Home Office

NON-TECHNICAL SUMMARY

Understanding serosal repair and internal scarring

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

scarring, therapy, abdomen, inflammation, repair

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?

To discover new medical treatments to prevent the formation of internal scars, or 'adhesions'. We will work out how cells inside the abdomen form these internal scars after an operation and/or infection and then use this knowledge to block the process.

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?

Adhesions are bands of scar tissue that join organs to each other and/or the inner body wall and are a huge problem with extensive health care costs globally. It is proposed that adhesions form in one out of every ten people who have open abdominal or pelvic surgery and incidence of small bowel obstruction due to adhesions post-surgery is 2%. The Surgical and Clinical Adhesions Research (SCAR) group found that 3·5% of patients in Scotland who underwent open abdominal or pelvic surgery were readmitted within 5 years of surgery for disorders directly related to adhesions, 17·6% for disorders possibly related to adhesions, and 13·1% for operations potentially complicated by adhesions. The cumulative, year-on-year, direct costs of adhesion-related re-admissions due to lower abdominal surgery in the UK are estimated to be over £787 million over a 10-year period. One US study reporting adhesion-related complications resulted in over 300,000 hospitalizations and almost 850,000 in-patient day admissions with a total cost of $1.3 billion (over £1 billion) in one year. Furthermore, in Finland, the annual direct hospital cost for postoperative adhesion-related intestinal obstruction was estimated as over £2million. Surgery, infection and ongoing inflammation are the main drivers of internal scar formation. Adhesions can result in severe abdominal pain and life-threatening bowel obstruction, as well as causing infertility in women. Unfortunately, there is no treatment once formed apart from further surgery, and present approaches to prevent their formation, such as degradable films and gel barriers used at the time of initial surgery, show limited benefit. Hence, there is an urgent need to develop better therapeutic ways to prevent internal scarring, thus minimising the considerable harm and financial burden associated with this condition.

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

This project will provide new ideas for better preventative therapies for internal scars and work out the best to deliver these therapies into the abdomen. Our findings will be published in appropriate scientific journals, discussed at surgical and basic science conferences and presented at science festivals so that new information about adhesions and their prevention is widely circulated to clinicians, scientists,

industry and importantly the public. In addition, we will share tissue samples, relevant data and reagents with others to progress new discoveries in the field of adhesions.

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

In the short-term, we will provide important information about how the abdomen responds to injury and why adhesions form which other scientists can use to modify and extend their own studies. Researchers with an interest in injury and inflammation, infection, endometriosis, peritoneal dialysis and cancer, will benefit from our results. In the longer-term, we will have identified biological targets that could be blocked with drugs and new ways to deliver them to prevent adhesions forming in patients. By working with surgeons and industry colleagues, our findings will help to develop new therapies to reduce scar tissue forming in patients having abdominal surgery, with infection or persistent inflammatory disease. Of importance, our discoveries will also be relevant to animals. Horses, in particular, suffer from adhesions which may cause their intestines to become twisted, and this requires immediate surgery. Therefore, these new therapies will also be of benefit to vets.

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

We collaborate with a number of research groups nationally and globally and present our findings at scientific conferences. Results will be published in relevant open access scientific journals and datasets and tissue banks made available so used by other academic beneficiaries for further data mining and as basis for future studies. Our findings will be of interest to industry and as such, new collaborations will be made in order to move from lab to clinic. In addition, colleagues from the surgical and veterinary community are closely involved in our research and so any new discoveries will be conveyed to a wide audience of end users. Importantly, we participate in public engagement events including science festivals and patient engagement forums to educate, inform and answer questions about our research.

Species and numbers of animals expected to be used

- Mice: 1000

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.

We will use adult mice as a part of our research studies as they have similar abdominal anatomy and respond to infection and surgical injury in a similar way to humans by forming adhesions. To better understand the processes involved, we will also use mice that have been genetically altered as this will help us highlight the cells and molecules to target to prevent adhesion formation.

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

Some mice will have an injection of factors into the abdomen that increases inflammation. These factors may include parts of micro-organisms (eg. bacteria and fungi) found in the bowel or chemical irritants present in peritoneal dialysis fluid and known to cause inflammation with internal scarring and possible, adhesion formation. To induce inflammation, mice will receive up to 7 injections of these inflammation-triggering agents into the abdominal cavity using published standard routes and doses. Mice will experience mild, transient pain but should experience no lasting harm from administration of these substances.

Some mice with have abdominal surgery with injury to the bowel and/or inner body wall and introduction of sutures to stitch tissues. In some instances, mice may have abdominal surgery and an injection of inflammation-triggering agent to understand how adhesions may form in a contaminated abdomen. Following surgery, mice will experience some discomfort and mild to moderate pain which will be managed with pain-killers. Animals will be monitored closely and should fully recover after 2-3 days.

Groups of mice that have undergone a procedure may also receive therapeutic substances delivered in a gel or solution to identify key processes involved in adhesion formation. On some occasions, genetically altered mice will be used where a gene of interest will be switched on or off to work out its role in adhesion development. To control the behaviour of the particular gene of interest some animals will be given substances by mouth, injection, or through food, that can target the particular gene and switch it on or off.

Animals will be humanely killed at the end of the study by schedule 1 method. Experiments will typically last for a week and no longer than 28 days as our previous data has found after an inflammatory episode, adhesions remodel over time and are stable by 28 days. The minimum number of procedures a mouse will receive is one and the maximum is four.

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

The mice undergoing abdominal surgery are likely to experience post-operative pain which will be controlled by appropriate anaesthesia and pain-killers and should last no more than 2-3 days. Mice are also expected to experience some weight loss (generally between 10- 15%) after surgical procedures, and this should return to pre-operative weight within approximately 5 days. Intra-peritoneal injection of inflammation-inducing agents (on up to 7 occasions) may cause some mild discomfort in mice after each injection but this should be transient. After the procedures, mice will be provided with soft bedding, a mash diet and kept warm. All animals will be closely monitored for any adverse effects and will be humanely killed if approaching severity limit at any point in the study.

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)?

100% moderate - mice that have either undergone abdominal surgery or injections of an inflammation-inducing agent into the abdominal cavity, or both.

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?

We have teased out some of the key events of internal scarring using cell culture systems in the laboratory. We have also analysed human adhesion scar tissue collected from patients while undergoing repeat surgery. However, more detailed studies looking at how factors, cells and signalling pathways interact are also needed. For instance, blood clots that form between damaged organs after surgery, and ongoing inflammation due to infection, are proposed to be the main triggers of internal scarring and adhesion formation. We can only successfully replicate these events in whole organisms to really understand which blood clotting factors are involved, how inflammatory cells move in and out of the abdomen and ultimately what makes adhesions form. Our mouse models will produce findings that we will be able to relate to patients as they involve mammals rather than non-mammals such as frogs or fish which do not have the same blood clotting or inflammatory systems.

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

We also use non-animal alternatives to help inform our animal studies. These include samples of human abdominal tissue collected from consenting patients undergoing surgery and cells isolated from waste peritoneal dialysis solutions that we can culture in the laboratory. We are also developing a human 3D abdominal wall model in a tissue culture dish that has the same features as the abdominal wall in a patient. This non-animal model will allow us to ask very specific research questions such as how cells communicate with each other and what triggers clotting factors to be produced and blood clots deposited. These studies complement and in part, will replace our animal studies.

Why were they not suitable?

We work alongside many experts including surgeons so that we are sure that we use the most relevant models and experimental techniques to mirror what happens to patients as closely as possible. Although non-animal alternatives are important to address specific research questions, they can not model the blood and inflammatory systems and anatomy of the abdomen to fully address how internal scars form over time. New developments and alternative models will be continually monitored and changes to experimental plans altered accordingly.

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 work closely with statisticians to ensure accurate calculations are performed to determine the minimum number of animals required to achieve our goals. We have performed these studies previously so have a good understanding of the number of animals required to produce statistically significant findings comparing treatment groups with non-treated groups. In addition, we have reviewed the work of others in the field to help inform our decision on the number of animals required in each group, dose and volume of agents to deliver and relevant controls to use. Calculations typically show that we need group sizes of 6-8 to achieve the quality of results we need. Cell culture studies, then pilot animal studies, will initially be performed for any new treatments using a reduced number of animals to help inform numbers required in each study to produce meaningful data.

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

We ensure that experiments are well planned before starting so that we are in the best position to generate statistically meaningful results. Every experiment has been designed to fulfil the objective of utilising the minimum number of animals that will ensure that statistically significant results are obtained with appropriate controls. NC3Rs' experimental design guidance and experimental design assistant (EDA) is used to inform our experimental design, improve our procedures and guide the statistical analysis of data. Experimental design includes bias avoidance strategies such as ensuring that animals are randomly assigned to different groups, surgery performed by the same individual for each study, endpoints analysed by researchers blinded to treatment and where measurements are subjective (for example adhesion scores, ultrastructural analysis), researchers will again be blinded to the treatment received. Mice will be house together and we will avoid gender bias by including both male and female animals at equal ratios and use genetically-altered mice including mice with normal behaviour or expression of the target gene (wild-type) as well as those with half the expression of the target gene (heterogenous) or no expression of the target gene (homogeneous).

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 get as much information as possible from each animal by collecting multiple samples – blood, fluid, tissues, cells - to be analysed. As similar cells exist, tissues and fluids may also be collected from the thoracic cavity as well as the abdominal cavity, in order to generate a tissue bank/resource for future studies and to share with other groups. In this way, we will gain a greater knowledge of the workings of whole body systems such as how body cavities communicate with each other by the movement of inflammatory cells and signalling factors. In addition, we will culture mouse diaphragms in the laboratory and these will replace some live animal studies. One diaphragm will be bisected, and one half used for the treatment arm and the other for the control so further reducing the number of animals required. As such studies using new therapeutic agents will initially be performed using the cultured diaphragm or new 3D human abdominal wall model before the most promising treatments are tested in vivo so refining the live animal studies performed.

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.

Adhesion formation in humans can be caused by injury triggers such as surgical trauma, foreign bodies like sutures, lack of blood flow (ischemia) as well as bacterial/fungal infection. At present, it is not clear whether all these triggers drive the same processes or signalling pathways in abdominal cells that lead to mature adhesion formation. We will investigate internal scarring and adhesion formation using two mouse model systems that we have previously used; the first represents inflammation-induced scarring based on the effect of chemical irritants or dead pathogens or components of them (Protocol 1) whereas the second represents surgery-induced scarring (Protocol 2) with inflammation driven by abrasion injury and the use of sutures. Scarring is known to be more extensive following surgery and infection and so in some cases, an infection-driven inflammatory trigger will be combined with a surgical injury.

Surgery-induced internal scarring involves injury to the surface of the inner abdominal wall by abrasion injury or restricting blood flow to a limited area, with or without abrasion injury to the bowel. Sutures are used at the site of trauma and to close the abdomen as would occur in patients undergoing surgery. The degree of injury is controlled using a tailor-made instrument to deliver a reproducible injury to the surface of the inner abdominal wall. Sutures are used that produce minimal foreign body reaction as in humans. Mice recover well from the surgery with pain relief provided and although adhesions form, these procedures do not lead to complications such as bowel obstruction or any lasting harm.

Inflammation-induced abdominal scarring involves injection of chemical irritants such as dialysis fluid and/ or dead or parts of microorganisms such as lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria or zymosan (a cell wall component of fungi) and/or other pro-scarring factors into the abdominal cavity. Commercially available inflammation-inducing agents will be used to ensure correct dosage, sterility and purity of products to generate sterile peritonitis, rather than using live organisms.

To control the expression of a particular gene of interest in GA animals, some animals will be given substances by mouth, injection, or through food that can control that particular gene and switch it on or off. This helps us to work out how adhesions form by telling us what role that particular gene plays in the scarring process. We may also use this method to express genes that colour code cells so we can track them as they move to the injury site or to delete specific cell types to see if they contribute to adhesion development.

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

Adhesion formation involves inflammation and blood clot formation between closely packed damaged abdominal organs and inner abdominal wall. Young mice do not have a fully formed immune system and do not have the same make-up of blood clots so would not be suitable alternatives. In addition, non-mammalian animals do not have the same anatomy, show the same type of immune cells and their clotting system is different from humans so not appropriate to use. The models described are well established, produce consistent results and findings have been disseminated in our publications. We will use tissue from culled mice in order to set up diaphragm organ cultures for experiments to study how abdominal cells communicate with each other and respond to specific triggers of adhesion formation.

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

We have extensive expertise already using these mouse models under our previous licences and will make several refinements based on our experience. These include re-grouping mice a day after surgery rather than keeping them individually housed. Welfare checks will be continuous throughout the studies and adverse effects such as weight loss, change in behaviour and pain scores reviewed at least daily using set template forms. We will explore the use of hydrogels as a means of slowly releasing therapeutic factors in the abdomen over time. We previously used Brewer's thioglycolate to induce a mild inflammatory response with only transient adhesions as well as heat-inactivated *Cryptosporidium parvum* (a gut parasite) to produce a more robust inflammatory response over several weeks with adhesions maturing 3-4 weeks post-injection. Using commercially available inflammation triggering factors such as LPS and zymosan as alternatives, we will improve reproducibility, standardisation and dosage. These agents have been used previously by many groups to induce experimental sterile inflammation in mice.

We work alongside many experts in their field including surgeons, vets and industrial colleagues so that we are sure to use the most relevant models and experimental techniques to mirror what happens to patients as closely as possible. New developments and alternative models will be continually monitored and changes to experimental plan altered accordingly.

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

We will follow published guidelines such as the PREPARE guidelines: http://journals.sagepub.com/doi/full/10.1177/0023677217724823 and :https://norecopa.no/prepare to ensure we plan and conduct studies in the most refined way. Other resources will be reviewed regularly including those from the NC3Rs and other published guidelines. In addition, local recommendations will be monitored via the animal facility newsletter, training opportunities and workshops.

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

We will regularly review the NC3Rs news and advice and are signed up to receive the NC3Rs newsletter. We will discuss our studies with the NC3Rs Programme Manager and attend scheduled

workshops. We will also review tissue engineering and surgery websites, databases and publications to remain informed of any developments in the adhesion research field.