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NON-TECHNICAL SUMMARY

Targeting endoglin function in cardiovascular diseases

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 following aims mentioned in paragraph (b)

Key words

Endoglin, Cardiovascular, Hereditary Haemorrhagic Telangiectasia (HHT), Preeclampsia, Genetics

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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?

To understand how human mutations in endoglin gene and changes in endoglin function cause or contribute to different cardiovascular diseases, and to apply such knowledge for therapeutic application.

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?

This project studies the function of a protein called endoglin, also abbreviated as ENG. It locates mostly on the surface of the cells lining the blood vessels. Changes in ENG function have been implicated in two vascular disorders, namely, Hereditary Haemorrhagic Telangiectasia (HHT) and preeclampsia.

HHT is a genetic disorder where the blood vessels, particularly capillaries, are not properly developed, resulting in distorted and weak connections between small arteries and veins which can lead to regular bleeding. HHT patients typically have red blood spots on the skin (telangiectasia) and regular nosebleed that may require blood transfusion, but it is the bleedings in the internal organs that are serious and can cause life-threatening haemorrhage. In addition to bleeding risk, many patients have distorted blood vessels in the lung, brain and liver, affecting the function of these essential organs. Type 1 HHT (HHT1) is caused by inherited mutations in ENG gene which lead to cells making less or faulty ENG protein, whereas HHT2 is caused by mutations in a gene called ACVRL1 which interacts with ENG. Mutations in ENG and ACVRL1 account for over 80% of all HHT patients. Average rate of HHT occurrence is 1 in 5000-10000 of the population worldwide.

Preeclampsia is hypertension (high blood pressure) during pregnancy and can be very dangerous for both the mother and the baby. During pregnancy, cell surface ENG is significantly increased in the placenta, and a small proportion of this can be cut and release a large fragment into the blood known as soluble endoglin, or sENG. Levels of sENG, along with another protein called sFlt1, are elevated by more than 10-fold in women with preeclampsia compared with those without. Current literature suggests that elevated levels of sENG and sFlt1 may directly cause preeclampsia, but more studies are required to confirm this.

There is no cure for either HHT or preeclampsia, and we still don’t know how the changes in ENG function cause these two vascular disorders. The aim of this project is to apply what we have learnt about ENG function at protein and cellular levels and perform further studies in mice to understand whether ENG changes are present in mice, whether they contribute to the development of HHT and

preeclampsia, and whether we can apply novel therapeutics to prevent and reverse the diseases. Such knowledge may help us find novel information in the blood proteins (called biomarkers) that can predict or monitor these disorders. It may also help to develop molecules that could restore the function of ENG as novel therapeutics.

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

This project has three objectives.

The outcome of objective 1 will reveal whether and how increased levels of sENG cause or contribute to the development of preeclampsia. It has been known for a long time that serum levels of sENG, along with sFLT1, are significantly increased by more than 10-fold in women with preeclampsia. The impact of such changes on the development of preeclampsia is not fully understood and our studies will reveal important insight into this. Such knowledge could lead to either new treatment targets or novel biomarkers for early detection of preeclampsia.

The outcome of objective 2 will be the results of testing novel therapeutics in mouse models of HHT1 – such mouse models mimic the human loss of ENG gene and mice develop some clinical symptoms similar to human HHT1 patients. Our potential novel therapeutics target the genetically identified pathway, aiming to correct the underlying cause of HHT1.

Objective 3, along with objective 1 and 2, will provide essential knowledge on ENG function at more detailed levels, for example, how endoglin interacts with different proteins and pathways in the blood vessel cells to affect blood vessel function. Such knowledge is crucial for a comprehensive understanding on how ENG achieves its normal function in the blood vessels and how the changes in its function contribute the two devastating vascular disorders.

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

In the short term, the knowledge gained from this project will be published in scientific journals and benefit other medical researchers. Such research will provide further information on how our body system controls blood vessel functions, thereby develop better therapies for the treatment of preeclampsia and HHT. In the middle to long term, such advances in PE and HHT research may lead to novel biomarkers and therapeutics, which will help physicians to better manage and treat patients, and provide benefit to the patients as well as their families, and our society as a whole.

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

We will disseminate our research findings timely and broadly, at scientific conferences and in scientific journals. We will make connections with patients' group whenever possible to explain our research. If any findings that can lead to a potential medical product, such as potential novel therapeutics, we will work with relevant drug discovery partners and translate our findings in a timely manner to provide patient benefit.

We seek amendments to refine the experiment protocols in order to achieve the proposed experimental outcome sooner and with reduced number of animals. The requested amendments are not expected to introduce worse symptoms than those in PE patients and are within the severity limit

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as proposed in the original application. Therefore, by reducing the number of animals used and achieving the scientific outcome sooner, the benefit of this amendment will significantly outweigh the potential harm of the whole project.

Species and numbers of animals expected to be used

- Mice: 6150

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.

For objective 1, we study preeclampsia which is high blood pressure during pregnancy that is resolved upon delivery, therefore we will need to use pregnant mice for the study. To determine whether the phenotype we observed is specific to pregnancy or not, we will need non-pregnant female and male mice as controls. It is not possible to use lower species animals for this purpose because we want to recapitulate the disease during the pregnant stages in human which are not present in lower animal species. It is essential to use a mammal and a mouse is the smallest, reliable mammal for this research.

For objective 2, we are modelling a human heritable blood vessel disorder HHT, manifested as malformed blood vessels in different parts of body. HHT patients are mostly adult, and many current literatures use new-born mice. We use a genetic modified mouse line that carries the hidden mutation in the gene that cause human type I HHT. We can allow the mice to grow to adulthood completely free of disease, and only induce the disease by giving an inducing agent called Tamoxifen which will delete the target gene and then mice will develop the disease symptoms over the following 5 weeks. It is this time window we will focus our research to study the pathological change and the resolution by potential new therapeutics.

For objective 3, we use these mice to help our research in understanding why the gene mutated in HHT can cause HHT-like diseases. These mice carry the hidden mutations that do not cause any disease in young mice less than 4 months old. We can generate cells from any organ and delete the HHT-disease related genes in cells isolated from different tissues after mice are euthanised, therefore we will not cause any pain to any live animals.

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

Injections and procedures will be kept to a minimum to achieve the required results. Tests and small-scale studies will be performed on new molecules and drug candidates to determine dose levels, dosing frequencies and the most appropriate route of administrations required to assess the effect of the molecules. We will also regular review published results and modify our experiment accordingly when required.

Animals will be kept under anaesthesia for the minimum time possible to achieve the required results. Sometimes we need to measure the blood pressure in the heart chambers and lung circulation as well as heart functions, as these are part of the disease symptoms we are trying to establish in the animal model. We use a method called catheterisation where we put an electronic probe into the heart under general anaesthetic condition. Typically, the catheterisation procedure takes approximately 30-40 minutes to complete. While under anaesthetic animals will be maintained at an appropriate temperature by using a heat mat or similar heating device. Animals will be euthanised at the end of catherterisation experiment.

Substances injected will be of known safe dose concentrations and administration routes. The administration of substances will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. Test molecules will be dissolved in solutions that have been tested and deemed safe to use.

Blood sampling via superficial veins (usually from the hind leg) will be kept to a minimum and safe volumes calculated based on estimated circulating volumes.

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

Genetically altered mice produced under this protocol are not expected to exhibit any harmful phenotype. Some animals will experience mild transient pain associated with an injection or blood sampling procedure.

For the two disease phenotypes we are modelling in this application, HHT and preeclampsia, animals might experience some human disease-like symptoms. For example, in HHT model, mice may develop enlarged hearts and weak blood vessels which can cause unexpected bleeding. For mice modelling preeclampsia, they may develop high blood pressure, proteins in urine samples and reduced placenta size.

Potential harm of IP injection in heavily pregnant mice may lead to stress or litter loss. We will monitor the animal and weigh them daily to ensure disease severity not exceed proposed in the project and humanely terminate the experiments whenever needed.

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)?

Mice Mild 50%

Moderate 50%

What will happen to animals used in this project?

- Killed
- Used in other projects

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 animal research in this project is essential to our understanding of several human vascular disorders including HHT and preeclampsia. We have performed over 10 years of research using non-animal alternatives, such as using proteins and isolated cells, to come up with the hypotheses and objectives to be tested. This project focuses on the disease model set up and testing therapeutic intervention. In order to translate our basic science research to patients' benefit, disease model studies using animals are an important step that cannot be replaced before clinical applications.

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

We will use non-animal alternatives whenever possible. We have used and are still using recombinant protein technology and structural biology to understand how ENG and its interaction partners achieve their functions under normal circumstances, and how mutations found in patients cause changes to protein functions. We also use cellular models to study how different proteins interact with each other inside and at the surface of the cells to influence the overall function of the cells. In collaboration with others, we will use patient samples and advanced cellular models whenever we can.

Why were they not suitable?

Pathological changes in human diseases are never limited to a single tissue or a single cell type. The disease-causing mutations in ENG affect blood vessels in multiple organs. To understand whether such changes are the cause or the result of a disease is crucial for clinical application. Non-animal alternatives will not be able to model the interactions between different tissues and organs. Furthermore, the impact of normal physiology, such as sex and age, during the disease onset and development cannot be tested without using animal models. Carefully designed animal studies allow us to detect the pathological changes in multiple organs simultaneously and over a period of time, thus we can test the effects of potential drugs or drug candidates in preventing and reversing the pathological changes. Such studies, which are proposed in this project, cannot be achieved without using animals.

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?

We have worked out protocols required in this project and estimated the numbers in each protocol based on published studies and test studies that we have performed so far. We will use the minimum number of animals required for our experiments whenever possible, and regularly consult a statistician for advice. We collect and review data from previous related work, from our own and from published work, to predict the expected results, and perform pilot experiments to allow the proper estimation of the number of animals required for each experiment. We used PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) to guide the design of the protocols.

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

We use online tools, such as the NC3R's Experimental Design Assistant (https://eda.nc3rs.org.uk/), to predict group sizes needed to detect differences with statistical significance based on the data collected during test experiments. Group sizes, gender, strain and age are matched for control and experimental groups. Sources of variability will be identified and minimised wherever possible. We will follow the PREPARE guidelines in our 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?

We will perform most efficient breeding strategy for each GA colony following Home Office guidance and NC3Rs guidance: https://nc3rs.org.uk/3rs-resources/breeding-and-colony-management. We will optimise and standardise the protocols whenever possible. For example, variability in vascular phenotypes following gene activation with tamoxifen is minimised by ensuring the optimised tamoxifen dose is used. Data is collected by researchers blinded to treatment wherever possible and with experimental details recorded following the ARRIVE guidelines. We will always perform pilot studies for each new protocol to allow refinement of the experimental design. When we finish our experiments, we take extra tissues when feasible and store them and will share the tissues with other researchers whenever possible.

We seek amendment to refine the protocol which will result in reduced animals needed for the experiments. For example, the pregnancy rate for timed breeding is 40-60%, which means nearly half of the mice cannot be used for the pregnant mice study. If we amend the protocol to allow performing the control non-pregnant study at the same time, we will also use these non-pregnant mice for producing results instead of wasting them.

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.

We use mice that can produce certain human disease features to understand the components that cause and contribute to the disease. The genetically modified mice that we use generally carry ‘hidden’ mutations, such that almost all of animals are completely healthy until they are given the inducer to activate the mutation, reducing any clinical effects to the absolute minimum necessary for the project.

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

We cannot use nematodes or fruit flies to model these diseases because they don’t have similar organs to the vertebrate and cannot provide the disease phenotypes observed in humans that we aim to investigate and treat.

Some of the diseases mostly manifest in adult human, hence we need to study these diseases in adult mice in addition to neonatal mice.

We study the disease progression over a period, from days to weeks, therefore cannot use terminally anaesthetised mice for this part of the work.

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

We will always use the minimum number of administrations where possible to achieve our scientific requirements in a study.

We will use the saphenous vein in the hind leg for blood sampling.

By using appropriate anaesthesia and pain-relieving drugs in the procedures to alleviate pain and discomfort, the protocols cause minimum possible discomfort to the animals. All Personal License holders (PIL) working under this Project License (PPL) will only work independently for any single procedure after their competency has been confirmed. Such information will be recorded and regularly checked using the online Mouse Colony Management System (MCMS).

Where animals are to be humanely killed for reasons unrelated to the scientific end of a procedure of the work described here, the Named Animal Care and Welfare Officer (NACWO) will be consulted to establish whether the animal tissues would be of value in other studies.

In terms of general mouse handling, we have moved to the tunnel method to reduce stress when removing a (post weaning) mouse from its cage.

From our previous experience, approximately 5 weeks following Tamoxifen injection to delete ENG gene in blood vessel cells, mice may develop enlarged hearts. A small proportion of the mice may also have bleeding in the lung. We have refined our protocol, performing daily monitoring after Tamoxifen injection, minimise the number of animals suffering and euthanise the animals when the clinical symptoms reach to the end points defined in the protocol.

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After the amendment and when we make changes to the experiment, we will enhance our monitoring and observation to record any impact, such as from increasing frequency and timing of IP dosing in pregnant mice, and take any humane measures whenever needed.

We have investigated different routes for compound administration in pregnant mice such as oral gavage, IP, IV and SC injections. For delivering protein molecules into the circulation, we choose IP route as the first choice because this has been successfully established and is used routinely by another group locally. Our pilot study suggests IP administration is safe in pregnant mice and causes minimum stress to the animals. Oral gavage will not achieve the expected dosing outcome for big protein molecules. Although IV or SC injections appear to be safer in pregnant mice, in practice it will cause more stress to the pregnant mice because mice will be more restrained when performing IV or SC procedures. We will keep IV and SC as options should we find IP route not suitable in future studies.

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

We will perform all the experiments in the most refined way that we can do. We will discuss care and husbandry with Named Veterinary Surgeon (NVS), NACWO and Named Information Officer (NIO) whenever necessary, and use internal 3Rs Search Tool to access additional websites for information. We will refer to our internal updated Tamoxifen Guidance whenever possible.

We will use the ARRIVE guidelines for the reporting of our research: https://arriveguidelines.org/

If we later need to perform recovery aseptic surgery, we will follow LASA Guiding Principles for preparing for and Undertaking Aseptic Surgery (2nd Edition 2017) (https://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf) for all surgical procedures.

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

We will regularly visit NC3R websites for updated information and subscribe to their newsletters. I am a member of NC3R Cardiovascular Network and will receive regular newsletter updates on 3R news and collaborative opportunities. We will use the experimental design tools on the NC3R website for each experiment. We will regularly discuss with the Named People at our facility to obtain any valuable resources for 3Rs advances.

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