Home Office

NON-TECHNICAL SUMMARY

Defining the molecular mechanisms underlying hypoxic ischaemic brain injury

Project duration

Years **5**
Months **0**

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

OBJECTIVES AND BENEFITS

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

What is the aim of this project?

The aim of my group's work is the efficient discovery of mechanisms underlying brain injury which occurs in babies suffering a lack of oxygen to the brain during birth, and translating these discoveries into novel neuroprotective therapies. Such an ambitious aim can only be achieved through a considered approach which integrates *in vitro* and *in vivo* strategies to enable rapid translation to clinic.

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?

Asphyxia (restricted blood flow/oxygen to the brain) during birth occurs in 2-3 term babies per 1000 in the UK, leading to the development of a condition known as hypoxic ischaemic encephalopathy (HIE) and permanent, life-long brain and motor disorders such as cerebral palsy. Following asphyxia, there is a delay of a few hours before the majority of brain cell death occurs providing clinicians with a valuable treatment window. The only available treatment, therapeutic hypothermia, is not always successful and new treatments are urgently required. However, therapeutic hypothermia does prove that intervening with treatment after the injury has occurred and within the treatment window can be effective.

This project is designed with the overall aim of identifying novel therapies to combat the devastating effects of this brain injury. Over the next 5 years, we will strive to improve our understanding of the cellular mechanisms underlying the evolution of the injury, enabling us to generate significant and realistic avenues for therapy development ready for preclinical testing. Primarily and most importantly, the success of this project will have far-reaching, long-lasting improvements on the lives of significant numbers of babies and their families but in addition, the basic science outlined in the project will be of substantial interest to all researchers in the field of brain development.

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

The outputs from this project are extensive and focus on identifying mechanisms leading to mitochondrial damage and using these data to provide new avenues for the targeting of treatments. Mitochondria are structures contained within all brain cells which provide the energy required for cell survival and which are susceptible to damage following birth asphyxia. Using a variety of methods, we will generate large datasets of candidate molecules and novel pathways leading to mitochondrial dysfunction and will make these available to the wider scientific community using the appropriate platforms. We will have generated a significant body of basic mechanism data contributing to the understanding of mitochondrial biology in the immature brain. We will also have provided a solid

foundation for the development of therapies aimed at preventing mitochondrial-mediated cell death triggered in response to this injury.

These outputs will be disseminated through publications and used to underpin further grant applications. If applicable, we will also consult with technology transfer colleagues to pursue any therapies in collaboration with pharmaceutical companies.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

Birth asphyxia is the second most common causes of death and disability in children under the age of 5 years in 2010, resulting in the loss of 50 million Disease Adjusted Life Years (DALYs). As there is currently only one therapy for this injury, which improves outcome for only 1 of each 7 infants treated, there is a critical unmet need. Our project is designed with the overall aim of identifying novel therapeutics to combat the devastating effects of term brain injury and there are wide-ranging short and long term benefits which may arise on successful completion of our project.

Short term, the basic cellular science outlined in the project will be of substantial interest to all researchers in the field of brain development and mitochondrial biology. We will also identify potential therapies through repurposing existing drugs or developing novel mitochondria-based interventions. Depending on the nature of these compounds, we will engage pharmaceutical companies in order to facilitate required testing prior to clinical trial.

Long term, and most importantly, the success of our project will offer far-reaching, long-lasting improvements in the lives of significant numbers of babies and their families who suffer the devastating consequences of birth asphyxia.

How will you maximise the outputs of your work?

We will maximise the outputs of this work in a number of ways.

We will provide our data to the scientific community through presentations at conferences dedicated to perinatal brain injury. These are necessary in order to generate new collaborations depending on the focussed areas into which our experiments lead. We will place our large datasets in appropriate repositories for use by the wider scientific community and will publish robust data (including any *in vivo* negative data) in well-respected, open access, peer reviewed journals in the field.

Predicted harms

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

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

Mice will be bred in social housing conditions and may be ear notched for identification purposes.

Typically a post-natal day 9 mouse pup will be anaesthetised and undergo surgery for a maximum of 5 min and then allowed to recover in a warmed recovery box until the rest of its littermates have been through surgery. The entire litter is returned to the mother for an hour, whereupon the pup is then placed in a warm low oxygen chamber for around 50 min. During this time, the pup may experience mild seizures similar to those experienced by the human newborn following birth asphyxia. However these do not last once the pup is removed from the chamber. The pup will then be left alone, given an injection of a neuroprotective drug and/or cooled down to 33C for 5 hours. This hypothermia does not cause the pup any pain or suffering. Pups remain with the mother until weaning when they are subsequently maintained for non-invasive behavioural experiments or until the experimental time point of interest. Each mouse will experience this protocol only once.

Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.

All pups undergoing surgery will be regularly monitored.

During surgery, exposure to hypoxia may cause seizure-like behaviour. However this very rarely lasts beyond the duration of the hypoxia (30-80min). Within the period of the previous licence, no animal experienced seizure activity following cessation of hypoxia.

Initially, following surgery, these mice will generally have a low level of weight loss but the weight gain trajectory is entirely normalised within a week. After this time, it is difficult to distinguish the experimental animals from the control, untreated animals.

What are the expected severities and the proportion of animals in each category (per species)?

All genetically altered mice that are being bred and maintained for the projects will experience mild severity only. Mice in protocol 2 will experience moderate severity as they undergo general anaesthesia for recovery surgery. However, subsequent recovery is usually in the mild severity category and the behaviour of mice is largely indistiguishable from their control litter mates. There are subtle behavioural differences, for example, forepaw preference can usually be observed in mice following the surgical procedure.

Application of the three Rs

1. 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?

Our project aims to understand the mechanisms involved in perinatal hypoxic-ischemic and infectious/inflammatory brain injury and to test neuroprotective strategies. As such, we need models that allow us to mimic human perinatal brain injury. Animals have to be used, as to validate a mode of action, experiments are required that cannot be conducted in humans for ethical and scientific reasons. In addition, interaction of the biological systems in whole organisms, with intact physiological barriers and excretion mechanisms, is key to inferring the potential of candidate therapies.

What was your strategy for searching for non-animal alternatives?

We have considered the feasibility of achieving our purpose by not involving animals at all, for example by using cell lines or in vitro recombinant methods, but no such alternatives are able to reproduce the brain injury we aim to investigate in this proposal.

However, where possible (for example, in altering gene expression *in vitro* or for testing the specificity of pharmacological activators/inhibitors), we will replace animal studies with primary cell preparations or experiments in appropriate cell lines (e.g. neuronal SH-SY5Y, microglial BV2, oligodendrocyte CG4 lines).

Why were they not suitable?

Our project ultimately aims to identify neonatal neuroprotective strategies formulated from evidence using *in vitro* cell systems. However, *in vitro* systems alone cannot mimic the unique and complex environment that exists within the neonatal brain. The brain is comprised of many cell types and *in vitro* systems cannot model the physiological interactions and communication between diverse cell populations. In addition, we are aiming to discover therapies beneficial to the neonatal brain, the environment and developmental trajectories of which are still being determined. Therefore to generate clinically relevant data, *in vivo* neonatal models must be used.

2. 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 used power calculations based on our previous licence and through refinements to the protocol.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

We regularly use PREPARE guidelines and NC3R resources such as the Experimental Design Assistant tool to make sure we are adequately powering our experiments while minimising animal numbers. We also use the services of the RVC chartered statistician and subsequently follow ARRIVE guidelines for publication of *in vivo* data.

What other measures apart from good experimental design will you use to minimise numbers?

The RVC has an efficient BSU with highly trained staff which will streamline the breeding and maintenance of genetically altered and wild type mice (protocol 1). In addition, the methodology of protocol 2 has been refined over a number of years and is in routine use in the lab. For neuroprotection studies we will plan pilot studies according to the guidelines on the NC3Rs website.

3. 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.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

We will predominantly be using mice at an early life stage. The brains of mice develop late in relation to birth and at postnatal day (P)9-P12, mouse brain development corresponds to the term human brain. Importantly, they share several important features with the human brain with regard to brain complexity and injury response in white and grey matter and thus can be considered a valid model in which to deliver the objectives of the project.

Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?

There are other animal models we could use, some in which the brain structure is more similar to that of a term human (primates, piglets). However, we have decided to replace the use of such animals with

mice, without detriment to the science. Mice are considered less sentient at an early age, easier to handle, breed easily and have a short generation interval. In addition, much is known about their genetics and physiology.

What are you going to do to refine the procedures (for example increased monitoring, post-operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?

Refinements we propose to test will include increased monitoring of vocalisation following surgery as a measure of distress in very young animals, in which standard characteristics of suffering or pain may not be so obvious. Equally, for young animals we will minimise rejection by the mother by rubbing the hands of the experimenter in bedding prior to handling the pups, to reduce transfer of unfamiliar smells. To this same end, routine monitoring will largely be through the side of the cage without disturbing the animals. Opening cages and handling animals will be limited to once daily as part of the standard observation and behavioural testing procedures within the project, unless a symptom of pain or distress is observed under which circumstance it may be appropriate to increase the frequency of monitoring.

How will you ensure you continue to use the most refined methods during the lifetime of this project?

We will stay informed through the NC3Rs website as well as taking advantage of the advice provided by the NC3Rs Programme Manager who will be based at the RVC one day per week.

Explain the choice of species and the related life stages

We are using mice for this project. Mice are easy to handle and a wealth of information is already known about their genetics and physiology which assists in the interpretation of data and the planning of future experiments. Brains of mice continue to develop after they are born so therefore we will be predominantly using mice at the life stage of post-natal day 9 as this is the point at which their brain development is closest to that of a human newborn at full term. The mouse model of hypoxic-ischaemic brain injury outlined in this protocol is highly relevant to the human condition as it was used in the development of therapeutic hypothermia, currently the only therapy available to babies who have suffered from asphyxia during birth.