Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 20

Projects with a primary purpose of: Translational and Applied Research – Human Respiratory Disorders

Project Titles and keywords

1. **Symptoms and pathogenesis of lung diseases**
    - Asthma, COPD, coughing, IPF, ALI

2. **Tissue Fibrosis**
    - Fibrosis, pathophysiology, therapeutics

3. **Modulation of murine lung injury by hormones, cells and growth factors**
    - Lung injury, sepsis, vitamin D, steroid

Project 1	**Symptoms and pathogenesis of lung diseases**	
Key Words (max. 5 words)	Asthma, COPD, coughing, IPF, ALI	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To discover the mechanisms that drive the development of airway disease and the associated symptoms.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits are to expand knowledge in order to identify novel mechanisms involved in the pathophysiology/symptoms of inflammatory airways diseases such as asthma and Chronic Obstructive Respiratory Disease and to profile possible disease modifying therapies to provide future treatments for inflammatory diseases of the airways.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice – 6,130 Rats – 2,400 Guinea pigs – 2,400 Over a 5 year time period	
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?	The expected adverse effects are similar to that experienced by patients suffering from respiratory disease i.e. bouts of shortness of breath and/or coughing. We expect the majority of the model systems to reach moderate severity. On completion of the study protocols the animal are culled, typically with an overdose of anaesthetic.	
Application of the 3Rs		

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	On careful review of the literature and to the best of our knowledge there are no satisfactory alternatives for the *in vitro* and *in vivo* experimental methods we propose. Although human cell culture experiments have taught us a lot regarding the biology of intricate signalling pathways it is essential to study the physiological relevance of these observations in an *in vivo* animal model and, currently, there is no replacement model which can provide this information. The majority of our use of these protocols is to carry out studies on agents which have been exhaustively studied in cell and in vitro based assays and require assessment of their anti-inflammatory activity of the substance in a relevant model of airways disease. The agent in question may already have been examined in vivo to determine drug levels in the circulation or drug breakdown parameters and any information gained will be utilised by our group when designing an in vivo study. This process is essential to provide the evidence which will allow an agent to proceed to assessment in the clinic (in patients) and cannot be achieved at present without the use of live animals. We are confident that our modelling systems can teach us about the clinical situation; we have many examples where the animal model mimics components of the clinical diseases. What is more we have shown that therapies that modulate responses in our models have similar effects in the clinic.
2. Reduction Explain how you will assure the use of minimum numbers of animals	These procedures have been established and refined by our group and although we have already determined how many animals/treatment groups are necessary to produce statistically meaningful results we carefully monitor each procedure for signs of reduced variability that would allow a reduction in the number of animals used. All studies are carefully set up in order to provide the maximum information from the minimum numbers of animals. Our studies invariably have multiple readouts where blood and many tissue samples are taken and many measurements are made to maximise the information gained from the animals we use. We have a group with many years of experience in the design of animal experiments and consider ourselves very well equipped to design meaningful experiments which will produce reliable results. Animal numbers/treatment groups are always an extremely important part of our design. We carry out detailed statistical analyses on all our studies

	including power calculations and using parametric or non-parametric t tests or analyses of variance as appropriate. These analyses prove a valuable aid not only to assessment of the current data but also to future experimental design where, for instance, a highly statistically significant result, denoting a reduction in variability in a particular protocol, may allow for a reduction in the number of animals used in future experiments.
3. 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.	The procedures we employ use mice, rats and guinea pigs. When developing new models the mouse is often the first animal to consider as there are many more analytical tools available. For example one reason for using mice is that one can use animals that have been genetically modified to lack certain molecules to prove or disprove a hypothesis in addition to a more traditional approach using drugs that block certain molecules and pathways. This approach is often necessary in order to confirm data obtained with certain drugs that may act on more than one pathway. However some studies are too technically difficult to perform in mice, where for instance insufficient blood or tissue could be obtained to perform an assay or surgery is difficult to perform due to their size. Furthermore certain species are selected because the responses being measured more closely resemble the human condition and are modulated by drugs that effect similar responses in human subjects. e.g. the Brown Norway rat is used for the majority of our allergen-induced late asthmatic response (LAR) work. Where possible we are fortunate to be able to parallel our animal tissue studies in human tissue to determine the relevance of the species we use and where results are similar this gives much added confidence that the choice of animal is correct and will provide meaningful results. The guinea-pig has long been established as the model of choice for cough studies as rats and mice do not cough, it also provides a model with a more highly developed lung structure (and nerve connections) which is closer to man than that of the smaller rodents and has frequently therefore been used to study various lung responses. Furthermore, it has been shown that guinea-pig and human isolated vagus preparations respond similarly to tussive agents e.g. capsaicin, suggesting that data obtained in the guinea-pig cough model can be compared extrapolated to the response in man.

Project 2	**Tissue fibrosis**	
Key Words (max. 5 words)	Fibrosis pathophysiology therapeutics	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Tissue fibrosis, where an organ or area of the body contains fibrotic (scar) tissue as a result of injury of inflammation, is a feature of many clinically significant diseases, including autoimmune diseases, liver and kidney diseases, lung diseases (e.g. chronic obstructive pulmonary disease (COPD)) as well as rare but fatal diseases like idiopathic pulmonary fibrosis (IPF). The fibrotic organs become stiff and lose function in the cells that allow them to perform their normal roles. For example in IPF, the lungs lose their ability to inflate properly during breathing and the outlook for patients is very poor. We are looking for new mechanisms that are involved in fibrotic diseases, so that we can identify novel treatments that could bring benefits to patients.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	We will increase our understanding of the way tissue fibrosis comes about in disease, and what makes it different to the fibrosis that occurs as part of normal processes like wound healing. This knowledge will enable us to identify new ways to treat these disease	

project)?	and we will go on to identify novel therapeutic agents which could become human medicines.
What species and approximate numbers of animals do you expect to use over what period of time?	We will mostly use mice, with the possibility of a smaller number of rats, in situations where mice are unsuitable. The total estimate over 5 years is 9480 mice and 5280 rats.
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?	The majority of animals are expected to develop tissue fibrosis in the lung, skin or liver as the result of a challenge with pro-fibrotic substances. Other ways to generate a fibrotic response could include development of autoimmune disease by injecting animals with proteins or cells. Kidney fibrosis may also be induced using surgical models which involve removal or destruction of kidney tissue. In some animals it is possible that they will develop fibrosis in more than one organ (for example skin and lung fibrosis could occur in the same model). We expect almost all of these studies to be conducted at or below the Moderate severity rating. A very small proportion of animals may undergo a protocol for generating lung fibrosis that means they may experience more than 20% weight loss. The expected adverse effects across all of these protocols would include impaired function (e.g. for lung this may affect respiration, for skin it may include hair loss and increased thickening of skin) and animals are likely to exhibit signs of ill health such as reduced grooming and decreased social interaction. At the end of all these studies all animals will be killed using humane methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although more complex *in vitro* models of tissue fibrosis are being developed, it is still not possible to reproduce the complex interplay of for example structural cells, immune cells, hormones and growth factors present in the living organs. We will always conduct as much work as we can *in vitro* before looking at *in vivo* systems.
2. Reduction	Experimental designs are reviewed by experienced scientists, and the statistical methods we use to

Explain how you will assure the use of minimum numbers of animals	determine group sizes are reviewed by qualified statisticians.
3. 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.	Rodents, and mice in particular, are well characterised mammalian species with similar (but not identical) organ systems and immunological responses to humans. Most of the models we use are based on established methods which have been refined over many years, and where new models are derived, we take steps to ensure welfare is maximised from the start, so that the model has high standards built in from the beginning. All animals are purpose bred for scientific use and kept in state of the art facilities to keep them healthy and clean until use. Anaesthesia and analgesia are used in accordance with best practice guidelines to minimise any pain or discomfort during the scientific procedures.

Project 3	Modulation of murine lung injury by hormones, cells and growth factors
Key Words	Lung injury, sepsis, vitamin D, steroid
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Broadly speaking the animal studies proposed aim to define if altering of hormone levels influences animal models of lung infection (pneumonia), abdominal infection (sepsis) and severe lung damage that can occur following these infections known as ARDS.

Using genetically modified or dietary adjusted mice we will determine if severity and duration of lung damage can be improved using cells and or vitamin d replacement.We will determine whether these approaches improve the models to be more reflective of human disease.

We have genetically engineered cells known as mesenchymal stem cells (MSCs) to over express an enzyme known as HSD-1. We believe these cells will help protect and promote recovery from the lung damage and inflammation induced in the different mouse models proposed.

Clinical applications would be possible following these experiments since we could generate human MSCs that over-express HSD-1 and undertake phase I-II studies once we have animal proof of concept that recovery is enhanced with tMSCs

To inform clinical trial design, we will also study the effects of replacing vitamin D in mice that have low levels of vitamin induced by a special diet. We will study how long after the start of the injury we can give vitamin D to try and determine how to treat patients with the drug,

Vitamin D replacement could be rapidly studied in humans as a phase II trial if vitamin D treatment improves the resolution of infection. Key unanswered questions are how long after the injury is vitamin D replacement effective and is there a threshold effect for the effect of vitamin D –ie what blood level of vitamin D provides the maximum benefit

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

We will potentially identify new ways of treating patients with sepsis / pneumonia and ARDS.

What types and approximate numbers of animals do you expect to use and over what period of time?

Mice Approximately 8000 over the 5 years – most will be colony for breeding.

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

Intratracheal administration of bacterial Lipopolysaccharide. This model we have used to generate our preliminary data in mice. LPS is a part of the cell wall of many bacteria. When injected into the lungs of mice it causes inflammation – with cells called neutrophils being recruited to the lung. The effect on the mice is moderate but they become unwell at 24-48 hours with increased breathing rate and a degree of weight loss. We ameliorate this by giving pain killers and fluids. The animals are closely monitored and any that show significant suffering are euthanaed.. Cecal ligation and puncture (CLP, severe severity). Many of the procedures undertaken in this model are similar to as outlined above. The injury model is a surgical model where anaesthetised animals undergo an abdominal incision, followed by tying off (ligation) of the caecum of the bowel. The caecum is then punctured with a needle to release faecal contents into the abdomen that causes infection and sepsis. As a result of CLP, there is a potential for pain if the anaesthetic depth is inappropriate, mice lose weight and may become unwell – lethargy starry coat, reduced activity. Animals are monitored post-CLP using a clinical scoring system that means that the severity of the model can be kept to moderate. Animals undergo humane killing if they reach a severity of 4 on that scale. There is a small risk of the sutures used to close the incision opening up post-operatively. Animals are given fluids and analgesia prior to the incision being closed to reduce the adverse effects, Pneumococcal Pneumonia model (moderate severity). Many of the procedures undertaken in this model are similar to as outlined above in the IT LPS model. This

model involves the intranasal loading of the pneumococcus bacteria. Putting the bacteria into the nose makes the mice sniff the bacteria into the lungs. The intranasal injection may cause stress due to restraint and transient discomfort from the tubing insertion and or anaesthetic injection. The animals do develop some respiratory distress and are assessed clinically using a scoring system outlined in to minimise the severity of the model which is moderate in severity. Analgesia and fluids may be given to reduce the adverse effects.

Application of the 3Rs

Replacement

The animal experiments are necessary to allow us to ascertain whether our proposed novel treatments for pneumonia, sepsis and ARDS have efficacy as a proof of concept in mice. We build upon our previous work in mice using these models. We cannot use fish for this work as they do not have lungs and there are no validated sepsis models.

We have already used other translational methods such as primary human alveolar macrophage and alveolar epithelial cell culture in order to minimise and reduce the number of animals required but we need the animal model to study the mechanistic implications of this project in more detail.

Reduction

The proposed experimental designs and methods of analysis of the results have been discussed with Dr Peter Nightingale statistician. Based upon preliminary data we have performed power calculations for the inflammatory mediator measurements. For most of the experiments, sample sizes will be set generally using a significance level of 5%, a power of 80%. The effect sizes for the inflammatory mediators and indices of permeability that we have calculated our samples sizes for are 25%.
Otherwise, we will use the least number of animals to provide an adequate numbers of cells for the purposes of culture, generally on the basis of previous experience (ours, or from the literature). In terms of the numbers of animals required, we expect that 8-12 animals per treatment group should be sufficient to obtain the required results.

Refinement

Why use Mouse lung injury models?

1. Mouse models have provided extensive insights into the pathophysiology of sepsis / ARDS and represent the most commonly used animals for this purpose worldwide.

2. The proposed murine models have been defined in the c57 black strain by ourselves / collaborators -providing data for appropriate power calculations to minimise the numbers of animals needed.

3. The ability to study genetically modified mice specifically bred e.g with HSD-1 deficiency allows us to address one of the central objectives of this project.

Justification of Animal Models Chosen:

We believe that it is necessary to look at a range of animal models as no single injury to mice reproduces the complexity and extent of human sepsis and ARDS.

Direct Lung injury- (IT) LPS challenge: The LPS responsiveness of the C57BL/6 mouse strain has previously been defined, with cellular inflammation peaking at 48 hours post inhalation. Mice receive IT 50 µg/mouse lose weight, develop pulmonary neutrophil influx, show impaired respiratory system compliance and have increased BAL total protein, all peaking at days 1-2. Appropriate controls are IT PBS.

Direct Lung Injury- Pneumococcal pneumonia models a clinically important direct infective lung injury. Utilising different strains of the bacteria allows for a variation in the severity and duration of infection / inflammation. Strains INV1041 causes an acute lung inflammation over a few days and bacteraemia, whereas strain 03-3038 yields a less severe, longer term infection which is ideal to study the resolution phase of the infection/ inflammation.

Remote Lung injury: CLP causes mild pulmonary damage characterized by neutrophil infiltration, increased levels of CXC chemokines, and oedema formation in 24 hours. This model causes abdominal infection with systemic septicaemia. To use appropriate controls we will use sham operated controls, who undergo laparotomy but do not undergo CLP.

We have refined the CLP model in our previous licence to ensure there are no deaths by only using a single pass of the needle to puncture the ligated caecum and to limit the post-CLP time points to within 24 hours to minimise the risk of mortality in these animals – this allows us to study early but not late sepsis.

Minimising Animal Suffering: For all models, we will resuscitate the animals at the end of the operation by injecting pre-warmed normal saline subcutaneously/ intraperitoneal/ intravenously. Post-operative analgesia and fluids (e.g. saline) may be given if clinical signs indicate the necessity, and any animals showing unexpected distress/problems related to the procedure will be terminated.