NON-TECHNICAL SUMMARY

Thrombopoiesis and application to transfusion

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.
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

platelets, megakaryocytes, transfusion

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?
Platelets are small circulatory cells that are essential to prevent bleeding. If platelet numbers are too low, one can transfuse platelets to decrease the risk of bleeding.

The aim of the project is to better understand how platelets are made in the bone marrow and how genetic defect lead to disease of platelets in patients. We will also apply this knowledge to the production of platelets in the laboratory and the testing of these platelets prior to human trials.

**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.**

What are the potential benefits that will derive from this project?

The sourcing of platelet from blood donors for transfusion to patients has several drawbacks: 1. Sourcing constant supply, 2. donor-derived infections (such as hepatitis, or HIV) and 3. Immune reaction of the patients against donor blood cells. Producing platelets in vitro would eliminate these 3 issues.

The project aims at better understanding how platelet are produced by their bone marrow mother cells (the megakaryocyte) to use this knowledge to produce the cells in the laboratory AND understand how certain proteins that control platelet formation can be targeted by drugs to reduce the platelet count in patients at risk of heart attacks and strokes.

In this project we will also test the platelet produced in the laboratory to make sure they function properly after transfusion. This will pave the way for applying to regulators for authorisation to carry out clinical trials with patients.

**Species and numbers of animals expected to be used**

What types and approximate numbers of animals will you use over the course of this project?

This project proposes to use mice. Approximately 3300 mice will be used over 5 years.

**Predicted harms**

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The animals will be maintained in state of the art facilities, general husbandry will be done by trained staff and experiments carried out by an experienced team. The facilities will also be a “barrier” to infectious agents (to prevent infections particularly in animal with a weak immune system). The majority of the animals maintained under this licence are not expected to show any detectable adverse effects. Most experiments will require the collection of samples after animals are killed either at the end of procedures whilst terminally anaesthetised or otherwise humanely killed. In some instances
animal will be administered drugs or cells via an injection in the tail vein or their tummy (or by mouth if appropriate). We may carry out blood test from the tail vein or one of the bigger vein in the leg. These will only cause very limited and short-lived discomfort.

In some instances, to be able to carry out the assay described in the project, the animals will need to have their spleen removed under anaesthetic. The effect from this procedure on the animal will be moderate discomfort and will be limited to the post-operative period.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Where possible, we minimise the use of animals by using platelets or stem cells from human volunteers from which we can grow the platelet mother cells (megakaryocytes). However, the experimental approaches that we can use with human cells are limited. Platelets cannot be stored for more than 1 week and therefore patients with specific disease/mutations affecting platelets would have to be bled numerous times in order to study their platelets in details. The number of stem cells that can be obtained from blood is also very small which means megakaryocytes can only be grown in limited quantities to study how they function. In contrast genetically modified mice will give us the opportunity to study the influence of specific genes on platelets and their production by megakaryocytes by giving access to fresh samples to carry out comprehensive laboratory studies.

Moreover, megakaryocytes and platelets do not act in isolation from other cells. Megakaryocytes need the bone marrow environment to produce platelets and platelets will interact with blood vessel and other blood cells to form a clot. Our analysis of this complex process combines experiments on platelets in isolation from other cells, with experiments in a whole animal setting. This is vital to allow us to analyse gene function in the setting and context of these other cells and to test the quality of the platelets we produce for blood transfusion.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Animal numbers bred for use on this Project will be minimised as far as possible by matching breeding to experimental requirements. Pilot studies and power calculations will be employed to refine the number of animals used.

The methods chosen will generate the greatest amount of data for the fewest animals used. We routinely expect to derive multiple data sets from a single animal, by extensive use of modern approaches that allow us to analyse very small blood samples. This will be complemented by a team approach allowing the analysis of several different samples from one animal.

The ability to perform the intravenous injections of cells under terminal anaesthesia will enable us to perform the tail transection much sooner. This will reduce the variability of the data and therefore fewer animals will be required.
Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Mice are the species of choice for the proposed investigations because they are a good mammalian model with a well-characterised bone marrow and blood vessels that are similar to humans. Studying megakaryocytes and platelets in mice therefore provides valuable information that will further our understanding of human biology and diseases. In particular testing of platelets produced in the laboratory in mice is a well-recognized quality control experiment without which we could not obtain the authorisation to go on to do clinical studies in human patients.

Genetically-altered mouse technologies are becoming increasing sophisticated, where genes can be turned off within specific cells when required, whiles leaving the rest of the animal unaffected. Such genetically altered animals will be used wherever possible in this project and will greatly reduce the risk of adverse effect to the mouse. This is because only the platelets will be genetically altered, whilst all the other cells in the mouse will be normal.

The ability to intravenously inject cells under terminal anaesthesia will mean that the mice are anaesthetised during the injection and therefore will suffer less.