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NON-TECHNICAL SUMMARY

Understanding endogenous protective mechanisms in osteoarthritis; towards a new approach for disease management

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

osteoarthritis, disease-modifying drug, protective pathway

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

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to establish and utilise a mouse model of knee osteoarthritis (OA). The model will allow us to selectively knockout the gene encoding our protein of interest in the cartilage of adult mice in order to ***define molecular and cellular pathways in joint tissues that protect against the development of OA***. This will help us to understand why some people suffer from symptomatic OA, whereas others do not, and will support our development of a new treatment for OA.

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

Why is it important to undertake this work?

Osteoarthritis (OA) is a serious disease affecting 1 in 3 adults over the age of 45 in the UK. Pain and loss of mobility in joints (such as the knee) prevent people with OA from carrying out daily activities, which has a huge impact on quality of life. In addition, OA is associated with increased risk of heart disease, mental health problems and reduced life expectancy. There are no drugs disease-modifying drugs for OA and more than 160,000 joint replacements are performed each year in the UK to relieve pain and restore function. People with OA have identified a major need for treatments to reduce symptoms and delay joint replacement surgery. Previous research has developed our knowledge of how joint damage occurs in OA, but we don't know why some people develop symptomatic OA whilst others don't. Understanding the inherent molecular pathways that inhibit the OA disease process will pave the way for new treatments for this debilitating condition.

We have identified a protein that is made in joint tissues such as the cartilage, where it has protective effects and reduces inflammation. We are developing a drug for OA that is based on this protein and harnesses its beneficial activities. We have shown that our drug candidate can protect cartilage from damage and reduce pain in rodent models of OA. The focus of this project is to use a gene knockout mouse model to determine how the protein regulates molecular pathways joint tissues, e.g. following the type of injury that can progress to OA in humans. Informative studies on the early stages of OA development cannot be carried out with human tissues, due to a lack of availability of suitable samples.

The proposed work will support the development of our drug candidate, by providing information on its mechanism of action. Together with other ongoing work, where we are using tissue donated from joint replacement surgeries to explore pathways in established OA, this project will also help identify molecular markers to underpin the diagnosis and treatment of OA, including personalised medicine approaches.

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

The research outputs of this project will include:

(1) A mouse model of osteoarthritis (OA) where the gene encoding our protein of interest is selectively knocked out in the cartilage of adult mice.

(2) Data from our mouse model showing how our protein of interest influences the onset and progression of OA, both spontaneously and following surgical destabilisation of the knee joint. These will include imaging data and joint 'damage scores' to demonstrate whether OA develops more quickly and with greater severity in our model compared to controls, data on joint mobility and data on the expression of genes and proteins that are associated with pathways that promote or inhibit the onset and development of OA.

(3) Insights into the mechanisms by which our protein of interest has protective effects in joint tissues and how our drug candidate harnesses these mechanisms in the treatment of OA.

We will publish our findings in open access peer-reviewed journals making them available to the OA-research community.

We will present our findings in the form or posters and oral presentations at conferences.

The project will support our development of a disease-modifying treatment for OA.

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

These outputs have the potential to benefit patients with OA - a very common and debilitating condition - by improving our understanding of the disease process and, ultimately, by supporting the development of a new disease modifying treatment.

The outputs will also be of value to others who are conducting basic and clinical research in OA by providing new insights into the regulation of disease-associated mechanisms.

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

We will maximise the outputs by:

Disseminating the outcomes of our work through conferences, seminars and publications, where we will publish the outcomes of both successful and unsuccessful approaches.

Continued collaboration with others in the OA field - e.g. by making our knock-out mouse model available to other researchers.

Working with our Faculty Media Relations team to disseminate outputs through the media.

Engagement with patients affected by OA and with clinicians to ensure that our work to develop a disease-modifying drug for OA is aligned with their priorities and needs.

Species and numbers of animals expected to be used

- Mice: 4000

Predicted harms

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

Explain why you are using these types of animals and your choice of life stages.

This project will utilise mice as a model of human osteoarthritis.

Use of a mouse model will allow us to carry out genetic modification to generate a cartilage-specific gene knockout in order to specifically explore our protein of interest in the knee joint during the development of OA. The generation of the gene knockout will require a breeding programme, which will use pregnant mice, embryos and all life stages from birth to adult mice.

Cartilage-specific gene knockout will be stimulated in young adult mice at the point when they have a mature musculoskeletal system.

We will use these knockout mice to explore the spontaneous development of OA over a timeframe of up to 18 months.

We will also use the DMM (destabilisation of the medial meniscus) model of knee OA, where surgical destabilisation of the knee joint causes OA to develop over a few months. This model is used by many researchers in the OA field and is well accepted as providing informative data on disease-associated pathways and the effects of therapeutic interventions. For example, we have previously tested our drug candidate in the DMM mouse model.

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

The generation of genetically modified mice will involve natural mating of animals. The mice will be kept in standard conditions for up to 15 months.

To induce cartilage-specific gene knockout, mice will be treated with tamoxifen. This will be administered either orally or by injection.

To induce OA, mice will undergo a precise surgical procedure (under anaesthetic) to destabilise the knee joint. Animals will be humanely killed at a series of endpoints after surgery (up to a maximum of 20-weeks) so that joint tissues can be examined for molecular and cellular changes.

Some mice will be kept for up to 18 months without joint destabilisation surgery and then humanely killed so that joint tissues can be examined for molecular and cellular changes.

Some mice will be treated by a series of injections (up to a maximum of 3 times per week) with our protein drug candidate.

As well as the endpoint examinations of knee joint tissues, the development of OA might also be examined using non-invasive imaging methods, such as x-ray and MRI (no more than once per month), and by observation of the animals' mobility (a maximum of once per week).

Control mice, e.g. without gene knockout or with mock surgery, will be included for comparison in all elements of the project.

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

No adverse effects are expected as a result of the breeding of genetically modified mice.

Short-term treatment with Tamoxifen can result in some weight loss, hair loss and signs of urinary tract infection; these effects will be short-lived.

Following joint destabilisation surgery, mice might experience some inflammation, discomfort and ultimately arthritis in their joint - i.e. a swollen joint and possibly limping. Our previous work in the DMM model has shown that, although we can see changes in the joint tissues at the endpoints of experiments, mice show very little sign of discomfort.

Injection with our drug candidate is not expected to have any adverse effects, based on our previous work.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The breeding programme is a mild procedure. No animals are expected to experience adverse effects.

The effects of tamoxifen treatment may be mild or moderate; this will be minimised by optimising the dose and route of delivery. Most animals will experience some effect.

The effects of DMM surgery may be mild or moderate. All or most animals will be affected.

The spontaneous development of OA in our gene knockout mice may be mild or moderate. We do not yet know the extent to which OA will develop in these mice.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The onset and progression of OA in humans is very variable. We consider that this is due to differences in intrinsic protective pathways within the joint. We have identified a protein that is made in the cartilage

and inhibits OA-associated processes. Defining the molecular mechanisms involved and how they influence joint structure and function will improve our understanding of OA and support our development of a new disease-modifying drug.

The use of a mouse model is essential to achieve our aim. Mice have a musculoskeletal system that is similar to humans and are well established as informative models of OA. Mice are also the ideal species for genetic modification. The generation of a cartilage-specific gene knockout for our protein of interest in young adult mice will allow us to specifically define the effects of this protein within the knee joint.

Mice mature much more rapidly than humans, so we will be able to look at the age-associated development of OA in our model over a relatively short timeframe (up to 18 months). In addition, surgical destabilisation of the knee joint in mice causes rapid onset of OA and is a very well accepted model of post-traumatic OA in humans.

In these mouse models we will be able to use non-invasive methods to assess changes in joint mobility and structure during the study timeframes. At the study endpoints we will also be able to quantify joint damage and determine the expression of genes and proteins implicated in OA. In the joint destabilisation model we will be able to gather data for very early time points (from 6 hours after surgery) through to a maximum of 20 weeks. Evaluation of precisely defined time points, from the point of onset and throughout the progression of OA, could not be achieved using human tissues.

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

We have considered using knee tissue samples from people with late-stage, symptomatic OA that are collected during joint replacement surgery. We have already used these to explore how our protein of interest influences the expression of other proteins known to be associated with cartilage damage and how our drug candidate acts to suppress cartilage breakdown.

We have considered using tissue samples from people who have suffered knee injuries or who have early-stage OA, for this project. We have also considered the use of cell culture models of cartilage, which we have used previously to generate mechanistic data.

Why were they not suitable?

The proposed work requires a model system that provides an accurate representation of the whole knee joint and where we can carry out analyses at precisely defined time points that represent OA onset through to late-stage disease. Human clinical samples are not suitable because they would not allow us to carry out genetic manipulation. Furthermore: (1) the population of patients having knee replacement surgery for symptomatic OA is very variable, (2) there are no good diagnostic markers for early OA, so it is impossible to identify patients at specific stages of the disease, and (3) patients are seen by clinical teams at very variable times following joint injuries. Therefore, it is not possible to carry out an informative study using human samples.

Cell culture models could provide the option of genetic manipulation to knockout the protein encoding our gene of interest. However, OA is a disease of the whole joint and disease progression depends on the mechanical loading of the joint, which can only be achieved in a live animal model.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated that up to 4000 mice will be involved in our breeding programme that will generate the genetically modified mice to be used for cartilage-specific knockout of our gene of interest. This is based on: (1) the number of experiments we plan to use these mice for, and (2) the conservative assumption that each litter of mice we breed will include one mouse with the correct genotype.

We have estimated that up to 1000 of the mice that we generate in our breeding programme will be treated with the drug Tamoxifen to stimulate cartilage-specific gene knockout. We will use up to 400 of these mice for our studies on the spontaneous development of OA. In addition, we estimate that up to 450 of the mice we breed will be used in the DMM surgical model of OA; this will include up to 50 mice as sham-operated controls. This is based on: (1) the different experimental time points we will use (in order to explore the effects of our protein of interest throughout the early stages of OA development), and (2) previous work by ourselves and collaborators using the DMM model, which suggests that each experimental group will need to include 10-12 mice in order to generate informative data. Approximately 50 control mice that have not been genetically modified will be used to generate baseline data in the DMM model of surgically-induced OA.

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

We have used the outcomes of work in our own and collaborators labs and taken the advice of a statistician to reduce the number of mice being used in each experimental group. We will use pilot studies to further adjust group sizes as the study progresses.

By using both male and female mice in the spontaneous OA and DMM-induced OA studies we will reduce the numbers of genetically modified mice that we need to breed. Furthermore, generating data in both male and female animals is important with regard to the translation of our findings towards human clinical studies, where OA is common in both women and men.

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

We will continually review the outcomes of experiments and use our data to optimise animal numbers as the project progresses. For example, we will conduct pilot studies to optimise the protocol for tamoxifen induction of cartilage-specific gene knockout and to determine the group sizes required in the OA model studies.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

This project will use a genetically modified mouse model to allow selective knockout of a gene in the cartilage of young adult mice. This model will be used to explore the effects of the gene deletion on the onset and progression of knee osteoarthritis (OA). We will determine whether OA develops spontaneously in the knockout mice over time (up to 18 months); in this case we anticipate that any OA-like symptoms will be mild and slowly progressing.

We will also initiate OA by surgical destabilisation of the knee joint in the DMM (destabilisation of the medial meniscus) mouse model. This is a very well characterised model, which we have used previously. We have observed that whilst mice develop OA-like damage to their cartilage, they exhibit little or no evidence of discomfort/pain over the timeframe of our proposed experiments.

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

OA is a complex disease that involves the whole joint and is influenced by factors including joint mechanics as well as cellular and molecular processes. In order to explore the onset and development of human OA we need to use a live animal model with a mature musculoskeletal system that is similar to an adult human. The adult mouse fulfils this requirement. In addition there are well-established methods for genetic modification and for surgical induction of OA in mice - both of which are essential for the proposed project.

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

The major welfare cost to the mice in this study is the development of OA-like symptoms. Since the purpose of this project is to study OA in the mouse knee joint, this welfare cost cannot be changed.

With regard to the Tamoxifen induction of cartilage-specific gene knockout in our mouse model, we will carry out pilot studies to determine the least harmful way of administering Tamoxifen, whilst still ensuring effective knockout (determined by genotyping). We will preferably use an oral method of administration. Tamoxifen treatment can cause some weight loss and signs of general malaise such as loss of appetite, hunched posture and piloerection, but these are typically transient.

With regard to the DMM surgical model of OA, we will continually monitor our procedures for pre- and post-operative care to ensure that these are optimised. For example, using sterile techniques to prevent infection at surgical sites and making adjustments to food, bedding and enrichment and using post-operative analgesics if required. In addition we will carry out pilot studies and order our experiments so

that the data we gather can be used to refine the numbers of animals being used at each stage, i.e. to ensure that all experiments are informative, but without using more animals than necessary.

As well as collecting data on joint damage and gene expression profiles at the end of each experiment, we will use non-invasive methods (such as imaging and observation of animals' mobility) during the course of each experiment so as to maximise the information we obtain, but without any additional welfare costs to the mice.

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

We will use published documents recommended by NC3Rs to ensure that we are using the most refined approaches in all our experiments. With regard to DMM model of OA, we will regularly review the scientific literature for work by other researchers in the field to identify opportunities for refinement.

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

We are registered with NC3Rs and subscribe to their monthly newsletters, which will keep us informed regarding advances in the 3Rs. We will utilise the NCRs Experimental Design Assistant as well as seeking guidance from our local NVS and NACWO to inform the implementation of any advances that can be applied to our project.