Home Office

NON-TECHNICAL SUMMARY

Musculoskeletal pain: mechanisms and treatment

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

Bone, Fractures, Pain behaviours, osteoarthritis, osteoporosis

Animal types	Life stages
Mice	adult, aged, juvenile

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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?

Long-term skeletal pain is the most common complication of musculoskeletal disorders. We need to better understand the mechanisms involved in chronic skeletal pain to develop analgesics directed towards more restricted pathways involved in bone pain and this is the aim of this project.

A retrospective assessment of these aims will be due by 05 May 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

Despite a huge increase in the burden of skeletal pain, there are to date no analgesics that target specifically this pain which impacts on the quality of life of millions of patients worldwide - and can eventually require orthopaedic joint replacement.

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

This research will first increase our scientific knowledge of the mechanisms of pain after osteoarthritis (OA) and fractures. This will lead to research publications, presentation of data at seminars, national and international conferences. We will present our data at targeted conferences which have clinical streams in order to facilitate interactions with clinical colleagues working on skeletal pain. We also hope that answers to our questions may provide indications for the use of new therapies targeting pain in musculoskeletal diseases. This proposal is also ideal for PhD training involving both in vitro and in vivo experiments and a large range of imaging, histological, molecular and pain methodologies.

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

The results from this project will be of interest to a broad range of scientific communities including skeletal biologists, neuroscientists and orthopaedic surgeons. This research is also of major interest for wider audiences concerned by fracture pain, OA pain and joint replacement surgery.

Due to the development of more standardized small rodent models of fracture repair, most research studies now use animal models to assess factors and treatments that affect fracture healing. Because pain is rarely reported in these studies, we hope that our studies investigating pain in these models will raise attention to the scientists performing fracture surgeries on rodents and help them to better choose post-operative analgesics. The development of tools to evaluate spontaneous and evoked behaviours which are indicative of pain in rodents, have rapidly expanded to classify the type of pain and quantify it to improve animal welfare. Those animal studies are transposable to humans and have clinical relevance in patients with musculoskeletal pain. This proposal will also look at treatments of skeletal pain, which has huge implications on animal welfare.

In the longer term, we hope that the results of this research project may be able to guide the selection of drugs that reduce skeletal pain. This may lead to clinical trials examining the advantage of targeting specific signalling pathways for decreasing skeletal pain and this will benefit animals and millions of patients suffering from musculoskeletal diseases.

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

Important findings will be shared with the scientific community through publications, seminars, webinars and newsletters. We will make sure to publish unsuccessful results too as these are important to the bone research community and avoid repetition of experiments. The applicant is part of an EU training network and outputs from this project will be shared with Europe-wide bone research networks and platforms. This project will benefit from this network though extensive collaborations, share of innovative models, specific topic conferences, training courses and invited speakers as well as contacts with the industry for drug developments. We have used results from previous studies performed using the same models to improve the design of our experiments and inform the development of our new scoresheet. Similarly, findings of those studies will help us to refine them.

Species and numbers of animals expected to be used

- Mice: We estimate that we will use less than 1200 mice in total.

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.

Rodents are appropriate species because their fundamental skeletal biology is very similar to humans. Mouse models in particular have become of increasing interest for skeletal research because their life expectancy is only around 2 years, studying skeletal diseases associated with ageing such as osteoporosis and osteoarthritis is easier as these diseases develop earlier and the effect of treatments can be seen in a shorter time period. Also, a broad spectrum of antibodies and gene-targeted mice are available. We generally use young adult mice which are not growing anymore (12 to 13 weeks) to perform our studies. However, because fractures, osteoporosis and osteoarthritis are higher in the

elderly population, in some conditions we may want to age mice until 40 to 60 weeks of age. These ages correspond to adult mature mice which are past development but not yet affected by senescence and do not show adverse effects in relation to ageing. Nevertheless, these adult mature mice show bone loss as it starts from 3 months of age and this is interesting for our project. In most of our studies, animal will be kept for 6 weeks after surgery. No animal will be kept until more than 66 weeks of age.

In this project we will use mainly female mice (more than 70%) as osteoarthritis and osteoporosis occur mainly in females. However, STR/ort mice develop degenerative changes of the knee joints resembling human OA, with the males being more severely affected than the females, so we will also use male STR/ort mice. We may also want to compare pain behaviors in males and females, so we may use male mice in all our protocols.

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

One typical experiment would use around 40 female mice as our statistical power analysis requires us to use around 10 mice per group. We usually have 4 groups, one sham group, one with surgery (fracture or removal of the ovaries to induce osteoporosis) or submitted to mechanical loading of the joint to induce osteoarthritis, one group with surgery plus treatment and one sham group plus treatment. These treatments could be novel analgesics, drugs that target ionic channels, neurotrophins and bone resorption. They can be injected (from daily to one or two injections in 6 weeks) or administered in the food or by oral gavage. Mice are usually kept for 6 weeks after surgery, the maximum being 12 weeks after surgery. Pain-like behaviors are measured at baseline before surgery and weekly from one week after surgery. They include measurements of spontaneous pain such as nesting, burrowing, locomotion, general activity but also responses to stimuli such as pressure, heat and cold that measure mechanical and thermal sensitivities. In addition, when surgery is performed in one limb but not in the other one, it is possible to measure the weight put on each limb. Blood can be collected every two weeks. Mice are usually euthanised 6 weeks after surgery.

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

All the models described in this project are widely used by ourselves and other bone researchers in the world. All mice in this project will receive appropriate pain killers after surgery and post-operative care. Because this project involves measuring pain after bone loss and fractures, pain -like behaviors in our project will be only measured 7 days after surgery to make sure that mice are receiving adequate pain relief after surgery. In case of removal of ovaries (ovariectomy) and sectioning the sciatic nerve (neurectomy) which are two surgical models that induce the loss of bone, analgesics are given for a minimum of 3 days post-surgery. In case of fractures, our previous data have shown moderate pain after fracture surgery which is maintained during the process of bone repair which lasts several weeks. We would like to study this chronic pain and assess the effects of new analgesics targeted more specifically at bone pain. Because pain after fracture surgery starts to decrease one week after surgery, analgesics will be given for 7 days after fracture surgery.

The possible adverse effects of surgery models (removal of ovaries, sectioning the nerve and fractures) are pain, wound infection (<1%) and risks of bleeding (1%). Wound infection and risk of bleeding are minimised by careful aseptic surgery. The animal can be expected to be at risk of mild to moderate degrees of spontaneous pain from the time of surgery to the terminal phase of the experiment. Pain will be indicated by reduced appetite and activity, poor demeanour and grimace observation.

There are specific adverse effects associated with fractures. Long lasting moderate pain could be present. Lameness is expected postoperatively but by day 2 animals should be ambulating freely with occasional limping and normal grooming. Weight bearing will be reduced but will increase daily and is expected to return to 70% of preoperative condition by 7 days postoperatively. Pin stability will be checked during surgery checkpoints.

The mechanical loading procedure (usually less than twenty five minutes) may also induce a temporary lameness, typically for no more than 30 minutes after the procedure has finished. Mild or moderate pain may also be recorded as indicated by in-appetence, lethargy, poor demeanour and grimace observation.

Occasionally, aging animals will be used (less than 5%) to mimic the loss of bone with age. Mice lose bone mass at 16-25 months. We wouldn't expect to see any associated harmful phenotype at these ages in our strains. Age-related conditions such as weight loss, lameness and respiratory difficulties will be carefully monitored for in these ageing animals.

Administration of substances by injection should only cause transient discomfort. Animals will be observed during the period immediately post-administration for any unacceptable signs of discomfort or distress. After gavage, animals will be observed for signs of stress and bronchial administration (<1%).

Some of the compounds injected may alter an animal's metabolism but should have no adverse effects on the welfare of the experimental animals. Animals will be observed during the period immediately post-administration for any unacceptable signs of discomfort or distress (such as decreased food and water consumption, rapid breathing, weight loss, hiding, self-mutilation, abnormal posture and appearance).

General anaesthesia: Anaesthetic complications are most uncommon (<1%) and will be minimised by correct dosing of injectable or inhalation anaesthetics, by accurate weighing and by good maintenance of body temperature. Post anaesthetic animals will be monitored to ensure they make a swift recovery and may have additional husbandry measures put in place such as additional warming, wet mash or high calorie diets placed on the cage floor.

The behavioral tests provoke very mild degrees of pain that do not persist after testing.

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)?

Our mouse models are mainly surgical (fractures, removal of ovaries, section of the sciatic nerve) or involve mechanical loading of the bone and joints and have been previously used and validated. All fall in the overall moderate severity level except for fractures that fall under a severe protocol but this will represent no more than 15% of our mice. Some animals (50%) on moderate protocols may experience moderate suffering that resolves after a few weeks, usually around 2 to 3 weeks. Animals under severe protocol are expected to suffer moderate pain that is maintained for several weeks. We use appropriate protocols and end points to manage pain and infection. To stop infection, sterile surgeries will be performed and we will use antibiotics if necessary. Pain is relieved by pain killers administered before and after surgeries and other procedures.

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

- Killed

A retrospective assessment of these predicted harms will be due by 05 May 2027

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

Our project investigates the mechanisms of bone and cartilage repair and of skeletal pain and requires the use of several animal models as these complex physiological systems cannot be effectively modelled in vitro. The integrated physiological environment of the living animal is indeed still essential to elucidate the pathophysiological mechanisms of skeletal conditions and of their associated pain. In this project, we will use well-established models of osteoporosis (ovariectomised mice), osteoarthritis (Str/ort mice that develop spontaneous OA, mechanical loading of joints) and fractures (osteotomies and bone defects). To investigate pain in these models, we use a set of behaviours both spontaneous and evoked.

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

Many aspects of our work are however achieved through the use of in vitro cell culture (bone formation and resorption assays, chondrocytes assays, culture of dorsal root ganglia) and ex vivo imaging together with molecular analyses.

We also started clinical studies in human patients to correlate fracture pain with biomarkers measured in serum of these patients. These markers are also quantified in mouse serum after osteotomy.

Why were they not suitable?

There are suitable as these in vitro techniques allow identification of the most likely treatments to be validated in vivo and this replacement reduces the number of animals used. The clinical studies allow us to translate some of our findings in animal models. The integrated physiological environment of the living animal is however still essential to study skeletal pain. This is because pain involves both the peripheral and central nervous systems and this is impossible to model it in vitro. There are no molecular readouts of pain that are appropriate to assess the effects of novel analgesics on pain.

A retrospective assessment of replacement will be due by 05 May 2027

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 often design our protocols in such a way that we have 4 groups, a Sham group, a disease group (surgery or application of mechanical loading) a sham group treated with a drug and a disease group treated with the same drug. Our experiments therefore often involve 40 mice as we usually have groups of 10 mice.

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

We always aim to reduce the numbers of animals we use. We adhere to PREPARE and ARRIVE guidelines to design our experiments and report our data. In this project, most of our experimental models have been previously validated and used and do not require pilot studies. However, pilot studies may be necessary to estimate the effects of drugs.

Fractures and Sham surgery are performed on one leg, allowing the use of the contralateral leg as a control when possible. This is also the case for mice submitted to mechanical loading of the bone which is performed on one leg only. Sham surgery includes the same surgical procedures as the fracture group except that there is no fixator placed and no generation of fractures. This allows to control for post-surgical pain unrelated to fractures. This is also the case when evaluating pain after removal of the ovaries and neurectomy.

In most of our studies, our main end point is the assessment of pain and the use of Sham animals is essential to differentiate between the pain induced by surgery and skeletal pain. However, in some experiments we will only assess the bone loss induced by hormonal and mechanical changes, nerve section and bone diseases. in these cases, non-operated animals can be used to minimize the number of animals used in sham surgery groups. In addition, if we repeat long series of similar experiments, we may be able to use information from historical controls from previous experiments to reduce our number of Sham controls.

We also aim to refine our power calculations after a series of similar experiments to make sure that our number of Sham mice is reduced to its minimum.

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

For completely new procedures, a pilot group (including only 4-5 rodents) will be used to determine the outcomes before generating all the data. All tissues that are potentially interesting for our research are collected from one single experiment in case we need them in the future.

A retrospective assessment of reduction will be due by 05 May 2027

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

In this project, we intend to use only mice and predominantly females as all our skeletal diseases models and our joint loading models were developed in this species.

The mouse is an appropriate species because its fundamental bone biology is very similar to humans, and, as mentioned previously, holds many advantages in terms of pathology, timescale for disease progression, space requirements and biological tools available. All the models described in this project are widely used by ourselves and other bone researchers in the world and have proven to be very useful for the advancement of clinical management and the prevention of musculoskeletal diseases. Our research aims at investigating pain associated with these musculoskeletal diseases and mouse behavioural models have been important tools to increase our understanding of the mechanisms of pain as well as identifying novel analgesics. Despite intending to assess pain behaviours during the progression of skeletal diseases and fractures, our strategy aims to minimise the animal suffering and this would be also further lessened by the use of appropriate analgesia protocols, where possible, for example after surgeries. However, we will not be able to use analgesics during all the duration of our studies as we want to assess pain-associated with bone loss and bone repair. Novel analgesics will however be tested in our models. Our models of skeletal diseases and fractures are mainly surgical and have been previously used and validated. These fall in the overall moderate severity level, except for fractures where long lasting moderate pain can occur. Our models were designed not to produce excessive trauma or suffering. For example, our model of osteoarthritis (OA) is non-invasive compared to other models of OA which are surgical. Our fracture model is the most invasive but we will minimise its use.

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

Clinical skeletal pain is a perception that is influenced by psychological and experiential factors but, despite this, considerable progress has been made in modelling and quantifying nociception in animals, particularly in mice. Unlike the clinical setting where patients can verbalise their pain level, preclinical studies in animals rely upon expression of nociceptive behaviours to assess pain levels. Consistent animal behaviours in response to noxious stimuli are referred to as pain behaviours and have been extensively used in mouse studies. Skeletal diseases occur in the ageing population and this can’t be mimicked in younger mice. We also mainly use female mice to reflect the clinical situation where osteoporosis and fragility fractures occur in women.

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

Animal suffering will be limited in our studies by our strict monitoring of actual severity limits. We provide a number of supportive measures for an effective pain management regimen: Peri-operative analgesics will be administered for at least 7 days after fracture surgeries. Animals will be monitored for pain and will be given analgesia until they begin weight bearing on the fractured limb and are able to ambulate freely. Stress-relieving environment will be provided (eg nesting material, enrichment). Nesting material has the potential to improve mice well-being. Cages will be filled with soft bedding. Easy to reach and soft palatable food and water will be provided.

To minimise variability and the number of animals used, most studies will only be performed in mice of the same sex, age and strain to limit variability in pain behaviours and bone healing. The same surgeon will perform all surgeries. Mice are habituated to pain measurements one week before the procedure and pain measurements are performed before surgery to measure pain baseline.

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

We adhere to ARRIVE guidelines for designing and conducting our experiments and LASA guidelines for substance administration. We also follow NC3Rs best practices for example for blood sampling. We attend training offered by our institution, the Home Office, UK academic institutions and EU collaborators working on bone pain.

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

We will attend 3Rs webinars and seminars as well as other training that is available to make sure that we stay informed. The NC3Rs website is a good source of information on 3Rs development and we will regularly consult it.

A retrospective assessment of refinement will be due by 05 May 2027

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?