Home Office

NON-TECHNICAL SUMMARY

Pulmonary Inflammation and the Circadian Clock

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

lung, inflammation, circadian, clock, asthma

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

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 why inflammatory lung diseases, for example asthma, are rhythmic in nature. We want to understand the underlying pathways controlling time of day changes in inflammatory lung pathology. The pre-clinical work outlined in this project complements the clinical cohort studies and drug trials being performed by the same research group. We hope to discover new therapeutic targets to treat inflammatory lung disease, and how to use existing treatments at the most effective time of the day (chronotherapy).

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?

Lung disease affects one in five people and is the third biggest cause of death in England costing the NHS more than £9 billion every year (Asthma and Lung UK/NHS England). Hospital admissions for lung disease have risen over the past seven years at three times the rate of all admissions generally.

The symptoms of chronic inflammatory lung diseases (e.g. asthma, COPD), often show daily variation in their occurrence, worsening during the night. Similarly, biomarkers used in the clinics to assess disease severity fluctuate over the course of the 24h day.

The circadian clock is a timing mechanism which synchronises animal physiology to the 24h environment created by the earth rotating on its axis. This biological timer regulates numerous aspects of physiology, including sleep wake cycles, feeding and metabolism, hormone secretion and the immune system. Disruption of the circadian clock is associated with increased prevalence of inflammatory diseases, including lung disorders (e.g. asthma, lung cancer).

The circadian clock plays a critical role in regulating the normal working of the immune system and ensuring appropriate inflammatory responses are mounted when the system is challenged. This work investigates mechanistic links between the biological timing and immune systems to understand the involvement of the clock in regulating lung physiology. An ultimate goal of these studies is to reveal novel therapeutic targets or improve existing therapeutic regimes to treat lung disease through the use of biological timing (chronotherapy).

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

A major output from this project will be an advance in our knowledge regarding circadian clock control of immune responses within the pulmonary system. More specifically, information generated from

these studies will contribute to our understanding of how the circadian timing system affects the development and progression of human pulmonary diseases such as chronic obstructive pulmonary disease (COPD), asthma and pulmonary fibrosis. This information will further our understanding of how circadian disruption (a consequence of rotating shift-work) may impact on lung function in both health and disease. Additionally it is predicted that data generated by studies outlined here will have a positive impact on the diagnosis and treatment of human pulmonary disease. An example here is the implementation of chronotherapy (for example timing therapeutic interventions with the peak of disease symptoms) to treat asthma.

The data generated from this project will be made available to other researchers in the scientific community at the earliest appropriate time therefore informing further scientific discovery within our research community.

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

Healthcare sector: Outputs from this research will be of benefit to the healthcare sector. There is a growing understanding of the importance of considering circadian time in the diagnosis and treatment of pulmonary disease. This work will further develop our knowledge of how the circadian timing system interacts with processes underlying human pulmonary conditions. It is hoped that these studies will promote further incorporation of "clock logic" into clinical practice in the long-term. That may be through standardising the time of day at which a patient's blood or sputum is sampled for a disease biomarker, or through recommending the best time of day at which to take medication.

Scientific community: Although the focus of this project is pulmonary disease, the pathologies and processes underlying these diseases are applicable more broadly in the field of immunology. Thus data generated in this project will advance our basic understanding of clock control of immunity and will be of benefit to the wider research community. Data will be disseminated at the earliest opportunity benefitting the scientific community as soon as possible.

Patients, carers and the general public: The importance of the circadian clock and good sleep hygiene for maintenance of health is becoming widely recognised by the general public. Information obtained through this project will be of interest to the general public, including people engaging in shift-work and patients suffering with pulmonary disease. It is becoming more and more evident the disruption of the circadian clock has negative consequences on health. Whilst sometimes circadian disruption cannot be avoided (e.g. shift-workers), for some individuals small lifestyle changes may have a positive impact on health. In order to engage the general public we will continue to reach out via public engagement events run by the Establishment.

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

Outputs from this work will be published in highly visible journals targeting a multi-disciplinary audience. The research group has an excellent track record of publishing data in high impact journals read by basic scientists and clinicians (e.g. AJRCCM, Thorax, ERJ). Furthermore, through the use of social media and the Establishment press office, we will publicise our results as widely as possible in order to engage with patients and the general public. In the past, this has led to opportunities to present our research on the radio, television and news websites. We appreciate that it is important to disseminate negative findings to minimise replication of experiments across research institutes. In

addition to publication of articles, ongoing work will be presented at international and national meetings aimed at circadian biologists, immunologists and respiratory clinicians.

To maximise the benefit of our research we will continue to engage with the general public and relevant patient groups through organised events. These activities are important for maintaining public interest in our research and also further developing trust in UK research. We work closely with leading charities for lung diseases in the UK.

Species and numbers of animals expected to be used

- Mice: 11200

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.

These studies will utilise adult mice, which may be genetically modified. Mice are the most appropriate species for these studies as the systems which we are studying here (the body clock and immune system) are well reproduced between mice and humans. Additionally, the mouse genome is tractable, meaning that we have a vast repertoire of genetically altered animals to allow the investigation of underlying physiological and disease mechanisms.

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

Animals may be monitored non-invasively for behaviour under normal or altered environmental conditions (alteration to light/dark lighting, typically for periods lasting 2-6 weeks). More rarely, animals may undergo physiological monitoring using implanted telemetry devices (intra-peritoneal, requiring a brief surgical procedure) or imaging (under recovery anaesthesia) or lung mechanics measurements under restraint. Rarely repeated imaging may be utilised (up to 8 times in one day). Changes to rhythmic biological signals (such as glucocorticoid hormones, melatonin hormones or microbial metabolites) may be instigated through implantation of hormone pellets under the skin (brief surgical procedure) or through application of antibiotics.

Approximately one third of the animals utilised in this project will undergo a procedure to induce pulmonary inflammation. To instigate pulmonary inflammation one of four approaches will be utilised, each modelling a different human chronic inflammatory disorder. (1) Inflammatory mediators (e.g. lipopolysaccharide - a component of the bacterial cell wall) may be applied acutely (20 minutes) via an aerosol to directly target the lung. (2) Allergic inflammation may be induced via application of a reagent that the animal becomes sensitised to, such as house dust mite. (3) Fibrosis (mild scarring) of the lungs may be induced through brief local application of reagents that cause local lung injury (bleomycin) resulting in tissue remodelling in the longer term (2-4 weeks). (4) Animals may undergo a surgical procedure (under terminal anaesthesia) where the lungs are mechanically ventilated for a

period of time. Prior to, or during these four procedures the immune system may be manipulated, this includes the application of reagents (a substance that causes a chemical reaction) to target specific pathways (e.g. through the use of antibodies). Additionally, reagents may be administered which target the clock or the immune system to establish their effects on inflammatory processes.

Animals utilised in these models of lung inflammation may be assessed using in vivo imaging, collection of small volume blood samples and/or assessment of lung function.

In addition to these procedures, mice will be used in this project for breeding, and for provision of cells and tissues for ex vivo studies.

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

Animals may experience mild adverse effects such as temporary stress or brief pain and discomfort. Temporary stress may be induced by periods of restraint (e.g. in order to administer an injection (seconds), or for non-invasive lung mechanics measurements (minutes)) or alterations in their housing environment (e.g. single housing (up to 8 weeks) or alteration of the light cycle (up to 6 weeks in constant light)). Mice may experience a brief period of pain and discomfort in response to dosing (e.g. injection or intra-nasal dosing) or blood sampling, or following surgical intervention to implant a telemetry device or hormone pellet. In these instances the stress and discomfort will be transient.

Acute models of pulmonary inflammation are associated with transient discomfort and stress due to application of inflammatory reagents and may result in brief periods of mild respiratory distress (less than 1 hour). The chronic models of pulmonary inflammation (allergic inflammation and fibrosis) may also be associated with longer periods of mild respiratory distress (hours), transient weight loss and occasionally loss of condition.

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 studies outlined in this project will result in a cumulative impact to the animals that are sub-threshold to mild (approximately 60 percent) or moderate (approximately 40 percent) severity rating.

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?

In the context of organ-level inflammation, it is currently not yet possible to replicate the complex multi-cell environment underpinning disease. Thus, in vitro cellular models and in silico models have limited application as a replacement for studies of pulmonary inflammation. In order to study complex interactions between the circadian timing system and immune responses occurring in the lung, mouse models are the most appropriate approach. It is necessary for us to use a model that has lungs (rather than gills) to most accurately reflect the human condition and allow investigation of physiological lung function.

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

In vitro approaches include the use of single-type cell lines or co-culture systems (where two or more relevant cell types are studied together). We utilise these methods to inform the direction of animal studies. In vitro assays can be used to test how genetic or pharmacological interventions alter the function of the cell intrinsic clock and/or regulation of inflammatory processes. For example, in vitro assays using cell lines or primary cells allow us to first test a diverse range of potential therapeutic interventions and identify a small number of candidate molecules with the most potential for subsequent use in vivo. However these approaches cannot replicate the complex environment of the lung and cannot replace the use of animals. Our research objectives will also be supported by clinical studies, where human immune cells are harvested from donors.

Why were they not suitable?

We have successfully utilised cell lines (such as Human Bronchial Epithelial Cells) to examine circadian control of inflammatory responses. Furthermore, ongoing projects in the laboratory involve exploring the potential of various cell lines in monoculture or co-culture to help address our research questions. However, it is critical that any cell lines utilised are circadian rhythmic. Our experience here however, is that cultured cell lines are often non-rhythmic, in contrast to the situation in vivo where they may be rhythmic. This may be an artefact of multiple passages of cell lines, or due to the absence of other signals (hormones or cytokines) that these cells would normally be exposed to in vivo. Thus their use is somewhat limited. Furthermore, in vitro assays cannot adequately model the complete array of inflammatory responses or address how systemic timing signals may modify these responses.

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?

Our grouping has extensive experience with the methodologies and approaches outlined here and of running projects of a similar scope. Consequently, estimates of animal numbers are based firstly on previous experience with the models to be utilised and the types of data generated, and secondly with careful consideration of the experimental design.

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

Throughout the duration of this licence, we will carefully consider the design of each experiment. As the project develops, and we build up further data sets, we will utilise this information to further refine the experimental design. When considering the design of experiments underpinning this project we have consulted with statisticians to gain specialist advice on the types of experiments that will be undertaken and the nature of the datasets that will be collected. We will continue to do this as the work develops. We will be utilising purpose written software (such as the NC3Rs Experimental Design Assistant) to further support experimental planning and randomisation and blinding.

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

Throughout this project we will continue to strive to optimise animal use wherever possible. This starts with efficient breeding of transgenic mouse lines, achieved through close monitoring of colonies which is facilitated by the use of specialist colony management software and effective communication between our laboratories and technical staff managing the colonies. We aim to minimise numbers of animals bred whilst still achieving adequately powered, age and sex-matched groups of experimental animals. When individual projects have been completed and data is being prepared for publication, breeding of relevant mouse strains will be minimised until a suitable time to preserve the colony by freezing down gametes.

Pilot studies are utilised to optimise experimental conditions when we are developing new approaches. Where it is appropriate we utilise technologies that permit longitudinal assessments in the same animal. Furthermore, we always look to maximise the amount of data that we can gather from a single sample using the latest technologies to their full capacity (for example using mass cytometry to assess expression of numerous proteins in one individual cell).

At the end of each experiment we carefully consider which tissues to collect with future studies in mind. By building a well archived tissue bank we are able to utilise existing samples in the laboratory to test new protocols or reagents (e.g. new antibodies) without the need to utilise further animals. We also make our banked tissue available to our collaborators.

Through providing our collaborators and the wider scientific community with access to data generated through our studies (through depositing large datasets in online data repositories) we aim to maximise the scientific knowledge than can be obtained from our animal studies.

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.

A substantial proportion of the methods that we will use in the project are non-invasive and involve environmental manipulations to study effects on circadian physiology and/or immunity, including manipulation of the lighting. These processes themselves are unlikely to cause suffering or distress. On occasion these manipulations may require animals to be single housed, to minimise potential distress in this situation, where possible we will supply mice with environmental enrichment (e.g. plastic tubes, plastic igloos, wooden logs or nestlets). Some animals may be subject to a brief surgical procedure to implant a telemetry device or hormone pellet. We have significantly refined our approach to glucocorticoid hormone replacement by establishing in previous studies that it is not necessary to surgically remove the adrenal glands (the major source of these hormones) prior to pellet implantation. This has reduced requirements for surgical intervention and improved recovery times.

Where we seek to administer reagents to experimental animals we utilise the most refined route of administration possible. For example, we routinely administer antibiotics in the drinking water rather than through oral gavage. Furthermore we always seek to minimise the numbers of doses of a treatment in order to achieve our objective, this may involve pilot studies to identify an optimal dosing regime.

This project involves the use of models of pulmonary inflammation. We chose to use the most refined models available to address our experimental objectives. For example in order to establish a local acute inflammation within the lung, we utilise aerosolised administration of lipopolysaccharide (a component of the bacterial cell wall) rather than dosing with bacteria itself (e.g. Streptococcus pneumoniae, which induces a more profound inflammation which can rapidly spread to the periphery). Other models we utilise include administration of a chemical to the lungs which causes local tissue damage and scaring, which has been optimised in terms of dosing route, dosage and time post-administration in order to induce a robust and consistent response whilst causing the least harm and suffering to experimental animals. Models of allergic inflammation utilised in this licence are relatively mild with the period of lung inflammation and animal experiences being minimised as much as possible whilst not compromising experimental aims. Further, assessment of respiratory mechanics in these models is performed utilising in vivo approaches which generates the most robust and reliable data and is performed under terminal anaesthesia (FlexiVent) or in a restrained mouse (Dual Chamber Plethysmography).

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

We cannot replace these studies in mice with studies in another species (such as insects or fish) to achieve our objectives, as they lack the complex immune and circadian systems seen in higher order species. Whilst we are able to carry out some of our studies in terminally anesthetised animals (for example lung ventilation studies) this is not appropriate for the majority of our pulmonary inflammatory

models as they take a prolonged period of time to initiate disease and/or we require longer term assessment of disease progression.

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

We routinely seek to minimise stress and discomfort to animals during our work and achieve this by ensuring all researchers utilise appropriate animal handling techniques (e.g. tube handling to remove animals from their cage) and by using environmental enrichment wherever possible, especially in instances where it is necessary to singly house an animal.

Occasionally animals may undergo a brief surgical procedure (e.g. to implant a telemetry device or hormone pellet). After surgery, animals are closely monitored and post-operative care provided (pain management with analgesics and provision of extra fluids). Analgesics may be provided in the form of a palatable gel to encourage voluntary ingestion.

Animals who develop allergic pulmonary inflammation or pulmonary fibrosis will be monitored frequently i.e. at least daily and the monitoring regime will be tailored to the stage of condition and risk of deterioration of the animal’s condition, including monitoring weight loss and general observation. Where transient weight loss is expected as a consequence of this condition, soft food (wet mash) will be provided on the cage floor as well as environmental enrichment.

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

To ensure that our experiments are conducted in the most refined ways we continually assess our experimental design and re-assess approaches if the opportunity arises. We use the PREPARE guidelines for planning our experiments. We stay informed about best practice guidelines by referring to information provided by Laboratory Animal Science Association (LASA) and NC3Rs. We use the ARRIVE guidelines for reporting our results in publications.

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

Our group stays informed about recent advances in 3R approaches by staying up to date with NC3Rs recommendations and developments. This information from the NC3Rs is obtained through interaction with their website, local seminars, contact with their staff and through social media. We also discuss further refinement opportunities with our NVS and NACWO and through interaction with colleagues at conferences, workshops and seminars