Home Office

NON-TECHNICAL SUMMARY

Zebrafish models to investigate disease processes associated with brain haemorrhage

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
- (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

zebrafish, brain haemorrhage, blood vessels of the brain, disease biology, drug discovery

Animal types	Life stages
Zebra fish (Danio rerio)	adult, embryo, neonate, juvenile

Retrospective assessment

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> 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 identify and investigate molecules that can regulate the stability of the blood vessels of the brain and/or regulate brain injury in models of human diseases associated with bleeding in the brain, such as stroke.

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?

Brain bleeds, also known as 'brain haemorrhages', are a type of stroke that account for almost 6% of all global deaths. We do not fully understand the biological mechanisms that cause blood vessel weakness that lead to brain haemorrhage and we have no specific medicines that target the brain injury for patients once the brain haemorrhage has occurred. This project will allow us to continue to investigate the disease mechanisms and also identify and test potential drug candidates for the treatment of diseases associated with brain haemorrhage.

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

We will generate new data that will expand our understanding of the disease biology associated with brain haemorrhages and related cerebrovascular diseases. Our work will identify new molecules that may be developed into candidate compounds for progression to clinical trials. We will also continue to produce a number of publications that describe our work. We will also present our data at national and international scientific conferences. In this project, we will engineer some new zebrafish with different genetic backgrounds which we might share with other researchers. The outputs from this project will allow us to continue to provide evidence that zebrafish disease modelling is a suitable alternative approach for pre-clinical brain haemorrhage research that can reduce the numbers of mammals required for these types of studies.

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

The immediate impact of these outputs will be for our research group. Longer term these outputs may benefit both the wider pre-clinical and clinical stroke research communities and zebrafish researchers. Based on subsequent development of candidate compounds identified in this project, patients and the

pharmaceutical industry may potentially benefit from our outputs. More broadly, animal welfare may also benefit, as we aim to reduce the numbers of mammals required for this type of research.

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

We will regularly publish our work in open access scientific journals and present our data at national and international scientific conferences. As we have done previously, all of our datasets will be deposited in open access online resources. We will continue to collaborate with researchers and funders in the UK and internationally and share our knowledge/material with them. Wherever possible negative results will be included in publications (e.g. through supplementary appendices) or deposited through online resources.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 46,500

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.

In terms of Home Office regulations, zebrafish are animals with the lowest neurological complexity that can be genetically modified to study human diseases associated with brain haemorrhage. Our previous project licence has allowed us to establish zebrafish as an excellent model for cerebrovascular disease research and has indicated that they could be used more widely to reduce the numbers of rodents required for this type of research. Importantly, zebrafish embryos, larvae and juveniles allow for live imaging and microscopy protocols to observe cellular responses throughout the entire depth of the brain in living animals - a technique that is currently not possible in mammalian models. Due to their small size and abundant numbers, zebrafish embryos are also required for drug screening protocols, where thousands of drug compounds can be tested in a relatively short period of time - which is not feasible in such a short time frame in mammals. Genetic modification is also very well established in zebrafish and more efficient than in mammals. Therefore, we can easily generate colonies of genetically modified adult zebrafish that can be bred regularly to generate embryos/larvae/juveniles for experiments.

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

Different procedures will be performed on animals at different life stages.

Genetically modified adult zebrafish will be generated through manual microinjection of nucleic acids into fertilised embryos and raised to adulthood in the aquatics facility. To confirm genetic modification, some adult animals will receive brief general anaesthesia so that a tail fin biopsy can be taken for genetic analysis or held in a fish net so that a surface skin swab can be taken for genetic analysis.

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To note, zebrafish aged up to 5dpf are not protected under the Animals Welfare Act - and during these ages are commonly referred to as 'pre-protected'. For most experiments, zebrafish that display brain haemorrhage during the pre-protected stages will be used. Brain haemorrhage will be induced at 2-3dpf either because of a genetic defect or through exposure to a chemical (e.g. atorvastatin). Both approaches causes the blood vessels in the brain to burst at only this very early age. We will use these animals to observe how the brain injury caused by the haemorrhage resolves over time.

In some cases, experimental animals older than 5dpf will be used to study brain injury through live imaging using microscopes, and some of these animals will be immobilised in low-melting agarose. If necessary (based on age of fish), immobilised fish will be placed in an imaging system that contains a water flow to maintain oxygen supply for the animals. During this course, chemicals may be introduced into the medium surrounding the fish.

In some cases, swimming behaviour will be recorded in animals. This will either be done in a tissue culture dish or in an observation tank. Animals will be briefly anaesthetised prior to assignment of experimental groups.

In some cases, zebrafish will be bathed in water containing a chemical/drug. Alternatively, zebrafish will receive a specified dose of chemical/drug by intraperitoneal, intravenous, intramuscular or retro-orbital injection, possibly repeated over several cycles, under terminal anaesthesia. In some cases, terminally anaesthetised zebrafish will receive an intravenous injection of a fluorescent tracer dye and prepared for live imaging. To note, the chemicals/drugs used will be chosen based on either their 1) potential to improve brain injury outcomes or 2) potential to weaken blood vessels of the brain.

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

Animals that are recovering from anaesthesia during the project might display abnormal swimming behaviour. If this behaviour persists for 30minutes after the removal of anaesthetic, then such animals will be humanely killed using a schedule 1 technique.

Any fish exhibiting abnormal behaviour or signs of infection following genotyping (where animals are briefly anaesthetised so that a small piece of tail fin can be dissected for DNA analysis) will be humanely killed by a Schedule 1 method. For most zebrafish tanks, this will occur in less than 2% of fish.

Some fish may have the potential to develop harmful neurological observable traits after a certain age, which would appear as abnormal swimming behaviour. In all cases, these animals will be humanely killed before reaching that age and before these signs start, unless moved on to another protocol as continued use for a specific purpose.

Fish exhibiting any unexpected harmful observable traits will be humanely killed, or in the case of individual fish of particular scientific interest, advice will be sought promptly from a Home Office Inspector.

Fish that display signs of suffering that affects their health and wellbeing such as abnormal swimming, slow growth or abnormal feeding will be immediately humanely killed.

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Some anaesthetised animals that undergo injections may suffer from bleeding, but any pain will be controlled by analgesia as advised by the NVS.

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)?

For obtaining sperm/eggs and breeding and maintenance of zebrafish = 80% mild, 20% sub-threshold. (NB: Sub-threshold is a severity level where no pain or suffering is observed).

For experiments to assess progression/recovery from brain injury in protected zebrafish = 100% moderate

What will happen to animals at the end of this project?

- Killed
- Used in other projects
- Kept alive

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?

Although experiments using cells or in test-tubes can be informative, these systems cannot mimic the complex interactions that occur between blood, blood vessels, brain cells and the immune system in conditions associated with brain haemorrhages. The only way we can perform the necessary experiments to understand the biological processes that occur in the brain is to use animal models– where the natural environment within the brain remains intact.

Historically rodents have been used to study haemorrhagic stroke. Zebrafish are animals with lower neurological complexity than mice and rats. As such, the use of zebrafish embryos and early-stage larvae can be considered as a partial replacement of the existing rodent models. Furthermore, many of the experiments performed in this proposal will be performed on fish embryos and larvae during the pre-regulated stages, which we know experience minimal distress and recover quickly after brain haemorrhage.

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

We consider and do regularly perform some experiments in cell culture models (e.g. human brain blood vessel cells) - to help verify discoveries made in the fish model. We can also use brain tissue obtained from people who died from brain haemorrhage in a similar way. To aid with drug development,

we can use computer modelling to help understand how a drug might interact with a particular biological target. We can also consider the use of existing clinical trial data to help support the relevance of any potential new treatments we make in the fish model.

Why were they not suitable?

We try to incorporate non-animal experiments into our research wherever possible. Although experiments using non-animal approaches can be hugely informative, these systems cannot mimic the complex interactions that occur between blood, blood vessels, brain cells and the immune system in conditions associated with brain haemorrhages. Furthermore, for drug development, candidate compounds must be first tested in animal models before consideration for human trials.

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 numbers of animals we predict to use is based upon feedback from our researchers of the numbers of larvae they predict to use and the number of parents we would require to breed to create the required number of larvae.

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

For specific experiments we will continue to work with statisticians to ensure our sample size calculations remain accurate. Wherever possible, we will use fish that are siblings as our control comparison groups. For example, we will be able to generate young fish that carry zero, one or two copies of a genetic defect from breeding parent fish. As these young fish are brothers and sisters, it means that we can make better comparisons between these animals for our experiments, whilst also reducing the need to produce additional young fish from other parent fish.

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

At the end of each experiment we will collect tissue wherever possible to share with other researchers. We will bank tissue from surplus embryos and store in the freezer for future pilot and optimisation studies. Researchers will share embryos from each breeding pair/tank for experiments to avoid unnecessary overproduction of embryos. However, regular breeding of adults is essential to ensure longevity and to avoid health problems (e.g. egg bound females).

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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.

Most of the work in this project will involve the use of pre-protected zebrafish larval models that exhibit brain haemorrhage between 2 and 3 dpf. We have shown that these larvae recover by the age of 5dpf. Therefore, minimal welfare issues are expected with this model.

Some of these animals will be grown beyond 5dpf into the regulated stages and assessed to better characterise the biology associated with the recovery process after brain haemorrhage. As these animals are recovering, we do not expect any significant welfare issues, but will monitor them closely to ensure no signs of distress or suffering are apparent.

Some of these animals will be grown beyond 5dpf into the regulated stages and assessed to determine if there are any weaknesses in the blood vessels of the brain. This may involve injection of drugs and/or fluorescent dyes into animals. These fish will be anaesthetised to control pain from injection prior to imaging. The animals will be killed immediately after the experiment to avoid lasting harm.

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

Zebrafish are the least sentient vertebrate species available that can be used for studying haemorrhagic stroke and related conditions. Wherever possible we will use pre-regulated embryos and larvae for our experiments. A large component of our project will involve live imaging in animals under terminal anaesthesia.

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

As the project progresses, phenotypes that are observed that are deemed to be associated with cerebrovascular disease will be recorded to allow us to categorise the clinical signs based on severity banding, to allow us to generate a future 'traffic light' system, as implemented for rodent models of stroke.

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

We will closely follow updates on practical guidance for zebrafish research published by the NC3Rs. We will also attend international Zebrafish conferences on an annual basis to receive the most up to

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date published best practice guidelines for experimental procedures. We will continue to design and complete experiments in align with the ARRIVE and PREPARE guidelines.

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

Our research has received past and current 3Rs-related funding and we have regular contact with our in-house 3Rs team. As such, we are regularly informed of the latest advances in the field with regards to the 3Rs and the use of zebrafish in scientific research. Wherever possible we will implement such advances in our protocols, for example as we have done for zebrafish skin swabbing as a refined protocol for genotyping. We also have internal 3Rs-based seminars and workshops, and good communication with our animal unit and other zebrafish lab users that we can discuss best practice with.

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