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NON-TECHNICAL SUMMARY

Sprayed slow-release analgesic for application during abdominal surgery

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Vesicles, Hydrogel, Analgesic, Peritoneum, Abrasion

Animal types	Life stages
Mice	adult

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 main objective of this project is to investigate the safety and efficacy of a single-dose painkiller (analgesic) formulation for its use during abdominal surgery, avoiding significant side effects of commonly used analgesics.

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?

Surgery on the abdomen is painful. It is usually treated by various painkillers (analgesics), which can cause secondary problems (drowsiness, constipation, poor mobility), leading to a slow recovery. Local painkillers can provide short-term pain control at wound sites but do not target the area of surgery within the abdomen. There is a need for better strategies for controlling pain within the abdominal cavity. Therefore, we will develop a long-acting pain relief spray that remains active at the surgical site in the abdomen. Such a spray should be administered as a single dose directly onto the surgical site and provide continuous pain relief for 5 days.

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

Our spray analgesic will provide constant pain relief during the whole recovery period following abdominal surgery. This technology can be adapted to any open surgical procedure or site of trauma and adapted for the slow release of other drugs, i.e., chemotherapy. Outputs from the research will add to the general knowledge about alternative methods for providing safe and effective pain relief medication; this information will be published in academic journals to improve scientific knowledge.

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

The primary beneficiaries from the outputs will be patients undergoing laparoscopic surgery for benign and malignant abdominal conditions. Our sprayed formulation will provide constant pain relief during their hospital stay leading to quicker recovery, fewer complications, and faster return to normal function. Secondary beneficiaries will be healthcare providers. It is estimated that our pain relief medication will help reduce the length of time that patients spend in the hospital from 5 to 3 days, resulting in cost savings of ~£1,300/patient or ~£1.7billion/year to the NHS.

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

We will disseminate the findings to relevant forums through presentations to surgical and pain associations in the UK and publish the results in peer-reviewed journals. The group will undertake various outreach activities to ensure that the general public is aware of the research. This will include presentations to “Pint of Science” and “Be Curious” events organised through the local research institution. To ensure lay people easily understand the relevance of the work, we will use modern forms of communication, such as Visual Abstracts. Engagement with public and patients will be central to the design and conduct of the research to ensure that it remains directly relevant to end-users.

Species and numbers of animals expected to be used

- Mice: Optimisation experiments will be performed on 40 C57BL/6 mice to determine the sample size for the definitive study. The final number of animals for the definitive study is unlikely to exceed 200 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.

Adult mice are a good choice for animal experiments because they have many similarities to humans in terms of anatomy and physiological responses. Less sentient animals cannot be used because they would not help us to assess the pain relief capabilities of the formulation.

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

The animals will go through a simulation of abdominal surgery. Animals will undergo laparotomy (opening of the abdomen) under general anaesthesia and a 1cm2 abrasion made to the peritoneum to simulate surgical trauma. Half the animals will have the analgesic formulation applied to the traumatised area; in the other half the area will be left untreated. The abdominal wound will be closed and the animal allowed to recover. Animals will be monitored to ensure their safe recovery and to assess their pain sensations with a validated scoring system. All animals will be euthanized at the end of the 5 day observation period to obtain tissues and fluid samples for further analysis.

A small number of animals in the optimisation experiments will undergo application of gel carrier alone (without painkiller) to confirm that application of the gel alone does not influence postoperative pain. This would be most unlikely given that the gel is a biologically inert material. Inclusion of a gel carrier arm has not been added to the main study as it is unlikely to add any meaningful information and would only serve to unnecessarily increase the sample size of the experiment.

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

Possible adverse effects include the failure to provide pain reduction, a bacterial surgical site infection, wound infection and dehiscence, and organ dysfunction resulting from surgery. We will closely monitor

for adverse effects and trained personnel will take immediate action to avoid unnecessary harm to the animals. A validated postoperative assessment scoring system will be used to grade the severity of complications and determine a humane end-point for euthanasia (described in the stop/go section). All the animals will be humanely sacrificed at day 5 to obtain different samples from fluids and organs for further analysis.

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 proposed methodology is of moderate severity because it involves abdominal surgery. We will require 40 mice for initial optimisation experiments to determine the sample size for a definitive experiment. It is not expected that the final number of animals for the definitive study will exceed 200 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?

It is only possible to test the safety and efficacy of our formulation in a living biological model which experiences pain. We have previously assessed some inflammation (pain) markers associated with wound healing in the laboratory using cells. These experiments have shown promising results without any bystander toxicity. We need to verify that the results obtained are reproduced in a biological model.

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

We have previously undertaken in vitro optimisation studies using human cells to detect different inflammation (pain) markers. There is nothing further we can do in vitro to advance our technology. We have considered the option of using less sentient animals, but these will not allow us to properly assess our primary end-point – pain reduction.

Why were they not suitable?

Human cell lines can only provide surrogate markers of pain control, i.e., reduced inflammatory mediators. While this can be considered a good indicator of pain reduction, it cannot be fully verified because pain is a complex sensation in biological systems. An animal model is now needed to understand the safety and efficacy of the formulation.

Reduction

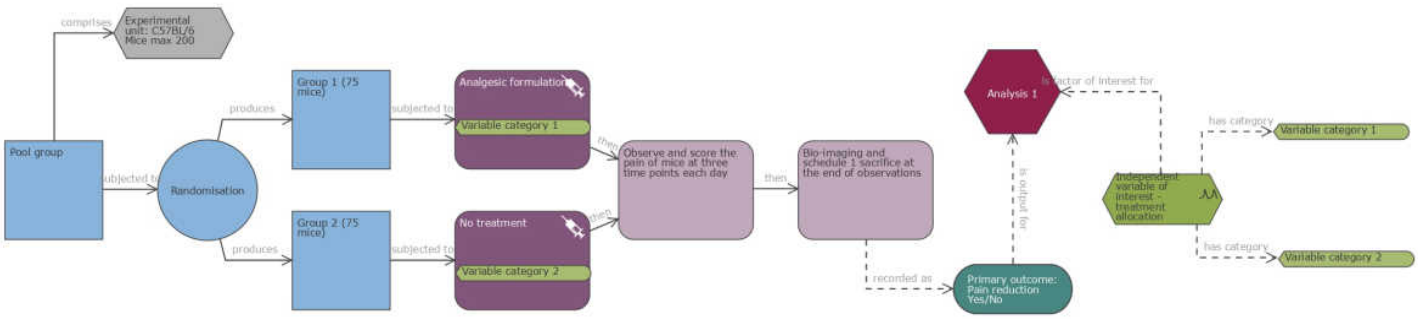
Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may 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 discussed the design of our experiments with biostatisticians to obtain reliable and reproducible data with the fewest possible number of animals. We will first perform optimisation experiments in 40 animals, which will inform the sample size for a definitive study. Based on a 30% reduction in Grimace score on postoperative day 1 or 2 in favour of the intervention group we anticipate a total sample size of approx. 150 animals (75 intervention; 75 control) to demonstrate a statistical difference at 80 power and 5% significance level.

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

We utilised the NC3Rs experimental design assistant to guide the numbers needed to demonstrate the safety and efficacy of the novel sprayed analgesic formulation in an abrasion mouse model. We have adhered to the 3Rs principles, and up-to-date ARRIVE guidelines to design the experiment. The ideal study design to evaluate efficacy is a randomised controlled trial. In line with the 3Rs of animal research, there will be two groups, the analgesic formulation alone and no treatment, to keep the numbers of mice to a minimum.



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

Training for the model will be performed on approximately 6 schedule 1 mice. Optimisation studies using 40 mice (20 intervention group; 20 control group) will establish the required number of animals for the definitive study. This will include a small number of animals to confirm that the gel carrier does not influence postoperative pain scores. The ideal study design to evaluate efficacy is a randomised controlled trial. In line with the 3Rs of animal research, there will be two main groups, the analgesic

formulation and no treatment, to keep the number of mice to a minimum. Randomisation & Blinding: Mice will be randomised using block randomisation through SealedEnvelopeTM at a 1:1 allocation ratio. All mice and samples will be labelled such that researchers assessing the effects of the treatment and analysing the results will be blinded.

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.

Mice have been chosen for the experiment because they are ideal animal models for biomedical research and comparative medicine. They have many similarities to humans in terms of anatomy and physiology. We will use an abrasion model for the experiments adapted from Brodsky et al. and Kraemer et al. This model allows us to simulate the trauma of abdominal surgery without causing harm to vital organs. It is believed this model provides the least pain, suffering, distress, and lasting damage to the fewest number of animals, whilst still enabling us to adequately assess pain reduction associated with our formulation.

Brodsky, J. A., Brody, F. J., Endlich, B., Armstrong, D. A., Ponsky, J. L., & Hamilton, I. A. (2002). MCP-1 is highly expressed in peritoneum following midline laparotomy with peritoneal abrasion in a murine model. Surgical Endoscopy, 16(7), 1079–1082. doi:10.1007/s00464-001-8335-z

Kraemer, B., Wallwiener, C., Rajab, T. K., Brochhausen, C., Wallwiener, M., & Rothmund, R. (2014). Standardised Models for Inducing Experimental Peritoneal Adhesions in Female Rats. BioMed Research International, 2014, 1–8. doi:10.1155/2014/435056

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

Less sentient animals would not help us assess the pain relief capabilities of our analgesic formulation due to their lack of nervous system. If the mice were terminally anaesthetised, we would not be able to measure the healing effects of the intervention at the required post-operative stage.

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

Animals will be reviewed regularly by the research team and staff from the Animal Facility to ensure animals do not experience excessive pain. A validated pain scoring system will be used - the Grimace Score. Oral analgesia (buprenorphine) will be given to animals, in both the intervention and control groups, with a Grimace Score of >/= to 3 out of 10 and the total daily analgesic requirement recorded. General animal well-being will be monitored using the validated Abdominal Surgery Post-operative

Severity Assessment. At the first sign of a point of no return (>20 points on the scoring system), the animals will undergo schedule 1 killing.

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

NC3Rs & ARRIVE Guidelines and guidelines local to the research institution will be followed.

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

We have reviewed the NC3Rs website regularly to ensure that our practice is up to date, for example, the recent change to the ARRIVE guideline recommendations (du Sert et al., 2020). We are also on the local animal housing mailing lists that distribute urgent updates that are reviewed regularly.

du Sert, N. P., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., … Würbel, H. (2020). Reporting animal research: Explanation and elaboration for the arrive guidelines 2.0. PLoS Biology (Vol. 18).