Home Office

NON-TECHNICAL SUMMARY

Molecular basis of infection-induced sickness behaviour

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

Key words

Trypanosome, Pathogenesis, Virulence, Neuroinflammation, Behaviour

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

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?

The aim of this project is to understand how African trypanosomes, the causative agent of Human African trypanosomiasis or sleeping sickness, induce metabolic disease and neurological disorders affecting behaviour.

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?

Human African trypanosomiasis is not only a devastating neglected tropical disease but can also be used as a tool to understand basic aspects of how our bodies combats infection. In our previous animal license, we generated significant knowledge regarding the mechanisms of skin colonisation, which is critical for parasite transmission. Additionally, we have generated a wealth of knowledge regarding the mechanisms underlying the damage to the brain caused by the parasites, including potential drug interventions. Moving forward, our work will shed light on how the parasite causes weight loss and sleep disturbances. This knowledge is critical to understanding this complex disease and has the potential to open up new possibilities for the diagnosis and treatment of infectious diseases. More broadly it can also provide insights into how our bodies fight infections, and the unintended consequences these processes might have on normal health.

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

Reaching the goals of this project will not only increase the current knowledge available regarding trypanosome infections, in the longer term our findings could lead to improved chemotherapy or disease interventions. This would greatly benefit, both socially and economically, the developing countries where this disease is endemic. Beyond trypanosomiasis the results of this project could shed light on other infection or conditions that affect the host during systemic chronic infections, including systemic metabolism and brain function.

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

In the short term, our results will have an immediate impact in the scientific community, including those investigating parasitic infections, as well as those investigating weight loss, brain function, and behaviour in response to infection. In the longer term, we also anticipate that our work will have an impact on disease modelling and potentially improved intervention strategies, including developing novel therapies.

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

Our work is collaborative and interdisciplinary, and so we anticipate that the outputs of this work will be maximised further through ongoing and future collaborations. We will engage with the scientific community in parasitology, immunology, and neurology to disseminate the knowledge generated from our work. Additionally, we will publish the work obtained from all the animal work conducted under this license (including negative data) to reduce the use of animals in duplicated work elsewhere.

Species and numbers of animals expected to be used

- Mice: 3,000

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.

We will use mice as they respond to infection in similar ways to humans and domestic animals such as cattle. Mice also have a well described immune system, genome and there is an array of mutant strains available, which will accelerate our research. The majority of trypanosome research studies have been based on this model and our results will be directly comparable to previous studies, maximising its impact. We will use adult mice (>6 weeks old) as their immune system will be fully formed and functional.

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

This project involves injecting mice with trypanosome parasites and monitoring how the infection progresses in the animal by routinely taking blood samples from the tail vein. Typically, wild-type mice will be infected for 1-3 weeks and may receive substances that can alter the function of the immune system via injection that may ameliorate the disease or shed light on the disease process. Mice will be closely monitored daily throughout the procedures and supportive treatments such as soft food will be provided, if necessary. Colonies of genetically altered mice will be bred and maintained, and then adult mice infected for 1-3 weeks via injection. Additionally, surgery may be performed to implant a wireless device to monitor electrical brain activity and skeletal muscle activity as a proxy to study sleep. In some instances, we will irradiate mice or treat with chemical compounds to modify the bone marrow, altering the immune system.

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

In the majority of cases infection by trypanosomes may induce mild adverse effects such as the transient pain associate with injection or periodic mild clinical effects of the infection, such as the development of a rough / stary coat or lethargy. In some cases, the mice could develop more moderate clinical signs including staggering when walking. If no signs of clinical improvement are noted during this period, the animals will be humanely killed. It is also possible that a few animals may decline considerably becoming moribund, if this does occur the animals will be humanely killed. In rare

instances, immunosuppression may occur as a result from irradiation. This could result in unintended side effects such as opportunistic infections. In such rare events, animals will be humanely killed to avoid unnecessary suffering.

Extensive experience of this infection model and a familiarity with the techniques used, allows us to recognise quickly any unexpected adverse signs. All mice will be closely monitored (at least every three days) throughout the procedures and recorded on the animal sheets and endpoints implemented as required. All animals will be humanely killed on completion of the experimental procedure.

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 20%

mice: moderate 80%

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?

Trypanosomes can be maintained in culture flasks and this technique will be used to create genetically modified parasite lines to study genes that may influence parasite survival and in initial drug investigations. However, trypanosome infection cause changes in multiple interconnected systems and organs within the host and neither the development of the disease nor the ultimate success or failure of potentially useful treatment strategies can be investigated fully in the isolation of a culture flask. For example, when the effects of trypanosomes were examined using an artificial model of the blood-brain barrier grown on a petri dish, no lasting damage to the integrity of the barrier was detected. When this was investigated using a mouse model of trypanosome infection, a progressive increase in barrier impairment was associated with disease development. This clearly illustrates a disparity between the mechanisms at play in tissue culture models and animal models of this disease.

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

Human tissue obtained from stem cells generated on a dish (known as organoids) that could be employed in very limited circumstances. We have been developing in vitro culture systems to model migration of the parasites through body barriers such as the vasculature, and have established

collaborations with other groups to validate the use of human skin explants generated in vitro to model how parasites invade tissues and dissemination to different organs. We have also explored the use of stem cell-derived brain organoids as a model to replace the use of live animals, with promising results. We will strive to employ these systems where possible.

Why were they not suitable?

Although these models are promising, as they stand, they do not fully recapitulate the responses obtained in animals. For example, brain organoids are devoid of innate immune cells, which limits its utility in the context of modelling infections in living animals. We are and will continue to invest efforts to keep developing organoids into more suitable culture models.

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?

Mice: During the 5-year licence period requested it is estimated that a maximum of 3,000 mice will used.

Analyses of the data gained from the experiment will usually involve multiple conditions (e.g., uninfected and infected animals that are either treated with a drug or with a placebo) to ensure that the greatest amount of information is achieved from a small number of animals. In general, the number of mice required to achieve the goals set out in this project licence has been calculated using an experimental group size of 6-7. Additional statistical support will be available when required to ensure that we gain the most accurate data using the minimal number of animals.

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

Working closely with a biostatistician, I have used data gathered over the past five years to estimate the number of animals required to achieve the expected level of significance for the most common experiments to be carried out under this license. The final estimates reflect the minimum number of animals required to answer the experimental questions behind this project license.

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 conduct pilot experiments to determine appropriate sample size and power calculations. We are also building a tissue bank repository in the lab, obtained from previous experiments, that can be

used as an alternative to conducting repeated experiments. Our breeding system ensures that littermates are used either in experiments or as breeders. As a general rule, we will maintain our colonies in low numbers and will only expand it when needed, and only for a finite period of time. At all times, we will follow the NC3Rs PREPARE guidelines and Norecopa guidelines.

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.

The mouse is the most suitable laboratory animal to use in this system as trypanosome infections can be manipulated to reproduce each of the disease phases that are essentially similar to those found in both human and animal trypanosomiasis. In addition, the mouse provides a standardised animal with a wide range of analysis reagents and genetically altered research lines available. When generating bone marrow chimeras, where possible, we will use chemical compounds such as Busulfan as alternative, less aggressive, methods to irradiation in order to manipulate the immune system.

Extensive experience in the use of this mouse model provides familiarity of handling and maintaining infections and in the consistent induction of the various stages of the disease. This also results in a reduction in the severity of the procedures performed and in the number of animals required to achieve the goals of the experiment. To minimise the impact to welfare all animals will be closely monitored throughout the procedures and, if necessary, for those animals that require it, supportive treatments, such as soft food will be placed within easy reach.

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

Trypanosomes are able to infect many vertebrate hosts, including mammals and fish. Whilst it is possible to infect zebrafish with trypanosomes, this requires the development of intricate infrastructure and husbandry of large fish such as carp to maintain the life cycle of the parasite. Further to this, it remains unclear whether zebrafish can be used to model the disease pathologies experienced by infected humans. Due to the extended nature of rodent models of infection, it is not possible to limit infection to an immature life stage. Moreover, most humans are infected during late adolescence or adulthood, when they have a mature immune system, meaning that juvenile rodents would not accurately reflect human disease pathology.

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

We have and will continue to adopt NC3Rs and local guidelines to handling methods, housing conditions, and environmental enrichment, to ensure the welfare of the animals in our license. Our

procedural approaches have been refined over the years to ensure that volume size, frequency of inoculation, and routes of administration (including needle size) do not cause distress to our animals. We typically monitor the animals after procedures to ensure there are not unwanted procedural side effects. We do not require analgesia but will maintain an open dialogue with the local veterinary team to make sure this is incorporate in a timely manner if needed.

Animal monitoring and pain management are an important part of all the procedures within the project. The need to minimise suffering is always considered when planning experiments and we routinely revise our experiments to reduce animal suffering. When using irradiation which can cause side effects in the gut, we will develop a more targeted approach thus improving the welfare of the animals, for example, by using alternative chemical reagents with less unwanted side effects (e.g., Busulfan). Of note, none of our experiments exceed a moderate severity level.

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

We will ensure our experiments are designed in accordance with the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs). Similarly, we will follow published best practice, eg Joint Working Group on Refinement.

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

We will engage with the local ethical committee to ensure the work conducted under this license aligns to ethical standards, maintaining the 3Rs principles at the core of our activities. To this end, we will participate in national meetings organised by the establishment and National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) in addition to in-house 3Rs meetings and events, to incorporate changes to our procedures in an effective and timely manner.