



Home Office

NON-TECHNICAL SUMMARY

Autophagy and other modulators of proteinopathies

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.

Key words

Neurodegeneration, Proteinopathies, Autophagy, Proteostasis, Physiology

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What's the aim of this project?

Proteinopathies are diseases where proteins within cells do not form and function correctly. This includes many of the major neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease. Although these diseases are caused by different types of mutations, they show a common feature in neurons and their support cells: proteins clump into large "aggregated" structures which are toxic and result in neuron death.

There is an urgent need for new drugs and therapies to halt, delay and prevent proteinopathy diseases. This project focuses on two areas that regulate proteins in cells. The first is the cellular process called "autophagy", which is a way that cells can break down protein aggregates and recycle them. The second is chaperone biology. Chaperones are a group of proteins that correctly fold other proteins so that they do not clump into aggregates.

Several studies have showed that modulating autophagy or chaperones can be beneficial to treat neurodegenerative diseases and help neurons to cope with protein aggregates.

Moreover, it has also been suggested by several research groups that, when autophagy or chaperones function less efficiently in a cell, the nervous system is more prone to develop neurodegenerative diseases.

Therefore our project has three aims:

1. To further develop the potential of increasing autophagy in cells, in order to remove disease causing proteins and aggregates, as a therapeutic strategy for the treatment of proteinopathies.
2. To investigate the physiological (normal) function of autophagy and how changes in it may lead to disease progression. This expands our understanding of how this process works and will provide new avenues to manipulate autophagy for therapeutic purposes in the future.
3. To further our understanding of chaperone biology with particular reference to how this might be perturbed in disease and how modulating the function of these proteins may be beneficial in neurodegenerative diseases.

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.

What are the potential benefits that will derive from this project?

At the first level, we are testing approaches and compounds for their therapeutic benefit directly in models of neurodegenerative disease. In the long term we hope that these studies will be used as the basis for further translational therapeutic trials in human patients.

At the second level, the main benefit from work carried out under this licence will be scientific advancement, particularly in the areas of autophagy and chaperone biology. This will be published and presented to the global scientific community in particular, providing pioneering information for

discussion and review. Overall we hope to identify new genes and compounds that allow us to better understand and manipulate these processes. This will benefit and guide future therapeutic studies.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We plan to study at least three mouse models for neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington disease. Moreover we will study several transgenic mice to understand the function of autophagy and chaperons in mammals.

Given the wide approach of our research, we plan to use up to 20 000 mice in five years. They will be disease models (transgenic) and wild type.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Over the period of the license, we may generate new mouse lines using standard protocols for superovulation and embryo implantation into pseudo-pregnant females.

We will also carry on breeding of genetically altered animals that will allow us to study the role of autophagy and chaperone biology in neurodegeneration. For this purpose, mice are likely to undergo mild to moderate procedures. In parallel to our *in vivo* studies, we will also generate primary cells, including neurons, glial cells and fibroblasts. For this reason, we will use mouse embryos or pups (P1-P3) that will be killed by Schedule 1 method and will not exceed subthreshold severity.

To investigate the role of new autophagy and chaperone modulating pathways in neurodegeneration, we will use mouse models for Huntington's disease, Alzheimer's disease/tauopathy and Parkinson's disease. All these models over-express mutant proteins found in patients with familiar types of the respective disease and show age-dependent disease relevant moderate phenotypes. We will administer compounds that modulate autophagy or chaperone related pathways through the least invasive method (intraperitoneal/ subcutaneous/ intravenous injection, food/drinking water, oral gavage, osmotic mini-pumps, stereotaxic injections) and perform studies to measure relevant disease phenotypes. Our approach will include measurement of the levels of the respective mutant protein, histopathological examination, behavioural analysis and ageing studies. Successful compounds will be further tested for their suitability for translation into patients. This will require studies to validate drug effects at concentrations relevant to those that can be achieved in humans. For those studies, the mice are likely to undergo subthreshold to moderate procedures.

Our previous work has identified novel pathways that regulate autophagy and chaperone biology. To further understand their role in autophagy and chaperone modulation *in vivo*, we will characterise genetically altered mice for the relevant genes or we will transiently modify gene expression by

injecting siRNAs or non-pathogenic viral particles. These mice will be tested by behavioural, biochemical and histopathological studies. We will also mate these mice with our mouse models for neurodegeneration or with autophagy reporter mice. For those studies, the mice are likely to undergo subthreshold to moderate procedures.

All mice will be killed at the end of the experimental procedures.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

We replace all possible elements of our mouse research with cell culture work and a variety of *in vitro* approaches.

However, there is no single alternative to assessing disease models in the context of a mammalian brain, especially if one wants to have a proof-of-principle model for testing potential therapeutics. As such, our cell culture and alternative model approaches guide all elements of our work that cannot be replaced. This includes both aspects of our aims for scientific advancement and therapeutic approaches.

Reduction

Explain how you will assure the use of minimum numbers of animals.

We use the minimum number of animals through careful planning of our experimental design and breeding the minimum number of animals to meet this target. We have a dedicated member of staff to identify the genetic background of every mouse produced to ensure we can correctly define and use each mouse experimentally. We also maximise our use of animals beyond their lifecycle by routinely collecting and storing tissues for later re-use in other experiments as part of histological and biochemical experiments.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

In addition to our mouse models, we also work with and are continuing to generate zebrafish models to replace our work with mice where suitable. However, mice are a powerful animal model as they are amenable to genetic and compound modulation. Our animal models of neurodegeneration develop either the clinical signs seen in human disease – such as our Huntington's model, or human pathology characteristics as in all our disease models. They show clear behavioural, biochemical and histopathology changes that we can measure and aim to modify. In the case of our Huntington's

disease mouse model, we have carefully established processes with monitoring to ensure that we minimise their suffering as a result of the disease.

Our emphasis is on understanding cellular processes in mice as a step towards modulating these processes for treating human disease. Our experimental approaches are guided by cell and zebrafish model work such that we carefully build on a solid scientific foundation when aiming to generate and modify new mouse models for understanding and modulating proteostasis.

Our project aims is to develop therapeutic strategies for neurodegenerative diseases and we are therefore testing potential drugs for amelioration of symptoms. We will choose the least invasive method for drug administration and careful experimental design, having as top priority animal welfare.

In some of our studies, we have to test a drug for prolong time to see it has a beneficial effect in neurodegeneration. To do that we need to perform a minor surgery to implant a small plastic pump under the mouse skin to deliver drugs. These surgeries are performed in aseptic conditions by trained staff and analgesia is provided. We will favour the administration of analgesia in a flavouring, such as Nutella, which mice will find attractive to eat. Whenever suitable for our studies, we will favour the use of plastic pumps that can be refilled without the need of replacement.