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Project summaries granted during 2013

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Environmental sensitivity of subclinical disease resistance and resilience in farm animals

Summary
This program of work assesses nutritional, genetic and environmental sensitivities of (the outcome of) sub-clinical disease in farm animals.
Rational
Drug resistance hampers disease control and we need to know whether alternative nutritional strategies depend on other factors. Feeding extra protein to sheep reduces worms but may be breed dependant. Feeding probiotics to young pigs improves gut health but may depend on diet composition and breed. Many plants may have anti-parasitic properties, and we aim to identify their efficacy. We also assess if climate change affect parasitism in sheep. Results will inform strategies in farm animals to achieve disease control with minimal use of drugs and better predict future disease risk from climate change.
Outline
Sheep will be infected with worms or left uninfected, and then dosed or fed with plant extracts, or fed different levels and types of protein. Sheep will be housed at different environmental temperatures and infected with parasites reared tinder different climatic conditions. Pigs will be infected with bacteria and fed probiotic containing foods with different nutritional compositions.
Animals use
Complexity of underlying interactions between digestive, immune and endocrine functions requires animal use, although *in vitro* studies will pre-test anti-parasitic plants.
Suffering
Our refined infection and nutrition protocols cause little or no harm, and experimental foods have high quality ingredients. Animals deliberately fed a little bit below their nutrient requirements simply grow slightly less or produce slightly less milk without suffering. Animals are daily observed, and quantifiable, clear end-points have been established to ensure that animals do not exceed a mild to moderate severity limit.
Why sheep and pigs
This program of work uses sheep and pigs both as model and target animals. Although many aspects of nutritional sensitivity of parasitism are being addressed in rodent models, demonstrating breed effects and underlying host responses in target animals remain required to predict impact of alternative disease control strategies. Furthermore, there are no suitable alternative rodent models to predict nutritional sensitivity of gut health for weaned pigs.
Animal numbers, procedure description, adverse effects
We may use up to 140 sheep and 160 pigs per year, which we minimise through lab-based studies and through statistical tests to identify the minimal number required to observe desired effects. Nutritional and infection protocols are used to study effects of 1) protein nutrition on worm control and immune responses in sheep, 2) anti-parasitic plants on worm infections in lambs, 3) environmental temperature and humidity on parasitism in sheep, and 4) probiotics on gut health in weaned pigs. Protocols are sufficiently refined to only expect mild to moderate adverse effects.
Benefits
We aim to learn how plants with anti-parasitic properties, protein nutrition, probiotics and climatic influences influence (the outcome of) sub-clinical disease in farm animals. This may better predict impact of climate change on disease risk and inform feeding strategies to achieve disease control with minimal drug use. This would benefit both conventional and organic farmers, but also people in developing countries, where infection and malnutrition often go hand in hand.

Proteins with short half-lives

We wish to generate and breed a novel line of genetically altered mice, in which the production of a specific protein can be changed in a very precise way. We intend to use these animals in our studies to determine what this protein does, and why it appears to be associated with some common human cancers.

Cancer is a very complex human disease, which is becoming more common in our ageing population. Cancer is caused by cells of the body that grow out of control. Our laboratory has discovered that a previously ignored protein may play a role in controlling how the cells of our body grow. It first came to our attention because it “turns over” very rapidly in cultured cells. Other proteins with this property are known to be crucial in controlling growth and division, hence our interest in this protein of unknown function. It is intriguing that high levels have been detected in samples from some common human cancers, suggesting also a link with abnormal cell division.

We shall continue to work with cultured cells wherever possible but these are not truly representative of normal cells of the body and it is also very difficult to engineer the complete absence of a target protein in them (and hence to determine what exactly it does). We have therefore elected to generate a line of mice in which the production of these protein can be shut off completely, either in the whole animal, or in a specific organ. We also intend to be able to control this functional deletion in time, by using techniques that will induce it only when it is required. We therefore expect to be able to maintain the line without the genetic alteration having any effect. We have selected the mouse as our model species, because the techniques of making such genetic alterations are very well-established and yet the mouse shares much genetic information and body functions with humans. We will have to cross our line with other mice in order to be able to make the tissue- and time-dependent alterations and expect to use a total of 5000 mice over the five years in this important background activity.

Once we have generated animals with these functional deletions, most of our work will be carried out in so- called primary cell cultures isolated from different organs after the animals have been killed humanely. Cells derived from a single animal can be used in several experiments, thus reducing the number that we have to breed and use.

Because the protein is of unknown function, we shall carry out some very simple procedures to learn more about the effects of its deletion on the whole organism. These will include blood sampling, to look for any significant changes in metabolic chemistry (e.g., blood sugar levels) and observational tests of normal behaviour. These interventions are not expected to cause any significant harm or distress.
Because the protein has not been deleted in mice before, we cannot be certain as to what the overall effects on welfare will be. By restricting the tissues and times in which the deletion can have an effect, we expect to be able to minimise any harm. We will use very small numbers of animals in the initial experiments (one or two breeding pairs at a time), before setting up longer-term breeding programmes, so that any effects are understood and are then avoided.

Other proteins that turn over very quickly but are present at high levels in cancers are now being actively pursued as targets for new cancer therapies. We believe that our studies of this novel protein will help in determining its role in normal and abnormal cell growth and division. It too may turn out to be another target for treating human disease.

Pathogenesis of lung injury and organ failure

This project is designed to investigate the pathogenesis of a number of important conditions, primarily focussing on sepsis and acute respiratory distress syndrome (ARDS), which cause the deaths of many people within intensive care units, reportedly up to 40,000/year in the UK alone. The general plan for this project is to utilise 'clinically-relevant' in vivo models of sepsis and ARDS (plus other conditions) to investigate the disease processes and the effects of treatment. A major aim of the project is to enhance translatability (to humans) by incorporating a variety of 'co-morbidities' (e.g. diabetes, obesity), which are very common within patients but their influence is very rarely studied.

At present, it is impossible to produce an in vitro replica of these conditions, which involve complex interactions between different organs and the immune system. We do however intend to utilise in vitro cell studies and ex vivo isolated organs to analyse some of the basic mechanisms more precisely and thoroughly.

As far as possible we will utilize animals as efficiently as is feasible, taking multiple 'endpoint' samples from each animal as long as this does not compromise experiments. The maximum number of animals requested is -32,000 over *5* years, although in reality the figures are likely to be closer to 10,000 over this period, 90% of which will be mice.
The mouse is genetically well characterised and numerous research tools (antibodies, reagents etc.) are already available, including genetically modified mice. In particular circumstances it may be deemed necessary to utilise rats instead of mice (e.g. where complex physiological studies are not possible in mice, or where multiple analyses could be carried out on larger tissue samples), but it is anticipated that this would involve <10% of the procedures.

We will be utilising a number of models of sepsis/ARDS, as clinically there are a wide variety of insults that cause these conditions. These models will include acid aspiration into the lungs, live bacterial challenge, burn injury and .mechanical ventilation, amongst others. For many of the models the majority of animals will be under general terminal anaesthesia, and thus subjected to minimal suffering. However, we will also be utilising more chronic injury models in recovered animals. With induction of injury animals may become unwell and show some signs of distress. In some cases development of these symptoms (e.g. reduced mobility, diarrhoea, weight loss and rapid breathing) may be necessary to carry out the objectives. However, many scientific endpoints are likely to be achieved by terminating sufficiently early that indications of substantial distress are not reached.

At the moment there is no therapy for sepsis/ARDS beyond supporting patients within intensive care until they either die or recover spontaneously. A better understanding of the underlying mechanisms is crucial to develop effective pharmacological interventions that may reduce morbidity and mortality for patients with these frequently fatal conditions.

The pathophysiology and therapy of renal failure

Summary of Project

This project will use established small animal models of renal failure to increase the understanding of the cellular mechanisms underlying the development of acute kidney injury (AKI) and chronic kidney disease (CKD), Ultimately this research will contribute to the development of new drugs for the treatment of these different forms of renal failure.

Scientific Unknowns

There is currently no pharmacological cure for renal failure, with dialysis and transplantation remaining the only effective (but less than perfect) therapies. A better understanding of the cellular mechanisms underlying the development and progression of renal failure is now required for the development of more effective drugs with which to prevent or treat renal failure.

Scientific Background

Oxidative stress involves damage to the kidneys caused by toxic oxygen and nitrogen metabolites which accumulate under pathological conditions. Such oxidant injury is a major cause of renal failure caused by cessation and return of blood flow to the kidney (ischaemia-reperfusion, e.g. during kidney transplantation). Oxidative stress also plays an important role in the renal failure caused as an unwanted effect of commonly used drugs (i.e. nephrotoxicity, e.g. caused by some antibiotics), hypertension and diabetes (diabetic nephropathy).

General Project Plan

Initial investigations on compounds of interest will be performed on renal cell cultures or renal slices in order to (I) determine the efficacy against the cellular injury and death caused by oxidative stress and (ii) to investigate any potential renal toxicity. Only agents showing protection against oxidative stress-induced renal injury at this stage will progress to into *in vivo* investigations. Following these preliminary studies, a small pilot study will be performed using mice or rats to determine potential renal toxicity and dosages. Once an appropriate pharmacological dose is reached without any evidence of alteration of renal function, an agent will he investigated in established animal models of AKI or CKD.

Why animals have to be used

The use of animals is essential for this research as renal failure involves complex physiological mechanisms which cannot he studied effectively using cell cultures or isolated organ systems. Renal failure is multifactorial in that there are many pathophysiological processes involved which occur simultaneously (e.g. expression of pro-inflammatory genes, release of cytokines). Furthermore, the kidney is influenced by several extra-renal factors such as blood pressure and mediators released from other organs in response to renal injury. Such elements cannot easily be replicated using *in vitro* or *ex vivo* systems.

Numbers of animals to be used

We have carefully considered the “3Rs” (replacement, reduction and refinement) in order to minimise the numbers of animals used in our programme of work. We have also used power calculations, based on our data from previous successful studies, to predict the least number of animals required to show a significant beneficial effect. We predict that we will need 1,200 animals per year to complete this project, however, it is hoped that substantially less will be actually used.

How we will minimise animal suffering/adverse effects

Preliminary *in vitro* and pilot studies will he used to ensure that only agents which are least likely to produce adverse effects are investigated in *in vivo* studies. During *in vivo* studies, animals will be monitored regularly (minimum daily observations) by competent and trained staff and if any animals display suffering or adverse effects then action will be taken (e.g. administration of pain relief). In extreme cases the veterinary surgeon will be consulted and the animal euthanised using a humane method if required.

Why mice and rats have been chosen

This program of work will primarily involve the use of rats which are the lowest vertebrate group on which well characterised animal models of renal failure have been developed. Rat models of renal failure are robust and reliable and as they have been characterised in many previous studies, the numbers of animals required to establish these models will be reduced considerably. The option to use mice as models of renal failure will allow us to compare any data obtained with those obtained in genetically modified mice, in which a specific gene involved in oxidative stress has been deleted (knock-outs) or replaced in a modified form (knock-ins)

Benefits/advances from achieving the project's objectives

Renal failure is an increasing cause of patient morbidity and mortality. Overall, this project will provide a better understanding of the cellular mechanisms involved in the development renal failure. This research will also provide pre-clinical evidence of the mechanism of action and efficacy of specific interventions in different forms of renal failure and subsequently, a rationale for the development of new drugs for treatment of patients suffering from this dreadful disease.

Mechanisms underlying depression and treatments

Depression is a very common and serious disorder, occurring in different forms. However, the mechanisms underlying the development of behavioural and physiological symptoms remain poorly understood. This project aims at further studying the mechanisms that contribute to depressive symptoms and in particular, the role of transmitters- substances that transmit information within the brain. The benefits will lead to a better understanding of the mechanisms underlying depression. It will also contribute to identifying new targets to develop medications to treat depression, and to understand the effects of treatments on several symptoms.

Major depression and minor depression, such as postpartum depression, have a negative impact on life quality. Symptoms include persistent sad or “empty” feelings, feeling of hopelessness, guilt, irritability and fatigue, as well as changes in appetite. Another key symptom is sleep disturbances, with more than 80 % of depression sufferers complaining about insomnia or excessive sleeping. These sleep disturbances are preponderant in women. Often sleep disturbances are unresolved by depression treatments and this is associated with an increased risk of relapse. The beneficial effects of antidepressants are believed to enhance the generation of new neurons in adults, thereby lowering the stress response. However, despite their relative popularity, antidepressants show a limited efficacy, as well as a potential worsening of depression and increased suicidal behaviour.

To study the contribution of specific brain transmitters to depressive symptoms and their treatments, we need to perform behavioural studies, sleep recordings and subsequent molecular, cellular and anatomical studies in the brain and other tissue, therefore ii is not achievable *in vitro.* This work is part of a research programme which includes studies on human volunteers and the use of human tissue samples. Animal studies are only performed when human studies cannot generate knowledge. We will use mice because they have a well-defined genetic background and have been well-characterised in this field of research. This provides the advantage of requiring fewer animals for experimentation. Mice will be subjected to a chronic mild stress protocol that is a well-established model of major depression. Importantly, severe stressors, such as intense or painful stimulus, will not be used. We will also use mice, lacking a certain type of transmission, which is an established model of post-partum depression.

We will record the brain waves to characterise sleep and wakefulness parameters, behavioural and cognitive functions using non-invasive tests. The effects of medications on behavioural parameters, including anxiety, motivation, social interaction, memory and sleep, as well as physiological parameters (inc. stress response, production of new neurons, and expression of transmitters) will be studied. The effects of different light conditions will also be investigated for their therapeutic potential, by varying the levels of illumination or the daily duration of light.

Overall, the research will contribute to our understanding of the mechanisms underlying depressive symptoms and their treatment. This may lead to a better understanding of the contribution of specific transmitter to brain function, mood disorders, sleep disorders, as well as the development of new treatments for these disorders.

Selection and development of novel drugs for the treatment of human cancer

This project is intended to aid the development of new, safer and more effective drugs for the treatment of cancer in human patients.

Presently, cancer remains a significant unmet clinical need with roughly 150,000 deaths attributed to cancer and roughly 250,000 new diagnoses of cancer each year within the UK. Our aim is to offer services evaluating new molecules or series of molecules to our customers and use our in-house expertise to conduct a series of scientific studies using cell culture and animal based studies to select the molecules with the best chance of treating cancer. Extensive studies using both cell culture and animal studies will be conducted in order to identify the best way in which to give the drug, the tumour target(s) against which it is most effective and if the new drug can work with other drugs to improve treatment of cancer further.

Although much of the work in evaluating cancer drugs is conducted using cell cultures derived from actual human cancers, in vitro cell culture does not reflect all of the unique properties of tumours. Therefore, in-vivo testing is always required by regulatory authorities prior to approval for use in patients.

In outline, new drug candidates are preliminary screened using a number of cell culture and other in vitro techniques to select the candidates The overall aim is to eliminate potential failures as quickly as possible and, thereby, minimise the numbers of animals needed. Once a candidate is selected, additional data will be needed in order to be able to select route and frequency of drug administration, tumour response to the drug, and if the drug can be combined with other anti-cancer drugs to produce even greater potential benefits to patients. Mouse models of human cancer will be developed by implanting cancer cells in mice and allowing tumours to develop. We then treat small groups of mice using different amounts of drug or by different routes or by different frequencies. Also, we will examine the potential of the new drug in combination with other therapies, in order to obtain the greatest therapeutic benefit and allow us to determine additional risks to patients.

Mice will be used for these studies as this species is the lowest evolutionarily developed laboratory mammal available. This species is also the most widely used in oncology research and is accepted as the gold-standard by regulatory agencies as a method of demonstrating proof of efficacy.

Animals will be provided with sterile bedding, food and water, and environmental enrichment will be supplied. Trained and competent staff that have experience with models of human cancer and are familiar with effects of anti-cancer drugs on mice will perform all procedures involving animals.

A maximum of 20,000 mice will be used in these studies over the course of this project. Animals will be closely monitored and any animal that is considered to be in discomfort or pain will be humanely killed. Anaesthetics and analgesics will be used as advised by the named veterinary surgeon.

In conclusion, therefore, we will be conducting multiple experiments using animals that aim to provide new drugs for the treatment of cancer that are safer, more effective and are complementary or superior to currently available treatments for a disease that kills a significant number of human patients each year.

Patient tumour derived models

This project aims to generate patient derived models of cancer to study tumour development in response to clinically relevant therapies, therefore providing a platform for a personalized approach to drug discovery.

There is currently a high efficacy failure rate of novel compounds developed to treat cancer patients. The generation of models that recapitulate more faithfully the disease in humans is a high priority. Patient derived tumour xenografts have been shown to retain the heterogeneity observed in patients and reliably predict clinical activity of novel compounds in a variety of tumours.

We will generate patient derived tumour xenografts, which will be expanded and stored. The established tumours will be subjected to therapy regimes that will mimic those occurring in the clinic. Throughout these experiments, molecular and histological analysis will be performed using state of the art technology including next generation sequencing. En this way we will be able follow the development of individual tumours that are specific to cancer patients being subjected to therapy. Our data will be used to inform patient treatment.

Animal models are required to fully recapitulate the properties of the three dimensional heterogeneous tumour tissues growing within specific organs in cancer patients. These properties cannot be adequately recapitulated *in vitro.* Similarly, the effects of drugs need to be tested *in vivo* so that the effects of the tumour microenvironment, drug access and target specificity can be assessed. Mice are the most effective choice of species for these experiments and the availability of immunocompromised strains allow for the grafting of human derived tissue with minimal rejection. *In vitro* cell culture and three dimensional tissue models are being developed and compared to the *in vivo* models in an effort to establish animal replacements.

Non invasive imaging and molecular profiling are used to follow the development of the tumour and minimise the number of animals required at different time points during the course of experimental regimes. These assays not only reflect what is experienced by cancer patients but provide a high level of information that will lead to a reduction in experimental repeats.

In most cases, the patient derived tumour tissue graft will be placed under the skin and subjected to therapeutic drug regimes. Adverse effects will be minimized by keeping grafts to recommended limits and using tolerable drug schedules following NCRI Guidelines.
There is an urgent need to develop novel treatments to combat cancer. This project is developed in close collaboration with clinicians to deliver a highly translational approach that has direct impact on patient cancer treatment.

Tissue engineering using a combination of stem cells and scaffolds

This project involves the creation of artificial organs for transplantation. Currently, there is a lack of treatments when we deal with patients that have missing, faulty or damaged organs. The best solution is organ transplantation, however, that is limited by issues of rejection, a need for immunosuppression, and organ shortage. We aim to assess the combination of different scaffolds and cells in being able to recreate the structure of an organ. We also aim to investigate the role of stem cells in building an organ over time and the effect of different growth factors in achieving this result. We have exhausted all the ways of testing the suitability of our scaffolds and cells outside a living body.

To be able to progress our work toward humans, we have to use animals. We will be using mice with altered genes so as to able to investigate the role of stem cells in tissue growth. We will also be using rats as they are the smallest animal that we are able to successfully reproduce the surgical procedures we wish to perform. We will first assess the immune response to scaffolds by implanting them under the skin. We then aim to seed them with cells and place them in line with the host's organs (for example the tissue- engineered intestine will be sutured between two pieces of the normal intestine). The risks of this last procedure involve infection, pain, trauma and bleeding. We will aim to minimise these by using antibiotics, analgesics and careful monitoring of the animal.

These experiments will allow us to test our cell-scaffold combinations before attempting them in humans. Success will help a number of people that are on the waiting list for organ transplantation.

Pathogenesis of and immunity to viral infections

We aim to study the diverse interactions of viruses with their hosts. This involves the immune responses to infection, ability of the host to clear the infection, the interplay of the virus and host that results in disease, the effect of host genetic background on this and the ability of the virus to alter the host's genome. Understanding these processes is central to the development of new vaccines and treatments to inhibit infection and development of disease. We will use model viral infections to study the role of the immune response in viral clearance and disease, study novel ways of making vaccines and determine mechanisms of genetic variation caused by viruses. Murine norovirus will be used to study immune responses to gastroenteritis viruses and why they can establish persistent infection.

This includes studying early immune responses to the infection including those triggered directly by molecules sensing infection and identifying which responses clear the virus. Vaccination studies will use antigens from HIV and the lentivirus field and will move into murine norovirus and lymphocytic choriomeningitis virus. These will study immunomodulators to induce the desired immune response as well as identifying protective subunits of viruses and often specifically engineering them to give correct structure. Lymphocytic choriorneningitis virus will be used to study the ability of an RNA virus without a DNA intermediate to colonise the host genome and to cause heritable changes in the host. The interactions outlined above are complex and only limited areas of study can be performed outside of animals. In some cases infections may be performed in tissue culture to allow increased levels of infection and to study particular cell types in isolation. These will be used to focus further studies in animals.

Experiments will be organised to make use of statistical analysis and if necessary pilot studies will determine variance of results, so that in larger experiments sufficient animals are used to give valid differences between groups. Most procedures are non-surgical and will cause only transient discomfort but some of the protocols will use anaesthesia where necessary for specific procedures to improve the performance of the procedure or to limit pain. The guidelines given by Laboratory Animal Science Association (LASA) will be followed for volumes administered unless otherwise stated. Most procedures involve administration of substances including viruses to animals although some will involve bleeding animals or breeding mice. Where animals are infected they will be monitored for adverse effects such as weight loss, hunching and piloerection and if these are not transient, animals will be humanely killed. The majority of the animals used will be mice as these have the required genetic backgrounds and are natural hosts for murine norovirus and lymphocytic choriorneningitis virus. For vaccine studies guinea pigs and rabbits will be used to give larger volumes of serum for analysis.

A greater understanding of mechanisms of disease will allow potential targets for intervention against viral induced disease to be identified and exploited in the future. Novel vaccine candidates and formulations will be tested for proof of principle before being advanced into clinical trials.

Cardiovascular effects of adenosine ligands

Summary
This project is concerned with assessing the ability of novel compounds to exert beneficial effects on the heart and vasculature, without causing other side effects.

Clinical need, scientific background and statement of benefits
Adenosine is a compound found in the body, which acts in many organs to protect against the influences of stress. There are a number of cardiovascular conditions, such as angina, where the use of compounds that activate adenosine receptors would be beneficial. Currently, drugs that target adenosine signalling pathways have many cardiovascular side effects that limit their use. Clearly, if new adenosine ligands could be designed that have the wanted effects on the cardiovascular system, with no other adverse effects, this will be a major advance, with potentially important benefit for many patients.

Why animals have to be used
A major part of the programme of work involves the development and validation of *in vitro* methods that will reduce the need for future *in vivo* experiments. But because this project requires the assessment of the integrated function of the heart and blood vessels, in the absence of the interfering effects of anaesthesia, it is necessary to use intact, conscious animals for part of the programme of work.

Outline of general sequence of work
The current programme of work will involve the development of a number of *in vitro* assays that will be compared and validated against data generated from *in vivo* experiments. Dose-ranging studies will determine the regional haemodynamic profiles of novel adenosine agonists. Full experiments will involve both short-term and long term studies with n=6-8 animals per group.

The experimental approach is that developed and refined over many years (in consultation with the NACWO, NVS, and HO Inspector), whereby, using appropriate anaesthesia and analgesia, rats are chronically-instrumented with implanted flow probes and catheters. Thereafter, when animals have recovered fully and are eating and drinking normally, the detailed delineation of cardiovascular effects of substances can be assessed by their administration through catheters, without disturbing the animals. Because the animals are closely observed at all stages (including inspection by the NVS), and because they are monitored throughout the experimental protocol, then the likelihood of adverse effects is reduced, and any such effects are detected rapidly, and appropriate action taken. The use of chronically-instrumented animals means that each rat can act as its own control, and this minimises the use of animals. The design of the experiments is such that very low doses of compounds are administered initially, and, hence, any unexpected adverse effects of the compounds can be avoided.

Choice of species
The rat is the animal of choice, since there is an extensive background literature on cardiovascular regulation in this species, the cardiovascular system resembles that of man in many ways, and implantation of the measuring devices is not possible in mice because they are too small.

Estimate of number of animals and typical experience
Across the lifetime of this project, it is estimated that a maximum of 840 rats will be used. For a typical rat in the full experimental schedule, surgery to implant flow probes (maximum of 3) around blood vessels, such as the renal and mesenteric arteries and the descending aorta, and to implant catheters in blood vessels will be carried out under anaesthesia with operative and post-operative analgesia. For the second protocol, rats undergo surgery to implant

radiotelemetric devices. Although these interventions cause transient discomfort, the animals recover well and at the time of experiment, the rats are certified fit by the NVS, are eating and drinking normally, and live in home cages with appropriate environmental enrichment.

Once the animals have recovered from the surgical procedure, they are dosed with the potential therapeutic drug of interest. The flow probes and telemetry transponders will detect changes to the animals' cardiovascular parameters. The signals from these sensitive instruments are passed to data recorders allowing very specific interpretation of data and thus increasing knowledge about the effect the drugs have on the animals.

In order to minimise animal use, short-term experiments run over a maximum of 4 days, with animals acting as their own controls, and being exposed to more than 1 compound, if appropriate. No unexpected adverse effects have been detected in schedules such as these over many years.

How people will benefit from this project
This Project Licence application relates to integrated cardiovascular studies which focus on understanding the cardiovascular effects of compounds that act at adenosine receptors. The novel approach described in the licence will help unravel the importance of ligand-receptor interactions, by measuring integrated regional haemodynamics. There will be parallel *in vitro* experiments which will enable data from particular signalling pathways in recombinant cells to be compared with *in vivo* responses, to determine which *in vitro* assays are good predictors of *in vivo* efficacy. It is expected that this will lead to a reduction in the need for *in vivo* experiments in this area in the future. The benefits of obtaining a fuller understanding of the cardiovascular effects of adenosine agonists is an exciting therapeutic target with potential for alleviation of a variety of cardiovascular conditions, but the complexities of adenosine receptor interactions need to be more fully understood for progress in this area.

Abnormal toxicity & Pyrogen Testing

The overall aim of the programme is to evaluate the pyrogenicity, abnormal toxicity or general safety of pharmaceutical and veterinary products intended for parenteral injection in order to ensure the safety of the product before it is released for use in humans or animals. The testing is primarily a requirement of the British and European Pharmacopoeias(BP, EP). Where testing is performed to satisfy non EU pharmacopoeias(e.g united States, Japan) the requirements will not exceed the requirements of the European Pharmacopoeias in terms of animal numbers and severity.

The project is necessary in order to ensure that pharmaceutical and veterinary products are free from extraneous contaminants or unacceptable levels of biological activity that could cause toxicity or fever when administered to humans or animals. The pyrogen, abnormal toxicity and general safety test may be performed as a requirement of a UK or overseas Product Licence. Additionally the tests may be required to satisfy a manufacturing licence requirement for items which may be used in the preparation of pharmaceutical/parenteral products.

Regulatory studies are requested by and performed in compliance with relevant UK & EU legislative bodies including the European Medicines Agency (EMA), the Medicines and Healthcare Products Regulatory Agency (MHRA) and the Veterinary Medicines Directorate (VMD) in the UK. The guidance given in the various guidelines of the International Conference on Harmonisation (ICH) are followed in the design of safety evaluation programmes and in the design of studies.

For studies requested for other worldwide authorities, for example the US Food and Drugs Administration (FDA), a scientific justification will be sought if the study requirements exceed the requirements of the UK or EU regulatory authorities for similar studies, for example by requiring repetition of a test already performed or a greater number of animals or a more severe test. Authorisation to perform such tests will be obtained prospectively from the Secretary of State and any valid scientific justification will be included within the records of the project licence.

The individual studies will be conducted in accordance with internationally approved methods which are specified in pharmaceutical monographs. Mice and guinea pigs will be used for freedom from abnormal toxicity(FAT) and general safety(GS) testing and rabbits for pyrogen testing(RPT). These are the species of lowest neurophysiological sensitivity that will allow evaluation of the specific endpoints. The number of animals used in each test will be the minimum number as specified in the relevant monograph. Animals used in the rabbit pyrogen test will have been previously used in skin or eye irritation studies and will be re-used wherever possible in multiple pyrogen tests.

The RPT will be used in parallel with the Limulus Amoebocyte Lysate (LAL) test. The LAL test will be used wherever validated for a particular product type. However if a pyrogenic effect would not be detected with the LAL test then it is appropriate to use the RPT. Currently, there are no validated *in vitro* tests that can be used as a full replacement for the RPT and there are no validated alternatives to the FAT and GS tests. In FAT/GS tests the product is administered by the intravenous, intra peritoneal or oral route to mice and/or guinea pigs and the animals are observed for signs of toxicity for up to 7 days. In the RPT the product is administered intravenously to three rabbits and their temperatures are recorded to identify products which may cause a rise in temperature.

Over the period of the licence it is expected that approximately 3500 mice and 2000 guinea pigs will be used on abnormal toxicity and general safety tests. Approximately 1000 rabbits will be used in pyrogen tests. Administration of the test item to animals in abnormal toxicity and pyrogen tests causes transient mid stress and discomfort.

The ability to conduct pyrogen and abnormal toxicity tests in animals will allow the identification of adverse responses associated with pyrogenic material, toxic substances present in the product or unacceptable levels of biological activity so that corrective action can be taken to ensure the safety of the product before it is released for use in humans or animals. The integrity of a parenteral product is essential as administration of a product containing extraneous toxic contaminants to a sick human or animals could have life threatening consequences.

Cell Senescence and Cell Death in Atherosclerosis

Atherosclerosis is a disease that causes thickening of the arteries, and is responsible for heart attack and strokes, the commonest cause of death in the UK. The primary goal of this project is to understand how cell processes such as cell death, cell proliferation and cell ageing (senescence) contribute to atherosclerosis, formation of an aneurysm (a localised expansion of the artery that may rupture) and vessel ageing. The research plan involves cell culture work to understand cell death, proliferation and ageing, to determine the genes and molecules responsible. However, the complex processes involved in atherosclerosis and aneurysm formation cannot be reproduced in the culture dish. We therefore need animal models to mimic human disease and to test the impact of novel drugs on disease development and complications in vivo.

The mouse models have genetic alterations to make them susceptible to disease, or have over- or under-expressed genes. The number of animals is minimised by careful experimental design according to extensive previous experience in the models, and is determined according to pre-defined and appropriate statistical analyses. In this project, we are trying to. If we can discover the molecules and mechanisms underlying heart disease and vessel ageing we may be able to develop both new strategies and new treatments that could reduce the burden of heart attacks and strokes.

Peripheral and spinal nociceptive processing

We aim to identify, in various painful pathologies, signalling mechanisms between various cells in different tissues and terminals of sensory neurons which innervate those tissues, and signalling mechanisms in the spinal cord.

1. Why is it necessary to use animals in this project; what alternatives are available; have been considered unsuitable and why?

   Signalling between various cells in different diseased tissues and terminals of sensory neurons which innervate those tissues, as well as signalling within certain areas of the central nervous system including the spinal cord, play a vital role in the initiation, development and maintenance of pain. Our current understanding on those signalling is limited. In vitro techniques have involved tremendously in the last decades. Still, due to the complex nature of the development of pain gaining new knowledge is impossible without using in vivo animal experiments.

   Various established disease models, including models of inflammation of tissues of various origins, for example burn injury- or irritant-evoked inflammation, as well as models of traumatic peripheral nerve injury will be used. The great majority of the models will be acute models, which means that the pathological state as well as the assessments on neuronal signalling will be done under general non-recovery anaesthesia.

We will also constantly monitor improvements in in vitro techniques and will implement relevant techniques immediately to reduce the number of in vivo studies.

2. What are the kinds of animals to be used, their approximate ages and numbers involved?

In this project, in addition to using in vitro studies on cells cultures and cell lines, we will use in vivo studies on adult rats and mice (maximum about 3500) with highly effective and modern approaches. By using robust statistical analysis we will make also sure that the correct number of replicates is used to achieve the required statistical power.

3. What checks are to be made on the animals and how frequently?

When several days are necessary for the development of the pathological state, animals will be regularly (at least twice a day) checked for any suffering or adverse effects

4. What will happen to the animals and what adverse effects will they suffer?

Animals with adverse effects (e.g. paralysis due to accidental motor nerve injury) will be sacrificed humanly by Schedule 1. The great majority of the models will be acute models, which means that the pathological state as well as the assessments on neuronal signalling will be done under general non-recovery anaesthesia.

Whenever it is plausible, pain will be assessed, under general terminal anaesthesia sufficiently deep and stable to ensure that the animal is insentient throughout, by using surrogate pain markers, such as studying changes in gene or protein expression, or electrical responses of various neurons. When pain-related behaviour such as withdrawal reflex must be measured on conscious animals, pain will never exceed moderate level and the animal will be free to escape from the stimulus.

5. What are the reasons for carrying out this project?

Pain is a vital symptom of impending or actual tissue damage. However, pain becomes a disease on its own when it persists. Persistent/chronic pain is, very often, intractable with any of the currently available therapeutic regimes and has devastating effects on the patients' quality of life and puts a huge financial burden on health systems and economies. Therefore, developing new analgesics is of paramount importance.

Persistent/chronic pain accompanies a wide variety of diseases. The cellular and molecular mechanisms involved in the development of persistent/chronic pain depend on the underlying pathology. Therefore, for the development of new analgesics, we must have a better understanding of nociceptive mechanisms occurring in various types of pain conditions.

This project will identify molecules will serve as putative targets for new analgesics. Hence, through drug development people as well as animals will benefit in the longer term. In addition to the health benefits, these findings will also increase our knowledge on signalling between non-neuronal tissues and neurons and between neurons in general which will be of interests of scientist working in various fields and used in further studies to improve health care.

Mucosal pathogenesis and host immunity

Mucosal infections are the most common infections in humans and are often caused by opportunistic fungal and bacterial pathogens. The impact of these infections on global healthcare and economic expenditures is large and of growing concern. For example, *Candida* and *Aspergillus* species are the two most common fungal pathogens of humans, with vaginal candidosis alone causing ~30 million infections/year and oral candidosis causing ~3 million infections/year. In immunocompromised patients (e.g. transplant, cancer and intensive care), *Candida* causes systemic infections with ~40% mortality equating to ~100,000 deaths/year globally, and *Aspergillus* causes ~80,000 deaths/year with ~60% mortality. Systemic *Candida* infections are rising and are now the third most common hospital-acquired bloodstream infection and can be considered as equally deadly as many bacterial infections including gram-negative septicaemias. The project will target host-microbial interactions, predominantly at mucosal surfaces, with one fundamental objective: To identify fungal factors that promote mucosal/invasive infections and the host immune mechanisms that protect against infection.

The mucosal epithelium is of immense importance in host defence and immune surveillance, as it is the initial tissue encountered by the majority of microorganisms. Each mucosal site is unique, retaining the ability to maintain homeostasis and health whilst at the same time enabling the host to discriminate commensal from pathogenic microbes and to initiate protective host defences. In recent years it has become apparent that the mucosal system recognises and protects us from the myriad of microbes, antigens and allergens in ways that are different from the systemic (or whole body) immune system. We aim to determine how the mucosal immune system protects us against both infective and non-infective fungal diseases.

A significant proportion of the studies will be performed using non-animal model systems e.g. we will study the response of epithelial and immune cells (derived either from animals or from human volunteers) in vitro. However, these studies, although providing valuable information, cannot replicate the complexity and function of the whole mucosal immune system. Thus, animals will need to be used for these studies. Also, determining whether vaccines are effective against mucosal diseases such as candidiasis, requires animal models where the environment can be carefully controlled and manipulated so that specific parameters may be examined. We will ensure that we work within the ethos and guidelines of the 3R's.

The mouse has been selected since its immune system is very similar to that of humans, and thus the knowledge gained can be directly transferred. Genetically modified mice may also be used, and these will allow detailed analysis of individual components of the immune response. The mouse has provided a wealth of information of direct relevance to many human diseases and it is envisaged that this will apply to the diseases studied in this project. This will help accelerate the move towards more effective treatment. Typically mice will be immunised against microbial factors and the immune response monitored either by removing small volumes of blood or by sampling mucosal secretions e.g. saliva. In some animals it will be necessary to determine the requirement of a specific microbial gene or protein by challenging the animals with genetically modified microbes or to confirm the effectiveness of the immunisation by challenging the animals with live wild type microbes.

Numbers of animals used will be kept to a minimum by analysis of variation of parameters under study, and power calculations in order to determine the numbers required to achieve statistical significance. Specifically, power calculations are carried out prior to any experimentation being performed. These calculations are generally based upon 95% significance and a Power of at least 80%. Depending on the experiment, for disseminated fungal infection this is achievable with a group size of 4-6 and for oral, vaginal, gut and colitis infections this is achievable with a group size of 6-10. Further statistical advice, where required, is available at King's College London. We estimate that fulfilment of all the objectives will require up to 9500 mice over the entire course of the project.

Mechanisms Regulating Vertebrate Limb Development

Summary

This licence describes a programme of work aimed at increasing our understanding of how normal limb development is regulated and to explain the causes of defects that lead to limb abnormalities in humans. The experimental work will involve the production of animal models of disease with the aim of understanding the biology of limb development and disease and with the longer-term goal of developing clinical strategies to prevent or treat diseases effecting the limb musculoskeletal system.

Background/Scientific unknown

Limb deformities are a common congenital abnormality found in human live births. There are many examples of inherited disorders in which affected individuals carry a mutation in a gene that is required for normal limb development. While in some cases these mutated genes have been identified, little is known about the function of these genes during limb formation. Our goal is to increase understanding of the genetic pathways that regualte limb formation.
Often genes mutated in inherited disorders, as well as many other genes, are also required throughout life for normal limb tissue maintenance and repair. With an increasing proportion of the population surviving into old age, the number of people affected by age-related degenerative diseases of the limbs is increasing steadily. An important clinical objective is therefore to understand the biology of diseases that affect the limbs that will enable the design of therapies to prevent or treat these conditions.

Scientific approach

Under this licence we aim to use and refine genetic methods available in animal models to investigate how genes are functioning in normal limb development and how mutations in these genes can cause limb defects. We plan to investigate the properties of genes normally expressed in the limbs using complementary genetic methods in developing mouse embryos. We plan to address gene function directly in vivo (1) by activating genes in a region of the embryo where they are not normally functioning. These experiments tell us what gene products are capable of doing. (2) by inactivating genes in regions where they are normally functioning. These experiments tell us how a gene is required for normal limb development and can serve as models of human diseases caused by gene mutations.
Benefits
The main benefits that we anticipate are;

1) Increased understanding of the biology of normal limb development and the genesis of limb abnormalities
2) Development of specific animal models of diseases that affect the limbs

Use of animal models

We are taking a multi-facetted strategy to identify and test the properties of genes that are required for normal limb development. The proposed work builds upon and will run concurrently with in vitro experiments and in vivo experiments done using other animal models, including zebrafish, chick and frog. We are also exploiting 3-D imaging methods to phenotype our animal models and develop anatomy computer databases. This resource can be used as a replacement for an experimental animal in some instances and will reduce the number of animals used in future experiments.

To investigate how limb tissues are formed and how these tissues interact during their formation and throughout the life of an organism it is necessary to use the whole animal model. There is currently no alternative as it is currently impossible to simulate these situations with in vitro or computer models.

Scientific advance

A comprehensive understanding of the mechanism controlling normal limb development provides a powerful position from which to understand how mutations in genes can lead to limb abnormalities observed in humans as well as to understand and develop strategies to prevent or treat degenerative disorders that affect the limbs.

Pharmacokinetic studies to support drug discovery

We are working to identify and develop new treatments for severe human atopic dermatitis (AD). To achieve this, we will need to complement our in vitro human cell and tissue based research with mouse models of skin inflammation which exhibit specific features and mechanisms seen in the human disease. Severe atopic dermatitis is not a common disorder, and the milder forms of the disease can usually be managed using topical treatments such as steroids. Patients with severe atopic dermatitis suffer from painful and itchy skin that causes significant discomfort and has a major impact on daily living, including chronic sleep disturbances caused by the itching. The inflamed skin is also susceptible to clinically serious infections. Severe AD can affect children as well as adults. Severe AD patients do not respond to topical steroids and so are treated using immune system suppressing drugs such as cyclosporine, which have use-limiting toxic side effects, or using UV phototherapy which can itself cause burns.

We are identifying novel treatments which will alleviate the symptoms of severe atopic dermatitis but with a much improved safety and tolerability profile. We will induce skin inflammation in mice, as this species has a well understood immune system. We will use models that mirror closely the mechanisms that we believe may be responsible for disease in man, and that cannot be reproduced in vitro, and then try to block the disease using our novel therapeutics. Some strains of mice may spontaneously develop human AD, or we may treat them with allergens such as house dust mite which is a cause of AD in man. The skin (for example the skin on the external part of the ear or the top of the back over the shoulder blades) will become inflamed and the mice may start to scratch the skin. Breaking the skin barrier itself will expose the tissue to the proteins allowing an allergic response to develop. Other models may involve applying chemical agents to the skin which are known to induce AD-like symptoms, or injecting substances which will cause an increase in scratching behaviour. AD disease will be monitored using for example clinical scoring systems, histology and automated video monitoring of scratch behaviour.

We will always seek to use models which induce the least pain and distress in the mice whilst allowing development of a clinically relevant pathology. Pilot studies will be performed to determine the variability of the models and then those data will be used for statistics based group size calculations when looking for treatment effects. We estimate that up to 2000 mice may be used over a 5 year period. Identifying new treatments for severe human severe atopic dermatitis will bring hope to the many patients and their families who currently struggle to cope with this significant unmet medical need.

Pre-clinical imaging

The general aim of this project licence is to use imaging techniques for target validation, lead optimisation and safety assessment, key processes in the discovery and development of new medicines to treat disease in man.

This licence will focus on 5 major therapeutic areas respiratory, neurology, cardiovascular, oncology and metabolic disorders. Other areas may be investigated as new therapeutic targets become available.

At all stages during this programme of work in vitro methods such as cell culture, enzyme and human tissue assays are used prior to conducting imaging studies using animals.

All animals used in this project will be either mice or rats, 2 species that have been used in research and have historically provided meaningful data leading to discovery and development of new medicines for the therapeutic areas this project will focus on. A total of approximately 5750 animals may be required during the 5 years this licence will be in force however there will be continued efforts to minimise these numbers.

Animals will be routinely anaesthetised for imaging and substances administered orally or by injection to induce disease states and also to treat the symptoms of those disease states thereby testing the effectiveness of new experimental medicines. Animals may have cannulae surgically implanted in to a vein to inject substances or take blood samples reducing the need for causing repeated discomfort to the animals. All surgery is conducted using appropriate anaesthesia and analgesia.

The imaging employed is non-invasive and combined with our experience in both imaging and research of new medicines any potential adverse effects will be effectively managed and minimised. A major advantage of non-invasive imaging is the ability to monitor disease and its treatment over time whilst also reducing the numbers of animals required to answer a scientific question.

At the end of studies animals will be been humanely killed and samples of various organs taken for further analysis. All samples taken will help to provide the maximum amount of information from the animals.