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NON-TECHNICAL SUMMARY

Pathology and treatment of lysosomal and related disorders

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Lysosomal disease, Inflammation, Central nervous system

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

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 purpose of this project is to investigate a set of rare diseases that affect a part of the cell known as the lysosome, a fundamental compartment in many cells. We will study the impact of the immune system on the central nervous system (CNS) during disease course to help develop new targets for future therapies in murine models.

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?

Lysosomal and related diseases are inherited genetic disorders which lack specific enzymes that breakdown complex sugars, fats or proteins in a compartment of the cell called the lysosome. These include progressive childhood metabolic lysosomal diseases such as mucopolysaccharidosis (MPS) types I, II, IIIA, IIIB, IIIC, IV, VI and VII, Krabbe disease, Wolman disease and many others. A build up of undegraded by-products can result in widespread inflammation, modulation of diverse signalling pathways, organ damage and in some cases severe damage to the brain resulting in behavioural difficulties and death before 20 years of age. Some of these diseases such as MPSIIIA primarily affect the brain making treatment development very challenging. All of these diseases primarily affect children or young adults and most have very poor existing therapies. As they are individually rare, it is important to find commonalities between the diseases to aid in the development of future therapies. Current treatments are unable to correct the brain damage seen in many forms of lysosomal diseases, especially MPSIIIA and B since enzymes are unable to enter the brain. Bone marrow transplant is curative for a small subset of lysosomal diseases, but not all. Aside from supportive care, there are no effective treatments for these severe diseases and therefore they represent an urgent unmet clinical need especially with regard to treating the brain. Furthermore, each of these diseases provide a huge cost to the NHS, with enzyme treatment for an average child with a lysosomal disease at £250,000 per year. There is a large gap in our knowledge of pathology in these conditions and where disease treatment thresholds lie.

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

Outputs from the project:

1) we will acquire a deeper understanding of the pathological mechanisms (with particular focus on the immune response) to LSDs in mouse models relevant to human disease.

2) we will understand if the immune response can be modified to improve neurological decline and neuropathology in LSDs.

3) we will publish the results in open access, high-quality scientific journals and present the work at relevant conferences.

4) we will share our results with collaborating scientists and ultimately make our data publicly available.

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

There are currently no adequate treatments for these conditions, as such the main benefits of this project will be to broaden the understanding of disease mechanism (with a focus on immunological pathways), and downstream of this the testing of novel treatments that could make a significant impact in the lives of these children. The work will assess complimentary treatment strategies which are targeted to the brain and help to bring them to clinical trial more rapidly.

These data will be of paramount importance for the development of a novel complimentary therapy for lysosomal diseases in the next 5-10 years and in the short-term will inform neurologists and immunologists both nationally and globally in the interim with our research findings on the role of CNS immunity in LSDs. The National Institute for Clinical Excellence has begun to reject enzyme replacement therapies, beginning with the Morquio drug Vimizin (later reversed), due to the poor cost benefit relationship of these products (£250K/annum/patient). These costs are not sustainable, thus alternative therapies are key to solving this crisis.

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

We will maximise outputs through several means. We will collaborate with groups both within the establishment and externally to generate and share knowledge of these diseases and models. We will look to publish all findings in open access journals, even when unsuccessful. We will disseminate knowledge through internal seminars, as well as national/international conference presentations and publications.

Species and numbers of animals expected to be used

- Mice: 1000

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.

Mice are the least sentient species in which one can still model human disease progression of lysosomal diseases. Mice used will typically be between two months of age and 12 months of age. Many of the diseases we work with are childhood diseases and therefore working with young mice more accurately represents the human disease. Though it is clear that differences exist between mice and human brains in terms of structure, function and geometry, there is still substantial similarity in the physiology, including the immune and inflammatory response.

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

Most animal models are bred from birth with a genetic disease, and many of these are not initially harmful to animals. Over a period of several months models of lysosomal diseases and immune models may start to show behavioural changes, reductions in joint mobility and evidence of inflammation, depending on the disease and most have a shortened lifespan. We closely monitor harmful effects on animals and introduce mitigating measures where appropriate or humanely killed animals where required. Typical protocols include:

1) Phenotyping (measuring behaviour, blood and tissue samples) to understand disease. Typically, 2-3 times for each of the following over the several months lifetime of an animal: an animal may undergo non-invasive behavioural tests, generally involving brief (10-60 mins) tests with no pain suffering and distress or food withdrawal for more than 16 hours. Intervals between tests will be at least 10 minutes and the maximum number of individual tests or trials will be five in any 24 hour period, with no more than 24 tests or trials in a month. Samples of bodily fluids (blood, urine) may be taken to analyse disease progression, effects of treatments and/or the health of the animal on up to 10 occasions. Animals may be killed under terminal anaesthesia to allow their tissues to be collected.

2) Delivery of multiple therapies: In addition to phenotyping tests above: mice may receive up to four therapeutic substances by multiple routes (oral gavage, intravenous, intraperitoneal, subcutaneous, intracranial, intracisternal) via up to 12 injections, where no more than 2 injections can be given in 24 hours. Therapies may also be given via a non-invasive route (through diet or drinking water). E.g: An animal receives an anti-inflammatory substance in drinking water (non-invasive), then up to 4 peripheral injections of a disease modifier, and receive 6 injections into the brain of a therapy under anaesthesia. They may then receive further injections of therapeutic substances. Typically, phenotyping will happen after these steps, but may happen prior to drug administration.

Animals will typically be injected with a range of drugs/treatments and followed up for up to a year. Animals are always monitored closely throughout the length of the experiment to ensure good health and any signs of pain, distress or ill-health are addressed accordingly (pain-relief, antibiotics, creams, etc). At the end of the experiment the animals are humanely killed and organs are harvested for further analysis.

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

Expected adverse effects include weight loss (weight loss will not exceed more than 15% of previous week’s weight), pain following surgery, lethargy and abnormal behaviour. Animals can experience stress due to restraint and handling which will typically resolve by the end of the procedure. Sometimes food withdrawal for up to 16 hours prior to a test may be necessary for one or two behavioural tests which can cause stress, again limited to the duration of the test. Typically, behavioural panels will be performed up to 3 times separated by several weeks. Where animals require anaesthesia, they will experience transient discomfort from needle insertion and/or anaesthetic injection or inhalation of gaseous anaesthetics (100% incidence). Injections directly brain or the cisterna magna, a fluid filled region at the back of the brain, are performed under anaesthesia, as a result animals have few associated adverse effects. Local bleeding is possible after surgical incision of the skin. Infrequently seizures or brain bleeds can happen in the initial hour after surgery. There is potential for infections.

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)?

Expected severities will be mild to moderate. In breeding protocols 50% of mice will experience subthreshold harms, 50% mild. In experimental protocols 40% will be mild and 60% moderate.

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 conditions we work on are complex and are influenced by variety of factors. Many of these conditions are neurological in nature but also have non-neurological components often including heart problems and bone disease. As such, non-animal alternatives, such as in vitro assays, may not be appropriate as they can only provide limited results. The mouse models of these conditions mirror the human disease closely, giving us not only physiological data but also behavioural outcomes, which cannot be achieved using cells in a dish, nor with zebrafish models. We need to assess that the therapies we develop have the ability to treat affected organs, in particular the central nervous system, so computer based modelling and in vitro assays cannot predict outcomes. There are no appropriate alternative methods to avoid the use of animals to assess the impact of drug treatment on CNS immunity, due to the complexity of CNS cell-cell interactions and more generally immune responses. Our therapies and understanding of pathology will be assessed with a variety of outcome measures such as behaviour and tissue sampling which can only be achieved with animal models.

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

We use cell-based tests in the lab to replace animals where possible (e.g. cell culture assays, High Content Screening technology), which we use to reduce drug candidates to viable numbers for in vivo testing by aiming them at specific characteristics - specific immune cell targeting. We have considered the use of organoids and brain stem cells from patient derived induced pluripotent stem cells (iPSCs) to select aid in selection of drug candidates, and while immunological and other biochemical/neuro-physiological responses can be measured, we would not be able to measure the impact on behaviour and other phenotypic outcomes. There are no suitable in vitro assays or alternatives that are therefore relevant and combine a model which allows us to assess delivery of products, immune system-CNS responses and behavioural outcomes.

Why were they not suitable?

The CNS is incredibly complex, and we often use behavioural evaluation of mice with disease to assess the effect of therapy which is impossible in tissue culture and/or computationally.

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 based these numbers on our previous experience working with these models and the numbers used on previous licences. Around 40% of our rodents will be used in breeding only and most of our rodent lines breed as heterozygotes typically yielding the proportion of one unaffected, two mixed and one affected mouse. All unaffected and affected mice will be transferred to other protocols. Typically, we will maintain up to five rodent lines at any time, the remainder being kept as frozen stock. From historical experiments, we can estimate that we will typically need 10-12 mice in each group (typically 3-4 groups) to see differences between treated and untreated mice (by analysing behavioural outcomes and biochemical markers/immune composition in bodily fluids/tissue). We also have an accurate idea of how many experiments we would like to complete over the course of the five years that this licence will run. We expect to evaluate the role of multiple pathways and therapeutic substances (targeting 5-6 immune pathways/cell types).

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

We will use statistical methods to calculate the number of animals needed for our experiments. We will also perform as much work as possible in non-animal models, such as cell culture experiments, to further reduce the number of animals needed.

We plan to share controls between experiments where possible by running multiple experiments in parallel.

We have extensive experience of working with animals and performing these kinds of studies.

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 breed animals in such a way that the number of animals from each mating is optimised. The number of animals born from genetically altered animals will be reduced by following good colony management strategies described on the nc3rs website, such as holding stock but not breeding, and intermittent breeding when necessary.

We will collect data throughout the lifespan of the animal to generate the maximum amount of data and reduce the number of repeat experiments needed. Where possible we will utilise sampling techniques throughout the lifespan of the animal, reducing the need to sacrifice animals. We have a number of collaborators, therefore, maximum use is made of animal tissues via tissue sharing across a number of different projects.

Pilot studies will be undertaken when starting new experiments to inform experimental design, i.e. power calculations, again optimising animal use.

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.

To achieve the objectives set out in this project licence we will need to breed different mouse models. These will include transgenic mice which allow manipulation of immune cell populations (cytokine/receptor deficient animals) as well as, but not limited to the mouse models of mucopolysaccharidosis (MPS) type III and Gaucher disease. These are the best mouse models of these progressive childhood metabolic diseases available, are the least severe models, and are used in preference to naturally occurring cat and dog models of the diseases. Each generates a distinct disease and despite similarities between the metabolites accumulated, display different behavioural and biochemical outcomes. Comparisons of these models will help us to understand which components (with focus on the CNS) are important in each diseases' progression. Immune cell/cytokine deficient models will in some experiments be bred with disease models to assess the contribution of various immunological pathways to disease progression. These disease models will also be used to test the therapeutic effects of candidate drugs, particularly aimed at modulating the immune response and highlighting potential mechanisms and therapeutic targets.

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

Rodents provide some of the best available models of human disease progression as characterised previously. Other models, such as zebrafish, are inappropriate as abnormal neuro-cognitive phenotype is a characteristic of the diseases we research and zebrafish poorly model these aspects. Although we could start by treating zebrafish to show proof of mechanism, this would not reduce the number of mice required to demonstrate behavioural changes when we deliver a therapy. Many regulators do not accept studies in zebrafish prior to clinical trial, we would need to use mouse models anyway so the use of zebrafish seems an inappropriate use of animals.

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

Surgical procedures are well tolerated (less than 1% mortality) in lysosomal disorder models and this is in part due to the efficiency with which we can complete the procedure given our experience with the model (typically under 30 minutes).

All procedures are undertaken using good aseptic technique to minimise the risk of infection. Post-operative care will involve increased monitoring in the weeks and months following procedures. Animals will be weighed and assessed for pain and distress. Analgesia (pain relief) and other treatments will be used if necessary and in consultation with the vet.

We always keep up to date on the latest guidance and will undertake training when required to improve how we handle and perform procedures on animals, which includes acclimatisation periods to researchers and test arenas.

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

All relevant NC3R's guidance and updates will be engaged with, including sign posting to published studies, e.g. FRY, D. 2014. Chapter 8 - Experimental Design: Reduction and Refinement in Studies Using Animals. In: TURNER, K. B. V. (ed.) Laboratory Animal Welfare. Boston: Academic Press and the ARRIVE guidelines for reporting animal research (Kilkennyet al., 2010) - Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving bioscience research reporting: the PREPARE Guidelines for reporting animal research. PLoS Biol 8:e1000412

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

We liaise with colleagues in the animal facility within the establishment who keep us up-to-date on advances in the 3R’s via a monthly newsletter. We also access the NC3Rs and other welfare driving bodies websites for training information. We also attend periodic seminars and other educational events hosted within our animal facility. All our experiments will be conducted following PREPARE guidelines, ARRIVE guidelines and OECD protocols to further this knowledge as well as reading publications and outputs of colleagues.