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NON-TECHNICAL SUMMARY

Role of microglia and blood-brain barrier in dementia

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

Microglia, Blood-brain barrier, Alzheimer's disease, Vascular dementia

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

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 aims of this study include investigating the roles of microglia and blood-brain barrier dysfunction in age- and metabolic disease-related dementia by generating dementia mouse models that closely resemble human dementia conditions; and performing preclinical drug tests in these dementia 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?

Dementia is not a natural part of ageing. The patient has an impaired ability to remember, think, or make decisions that interfere with everyday activities. Alzheimer's disease (AD) and vascular dementia (VaD) are the two most common forms of dementia. Though these conditions affect millions of people worldwide, there is currently no cure or prevention. AD and VaD are complex and progressive diseases in which they are not separated but overlap in nearly 50% of the cases. Researchers have discovered that abnormal immune responses and blood vessel injury are possibly common conditions in AD and VaD. To develop effective treatments, we need to understand the intricate mechanisms underlying the disease. Understanding the importance of immune cells and the damage of the blood-brain barrier in dementia is crucial.

Immune cells, in particular microglia, play a vital role in the brain's defence against harmful invaders and help maintain a healthy brain environment. However, in dementia, these immune cells become overactive or not functioning properly. This contributes to inflammation and damage in the brain. By studying the immune cells, researchers can gain insights into how these cells go awry in dementia and potentially find ways to control their functions to protect the brain. Moreover, the blood-brain barrier (BBB) is a protective barrier that separates the bloodstream from the brain tissue. It acts as a gatekeeper, allowing only essential nutrients to enter the brain while keeping harmful substances out. Damage of the BBB is common in many forms of dementia, including AD and VaD. When the BBB becomes leaky or compromised, toxins and inflammatory molecules can enter the brain, further damaging brain cells. Understanding how and why the BBB breaks down in dementia is essential for developing strategies to strengthen and protect this barrier.

Many things can increase one's chance of developing the disease, known as risk factors, including genetics, ageing, obesity, metabolic diseases (e.g., diabetes, cardiovascular diseases, hypertension) and infection. In clinical observation, a combination of risk factors that occur in a person at the same time may change the immune responses and damage blood vessels in the brain. Our research team has been focusing on how these risk factors, including APOE4 carrier, ageing, and obesity-induced diabetes, change the immune cells and blood vessels in the brain that eventually cause AD and VaD. In this proposed project, we will develop different mouse models that have a combination of these factors and study the linkage between the risk factors and changes in immune responses and vessels. We will also use these mouse models to identify diagnostic targets and potent preventive and

treatment methods in order to combat AD and VaD. Lastly, we will examine the efficacy of combining newly identified drugs with the current treatment (amyloid antibodies) to advance the AD treatment strategy.

In summary, studying immune cells microglia and investigating how the blood-brain barrier is damaged in dementia is critical for advancing our knowledge of the disease and developing effective treatments. These aspects are like pieces of a puzzle, and by understanding how they contribute to dementia, researchers can work towards finding ways to slow down or even prevent the progression of this devastating condition.

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

The findings will be published, focusing on understanding the early events of Alzheimer's disease (AD) and vascular dementia (Va) using unique mouse models. The project will identify therapeutic targets and invent potential treatment methods that could lead to new patents and applications in clinical studies.

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

In the short term, this project aims to confirm if metabolic diseases e.g., type 2 diabetes and cardiovascular issues lead to immune responses and vascular inflammation that contribute to dementia. Special mouse models will be created to mimic different metabolic conditions, providing a new tool for dementia research. Human samples from the UK Biobank are limited to fluids (e.g urine, blood,) or genetic data with no brain specimen. Post-mortem brain samples are not suitable for studying the progressive change in the brain. Therefore, these mouse models can help study both late-onset Alzheimer's disease (AD) and vascular dementia.

Looking ahead, in the medium and long term, studying the immune responses and blood-brain barrier can identify targets for better early diagnosis and treatment of late-onset AD and vascular dementia. The mouse models can replicate specific features in human AD brains, making them valuable for discovering potential treatments and drug testing. Additionally, these models are useful for assessing the effectiveness of newly approved treatments, such as amyloid antibodies.

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

The research findings obtained from the investigation of microglia and blood-brain barrier functions in a late-onset Alzheimer's disease mice model hold great promise in advancing our understanding of the disease and potential treatment strategies. To ensure that the benefits of this research reach a wider audience, we have a comprehensive dissemination strategy which includes but not limited to:

- Publication in Peer-Reviewed Journals:
- Conference Presentations
- Engaging with the Alzheimer's Research Community
- Press Releases and Media Outreach:

- Open Access Databases:
- Collaborative Partnerships with pharmaceutical companies, medical institutions, and research organizations

Species and numbers of animals expected to be used

- Mice: 6000

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 commonly used as animal models in scientific research, including studies on aging and age-related diseases like dementia, for several compelling reasons. When it comes to aging research, aged mice are particularly valuable because they allow scientists to investigate the effects of aging on various physiological processes and to develop potential interventions. Mice are used as animal models in aging studies because:

- High Genetic and Physiological Similarity between mice and humans
- Well-developed methods of behaviour and pathophysiological studies in mice
- High level of reproducibility
- Genetically Manipulability for specific gene study

In this project, mice carrying different risk factors (e.g. genetics + obesity) from the young adult stage to the old stage will be chosen to study the early pathophysiological changes. A wide range of life stages is chosen as it is relevant to human aging. Age-related changes and diseases take time to manifest. We will employ this wide range of age groups to study how the risk factors change the immune responses and blood vessels during the early pathogenesis, and how these changes lead to the development of AD and VaD in older age groups. Aged mice are essential for conducting long-term studies that closely mimic the chronic nature of conditions like dementia. This makes them suitable for investigating late-stage aging conditions like dementia. Moreover, aged mice can be used to assess the effectiveness of potential interventions or treatments for age-related diseases. Researchers can study how these interventions impact cognitive decline and other age-related changes.

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

To develop mouse models of AD and VaD:

1. We will induce obesity and diabetes by treating transgenic mouse models with a high-fat diet chronically (6 months to 12 months). Mice fed with a normal diet are the control groups. Aged mice will be kept up to 24 months old.

2. About 10% of mice will also be injected with immune stimulants to mimic the combined risk conditions in dementia.

3. About 10% of mice will be induced with vascular dementia by surgically narrowing blood vessels under anaesthesia.

4. For mice aged over 15 months, mice conditions (e.g. blood pressure) will be monitored frequently (at least once per week) by non-invasive device (e.g. telemetry or tail cuff sensor) to ensure animal welfare.

To test potential drugs for treating AD mice:

1. The mouse models generated above will be treated with drugs by intraperitoneal injection, tail vein injection or oral feeding from 2 weeks to 3 months, depending on the type of drug). A combination of drugs may be tested, meaning that the mice may be treated with more than one drug using different administration routes.

Analysis:

1. Mice will then have behaviour tests and imaging under anaesthesia.

2. Mice will be killed humanely by schedule 1 procedures for brain tissue collection.

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

- Mice will become obese after high-fat diet administration. High fat diet/ Western diet are expected to cause a greasy coat (100%), which may lead to over-grooming (~25%) and as a result, possible skin inflammation/ulceration and infection (<5%). If the level of obesity becomes profound, the animals may become less active and a small proportion (1-2%) may experience difficulties in grooming certain regions. This however is not expected to affect the health and well-being of the animals.
- A portion of mice will be aged until 24 months and may develop memory deficits.
- Aging mice may develop high blood pressure.
- Mice that have undergone surgery to narrow the carotid vessel may have a slight weight reduction (usually less than 5%) in the first two days after surgery. These mice will develop vascular dementia under chronic reduction of blood flow due to narrowing of the carotid blood vessel. These mice will eventually develop memory deficits.
- Mice undergoing behaviour tests may have mild stress during the test but will be recovered after the test. For mice undergoing Water Maze tests may experience moderate stress during the test

but will be avoid with escalated handling and post-test measurements, to reduce stress during and after the test. Mice will usually be recovered quickly after the test.

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)?

The adverse effects are considered mild and moderate throughout the lifetime and during experiments.

75% of the mice will experience mild severity. 25% of mice will experience moderate severity.

What will happen to animals used in this project?

- Used in other projects
- 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?

Dementia is an age-related cognitive disease involving complex cell-to-cell and tissue interactions. The complexity of regional interactivity in the brain is such that in vitro approaches are inadequate to mimic, as cells in culture dishes cannot maintain full anatomical and functional connectivity. This approach would fail to express the cognitive features that drive behaviour. The interactions between the brain and the periphery tissues e.g., hormonal control, blood-brain-barrier functions, and immune cells between the brains and the periphery that underlie the development of dementia can only be assessed adequately in vivo using animal models.

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

There is a case for in vitro approaches that use brain slices for pharmacological screening, which actually becomes advantageous. Brain slices from one brain can help reduce the number of mice and refine the welfare. In another in vitro model, primary cells may also serve for the purposes of molecular studies within one cell type.

Moreover, some primitive and non-protected models, such as zebrafish larvae and drosophila, have been considered as an alternative, but it is impossible to induce obesity in zebrafish larvae. Zebrafish larvae are too early in the life stage, so they cannot be used as a model for studying aged-related disease.

Human tissues including post-mortem sections from patients only allow studying the disease pathology at the end-stage. We cannot study pathogenesis in the onset of the disease.

Computer Modelling and Simulation: When applicable, computer modelling and simulation techniques can be employed to predict outcomes and test hypotheses without the need for live animals. Computational experiments and modelling can reduce the need for in vivo experiments as a first screening of drug effectiveness. This can reduce the number of potential drugs for animal tests.

Why were they not suitable?

In the project, we will use these culture models to replace the use of animals in some experiments. However, these models cannot replace using mice to study age-related diseases. These in vitro models cannot replace the study of the interaction between the periphery and the brain, as well as the association between metabolic disorders and dementia. Computer modelling can only be used as a preliminary study but cannot replace the use of animals in drug testing.

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 will ensure that we use the minimum number of animals through careful design of studies, minimal animal handling by researchers to reduce stress, making sure that animals are accustomed to any testing arena before a study begins, and providing good researcher training. We will monitor the reliability of our studies closely and alter group sizes as appropriate and in consultation with statistical experts. We are working closely with colleagues to develop behavioural tests that improve data yield to reduce animal numbers further by minimising the potential negative effect of animal handling in our studies.

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

During the experimental design phase of this project, several steps were taken to reduce the number of animals used, aligning with the principles of Reduction (one of the 3Rs) and ethical considerations. We will make use of:

- NC3Rs Experimental Design Assistant
- Statistical Analysis Plan and Biostatistician Consultation

- Optimal Experimental Techniques
- Sample Collection and Sharing

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

In addition to good experimental design, several measures will be implemented to optimize the number of animals used in the project, ensuring that animal welfare is prioritized while minimizing the overall number of animals involved. These measures include:

1. Efficient Breeding and Colony Management

2. Use of Pilot Studies

3. Computer Modelling and Simulation

4. Sharing of Tissue and Data

5. Adaptive Study Design for the modification of sample sizes and research protocols based on interim results

6. Use of different Non-Invasive Techniques such as imaging, behavioural observations, or remote data collection, allowing different analysis on the same batch of mice

7. Longitudinal study of small cohort to gather preliminary data before large cohort.

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.

In this study, mouse models of dementia will be used due to their genetic similarity to humans and ease of genetic manipulation. The methods will emphasize non-invasive behavioural assessments and cognitive testing. Minimization of distress and pain will be achieved through careful experimental design, such as habituation to testing procedures and the use of positive reinforcement techniques. Behavioural assessments will be non-invasive and refined to minimize distress. Observations will focus on natural behaviors, and any potentially distressing procedures will be avoided or conducted with the utmost care and consideration for animal welfare. Training will be provided to staff and students who will perform oral gavage, intravenous and intraperitoneal injections of testing drugs, and anaesthesia/analgesia with proper techniques to minimise stress and suffering. Transfer of the mice

into the testing arena will be done with the cupping technique to minimise distress. Mice will also be handled by the same person for at least 7 days before behavioural tests are performed. When required, appropriate anaesthesia and analgesia for any invasive procedures will be strictly followed to minimize distress and reduce pain.

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

Using less sentient animals or animals in immature life stages, such as embryos or neonates and zebrafish larvae, may be scientifically unsuitable for dementia research. Dementia primarily affects adult or aged individuals. Therefore, using animals at more immature life stages with significantly different physiology and brain development may not accurately model the condition. Research findings may not translate to the understanding and treatment of dementia in humans. Drosophila is a good model for studying the basic biology of neurodevelopment and protein functions but not for age-related and pathological studies. Using animals that are not biologically relevant to the research question can lead to inconclusive or invalid results. Scientific rigor and validity are crucial in dementia research to ensure that findings can be extrapolated to human patients. In dementia research, it is crucial to strike a balance between the ethical treatment of animals and the scientific validity of the results. Using appropriate animal models, typically adult or aged animals, ensures that the research is ethically conducted and that the findings have practical relevance to understanding and treating dementia in humans. Researchers are encouraged to adopt the highest ethical and scientific standards to pursue this important research while minimizing harm to animals.

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

To achieve this, the following steps can be taken:

1. Review existing procedures to estimate the potential sources of stress, pain, or discomfort for the animals.

2. Pilot studies to identify potential issues in the procedures

3. Use of anesthesia and analgesia: to minimize pain and distress during procedures

4. Explore alternative techniques that are non-invasive to minimize the need for invasive procedures

5. Refine experimental protocols to identify opportunities for improvement e.g., adjusting dosages, refining timing, or minimizing the duration of procedures.

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

We will follow guidelines and recommendations from professional organizations associated with the specific field of research, such as the Laboratory Animal Science Association (LASA) and the Animal Welfare and Ethical Review Bodies (AWERB 2.0), to ensure the experiments are conducted in the most refined way. We will also refer to published guidelines of substance administration by Jenning M. et al., (doi: 10.1258/la.2008.007143.), and the NC3Rs

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

To stay informed about advances in the 3Rs (Replacement, Reduction, and Refinement) principles and implement these advances effectively during the project, the following strategies will be employed:

1. Regular Literature Review on the latest research articles, publications, and scientific journals related to the 3Rs principles.

2. Participation in Workshops and Conferences focused on the 3Rs provides a platform for learning about cutting-edge techniques, technologies, and ethical considerations in animal research

3. Consultation with Ethical Review Boards e.g., AWERB

4. Engagement with Regulatory Bodies with updated guidelines and regulations to reflect the latest 3Rs principles and compliance with the updates