and haemostasis in vivo. The murine model has the added advantage of being amenable to genetic manipulation allowing the functional characterisation of specific proteins.

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

The mice in this part of the project will undergo an altered feeding regimes for up to 16 weeks and then blood harvested under anaesthesia. The procedure will be performed once per animal followed by termination under an approved schedule 1 method.

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

There are three areas where adverse effects maybe anticipated.

1. Dietary manipulation - on rare occasions some mice find high calorie diets unpalatable and begin to lose weight. Mice will be monitored daily for their general well-being and weighted on a weekly basis. Should an animal show signs of distress they will be removed from the study returned to a normal diet. If the distress continues the animal will be culled by a schedule 1 procedure.

2. Administration of metabolites/drugs - There could also be potential impacts of administration of substances designed to influence the recruitment of circulating platelets and/or thrombosis. Substances will only be administered where dosing and potential toxicity data are available in the mouse or similar species. Such substances, such as established drugs or metabolites, are therefore not expected to produce adverse side effects at the concentrations administered. It is anticipated that the drugs used in the proposed experiment will be given intravenously on three occasions prior to harvesting of blood. If adverse effects are suspected the animals will be withdrawn from the study. If the adverse reactions persist the animals will be killed by a Schedule 1 or other method stated in the protocol immediately. As data is collected we will review the protocol to ensure that the severity limits are appropriate.

3. Recovery during anaesthesia - The severity of this procedure will be controlled through use and careful monitoring of general anaesthesia throughout. There a possibility the animal recovers prematurely from anaesthesia, although this would be a relatively rare occurrence. To prevent premature recovery from anaesthesia there will be continuous monitoring of the depth of anaesthesia by testing the limb flexion/withdrawal reflex and/or the corneal reflex, which will be supported continual monitoring of heart rate and body temperature. Should there be suspicion that the animal is recovering

prematurely additional anaesthetic will be administered by inhalation. However, it is noteworthy that this has not occurred during the procedures performed in the last five years and therefore we do not anticipate this to be a common occurrence.

In our experience all of the possibilities highlighted above are rare.

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 severity level for this protocol for all mice is mild and performed under terminal anaesthesia.

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?

The study of blood platelets is dictated by three limiting factors.

1. There is no reliable in vitro tissue culture models for the generation of platelets from primary megakaryocytes or cell lines hence the only source of platelets is from an animal model.

2. Platelets are not amenable to genetic manipulation since they lack a nucleus. Therefore, transfection studies used by many other researchers as an alternative to animal experiments that work with nucleate cell systems are not possible.

Therefore researchers commonly use genetically altered mice to identify and delineate the pathways involved in platelet activation. This laboratory is committed to perform work with animals only when the potential biomedical advances warrant this. We are interested in developing new approaches that would reduce the use or generation of genetically altered mice to study platelet function, and we are monitoring closely studies in other laboratories that have begun to examine ways to produce genetically modified platelets (using siRNA technology) in vitro from bone marrow culture. Should this technology become available will look to adapting it for our own studies.

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

None

Why were they not suitable?

They lack recognisable platelets.

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?

In the types of studies, we perform the anticipated response is usually “all or nothing’, that is the genetically modified mice fail to respond to stimuli when compared to the wild type. Therefore the numbers for *in vitro* studies of our programme are calculated based on the numbers of samples we can prepare per mouse. For example, the blood from each animal provides approximately 1 ml of washed platelets which is sufficient to prepare for four samples. With this knowledge we can extrapolate to the appropriate number of mice required by determining how many samples are required for each series of experiments within our project. Under these controlled conditions we aim to use approximately 6 mice per strain per assay (and 6 control animals), since platelet responses are generally very reproducible. In general we use between 10 and 15 assays during our functional evaulation.

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

Our experimental design is driven by studies performed in human platelets, which act as a guide to the potential importance of key proteins in the function of platelets. Careful analysis of the data emerging from these experiments will determine the need for experiments in animals. It is noteworthy that the experiments performed under this licence are *discovery science* and so very little information is available to guide our calculation of numbers. However, given the "all or nothing responses" expected, allows us to ascertain with relatively low numbers of mice whether extensive experiments using multiple in depth assays are required.

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

We have a number of strategies for optimising animal numbers.

1. The key step is the generation of pilot data from *in vitro* functional assays, lack of phenotype in these assays (usually between 10 and 15 assays) prevents mice being used in experiments that require dietary manipulation and reduces the breeding of mice.

2. All tissues from mice sacrificed under any of the protocols are available for other researchers within our institute.

3. All genotyping is performed by a commercial supplier to allow for industry standardised protocols. Correct genotyping is critical to implementing a targeted breeding strategy that allows for good colony management.

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.

Currently the mouse haemostatic system is considered a strong model for that found in humans and has the advantage of genetic manipulation. Therefore the project will use mice for the proposed studies.

The project uses two key steps to determine how specific proteins affect platelet function, all of which have been designed to avoid suffering and distress in the mice

1. Dietary interventions are mild and therefore do not induce an extreme metabolic phenotype.

2. Harvesting of blood is performed under anaesthesia and without continued use, which again minimise suffering to the animals

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

Non-sentient animals cannot be used for our studies since they lack recognisable platelets.

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

The major refinement to our protocols is in the area of blood harvesting from mice. We have now moved away from the use of cardiac puncture and take blood from the inferior vena cava. The success rate of harvesting blood using this method is significantly higher and more reproducible, thereby reducing the numbers of mice required for experiments. Furthermore since this procedure is performed under terminal anaesthesia it minimises suffering in the mice.

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

Major guidance through the project is taken from the NC3Rs, whose website (hubs and microsites) provides a vast array of resources on the general principles underlying the experiments highlighted in this project; this includes anaesthesia, breeding strategy and numbers, and experimental design.

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

The applicant's laboratory is conversant with the 3Rs and in continually examining ways that the principles are implemented. To ensure this

1. Group members are encouraged to check the NC3Rs resources page

2. We are kept updated by the NIO on a regular basis regarding changes in best practice, courses and additional training.

3. We are aware of the scientific literature in the haemostasis field, where refinements to these types of procedures are reported.

Where changes/advances in practice have been reported we aim to up-skill either through training courses or visiting laboratories to receive guidance.