Home Office

NON-TECHNICAL SUMMARY

Identification of cellular mechanisms important in responding to, and repairing, joint surface damage in an ovine model.

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Joint, imaging, sheep, repair, osteoarthritis

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

Osteoarthritis (OA), is an untreatable degenerative condition of joints that affects both man and animals and our research group is part of an international effort to understand the causes of the disease and to identify potential treatments. Damage to the surface of joints is known to cause OA. One of the key problems with developing effective treatments for OA is that the specific cellular processes that can respond to damage to the joint surface are unknown. The aim of this project is to indentify and investigate cellular mechanisms that are important in responding to, and repairing, joint damage in a sheep, an animal that has joints similar in size, shape and function to man, so that this information can be used to inform research into human and animal OA. We will also evaluate how proposed treatments for suface defects (for example drugs and cells) affect these cellular processes. As part of this project we will also develop new ways of imaging the cellular processes in the joints during the response to, and repair of, joint surface detects.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

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

Osteoarthritis (OA) is a significant disease of man and animals, causing degeneration of joints, pain and reduced mobility. Studies suggest that up to 50% of adults developing symptomatic knee OA by the age of 85. At the current time, there is no cure for OA – treatment strategies revolve around providing pain relief with severe cases proceeding to joint replacement.

This project will produce a detailed understanding of the cellular mechanisms in the joint during repair and in the development of early osteoarthritis and the response of these cells to a number of treatments. In order to achieve our aim we are developing novel, non-invasive, methods of imaging cells and cellular mechanisms within living animals using labelled cells that will allow us to track cells within joints of sheep during joint damage and repair.

Species and numbers of animals expected to be used

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

Sheep, total of 250 animals over 5 years.

Predicted harms

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

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

All the animals will undergo a short (20 minute) surgery on one of their knee joints. During this surgery a small (less than 1cm) hole will be made in either the joint surface or the meniscus (shock-absorbing structure within the knee). This is done to cause direct (surface hole) and indirect (meniscus hole) damage to the joint surface to enable the cellular responses to this damage to be studied. In about half of the animals, having created the damage, a treatment, such as using 'stem cells' (cells with the capacity to repair tissues) or a 'scaffold' (an artificially created structural support that is compatible with biological tissues) will be applied into the damaged area to investigate how the cells of the joint respond to these therapies.

The surgical procedure will cause the sheep to experience discomfort and pain immediately after surgery – in most cases this is of short duration (2 to 3 days) and this is minimised by pain relief in much that same way as for human patients who undergo joint surgery. A few animals (2%) may experience infections/ wound problems and, if so, the veterinary surgeon in charge of their care will treat them with antibiotics. Also a very few animals (<2%) may experience dislocation of the knee after surgery. This will be confirmed by taking X-rays and immediately repaired.

As part of this work, we want to develop new methods of imaging joints and the cellular response to damage and repair. Therefore approximately 30% of the sheep in this study will also have anaesthetics to allow for non invasive (MRI imaging) and invasive (using a small 'needle' microscope). These animals may have up to 6 anaesthetics over the course of the study, which can last up to 52 weeks, although most studies within this project last between 3 and 6 months.

Animals may also have blood samples taken during the course of the study – this procedure causes no more than transient, mild discomfort. Also approximately 10% of the animals will have cells harvested from their bone marrow anaesthesia that will cause transient, mild discomfort post-surgery. These cells are 'stem cells' that will be injected back into the operated joint in order to assess the response of the cells in the joint to the introduction of this different type of cell.

The expected adverse effect of our studies is lameness. We do not expect lameness to exceed a mild lameness i.e. the animal will stand and walk normally but the lameness is apparent when the animal runs. Regular clinical monitoring, with particular attention to lameness is performed on all our animals. We also measure how much weight is being taken on the operated leg by walking the sheep over a pressure plate before and after surgery (performed at least monthly). In addition, animals will be fitted with individual activity trackers onto collars so that the length of time they spend resting/lying down can be monitored and assessed. All of these methods of monitoring the animals will lead to early detection of lameness. Where lameness is detected, the veterinary surgeon in charge of their care will be informed and a course of treatment begun. If the animal does not respond to treatment or if it gets worse it will be humanely killed.

At the end of the experiments all animals will be humanely killed and tissues taken for further analysis.

Replacement

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

The project seeks to understand the interactions of cells in cartilage and bone with tissues over time and how this controls the repair of defects created directly and indirectly on the surface of joints. These interactions are complex and there are no adequate laboratory system to model this process. We also intend to determine the most suitable strategies for repair, for example using materials such as scaffolds, and the cellular mechanisms that underpin these repair strategies. Identifiying the most suitable strategies for repairing joint surface damage will allow us to progress towards clinical trials in humans. The use of live animals remains the only method to carry out these investigations as the joint/cartilage/body system combination cannot be adequately modelled with cells in a dish in a laboratory.

The two alternatives to work in animals are computer modelling and cell culture systems in the laboratory. Whilst both methods continue to become more sophisticated there is still a huge divide between what they can tell us and the actual events that take place in the body. We do conduct a large number of cell culture systems iin the laboratory prior to conducting any animal experiments. For example we carry out preliminary studies of the interactions between cells including detailed studies of cartilage cells and bone cells to determine what the cells are capable of. We also carry out studies to ensure that the material used to make scaffolds used for repair are not toxic and support the growth of the cells which they will contact following implantation.

Finally, we carefully save all of the cartilage/bone samples taken from the sheep used in experiments and these samples are available for further studies in the laboratory as required. In this way we have built up a significant archived tissue bank of sheep joint tissues that is available to other researchers both in and outside our group.

Reduction

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

We will always seek to use the minimum number of animals necessary to achieve the objectives of this project. Firstly, before beginning animal experiments, we will investigate the interactions between key cells in the laboratory. We will also identify suitable scaffolds and compounds that will affect repair and ensure that they are not toxic to joint tissues in laboratory experiments

We have considerable experience of the response of bone and cartilage in sheep using similar systems and we have conducted statistical analysis to suggest appropriate numbers. We also cross check these with studies published in the wider scientific literature. Our experiments are conducted in a randomized manner. For example we randomise which animals get which procedure/treatment and in what order they undergo procedures. In addition, we ensure that the experimentor is unaware to which group an animal is allocated such that analysis of data so that we do not introduce bias into our experimental interpretation. We use coding systems to anonymise data so that the person working on interpretation of data does not know the background of the data, which can lead to unintentional modification of the results. Indeed, in some instances, we use research workers outside our immediate group to analyse the final results of a study. We believe that these methods contribute to the robustness of our data interpretation.

The design of each experiment will be based on the specific research question in order to determine the experimental groups for comparison and the appropriate time points at which to determine outcome.

Again our previous experience and those of other research groups will be used to inform this process. All experiments will be hypothesis driven with the comparisons to be made and the statistics used determined before the study. Statistical advice has been sought prior to starting these experiments

Refinement

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

The sheep is considered to be one of the most suitable animal species for the evaluation of responses to joint damage and repair. It has joints of a size that are similar to man and the forces in and on the joints are more similar to humans than smaller experimental animals that could be used for these studies. In addition, the healing capability of the sheep articular joint surface is similar to humans i.e. poor. In contrast small animals, particularly rabbits, have excellent healing capacity, likely indicating that the mechanisms that underlie the response of the joint to damage and repair is different to that seen in large animals such as sheep. There are also enough published studies using sheep joints which can be used to improve understanding of the responses seen and to allow good interpretation of the results gained in this study.

We are constantly seeking to refine our use of animals. We have pioneered the use of activity tracker monitoring, using small monitors attached to a collar (which allows us to track, for example, the distance moved and the speed travelled) in sheep used for orthopaedic research. This tracking is done 24/7 and allows us to evaluate this functional behavior of the sheep without the presence of humans. This is particularly important as the presence of humans may influence the way a sheep behaves and potentially influence study results. This tracking can be used both to monitor the welfare of the animals based on movement activity – we will carefully examine and evaluate any animal that is resting more than the others or than it used to before the experiment started.

Other examples of the work we have undertaken to refine procedures include working closely with animal husbandry staff to optimise the regime surrounding fluid and food availability in the run up to an anaesthetic to minimise the risk of regurgitation and associated complications as far as possible. Sheep are animals with 4 compartments to their 'stomach' and this means it can be be very difficult to achieve an empty stomach before an anaesthetic (as is routine for human patients). There is a risk of regurgitation of stomach contents when a sheep is under anaethesia, which can lead to possible development of pneumonia (or death) should fluid be inhaled into lungs, therefore the chances of this happening must be reduced as far as possible, without compromising the welfare of the animal.

Our sheep are handled routinely to ensure that they are familiar with the presence of humans and we train them to walk over the force plate for measuring weight bearing on the operated limb and thus data collection in this regard is minimally stressful for sheep. We also have a ‘tame’ sheep that lives with the experimental group, who reduces the stress amongst the other animals in the presence of humans and is used during weight bearing testing to ‘buddy’ the experimental animal i.e. trained sheep walks over force plate and experimental sheep follows.

Following surgery sheep are housed indoors in purpose built buildings with appropriate feed,bedding and companionship and checked for lameness and ill health at least twice a day. When they are assessed as fit to have more exercise they are turned out into fields. The sheep live for the majority of the experimental period, which is between 3 and 6 months, outside in the fields in one stable group of animals. This allows them to exhibit their natural behaviour.

Amendment October 2020 We are developing methods of tracking labelled cells within joints that are less stressful for the sheep than standard methods such as MRI. MRI requires a general anaesthetic and we are going to use a new imaging method (MSOT) that is done quickly in conscious sheep. MSOT is performed using an ultrasound probe. It provides very sensitive information on inflammation and tissue structure in joints and will allow real-time analysis of labelled cell position within joints.