Home Office

NON-TECHNICAL SUMMARY

The link between diabetes, leaky gut and severity of acute pancreatitis

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

Acute Pancreatitis, Diabetes, Insulin, Gut barrier function, Antibacterial

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

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?

The over-arching aim of this project is to investigate the link between diabetes, leaky gut and the severity of acute pancreatitis. Diabetes is due to a loss of insulin secretion from pancreatic beta-cells leading to high blood sugar. Leaky gut is when bacteria leak from the gut into the blood due to a loss of the protective barrier function of the gut. Acute pancreatitis is an inflammatory disease in which the pancreas digests itself and originates within pancreatic acinar cells that secrete digestive enzymes into the gut.

This project stems from our recent discoveries that insulin has a direct action on pancreatic acinar cells, resulting in:

1-A boost in cellular energy, normally depleted during pancreatitis. This prevents cellular injury and death associated with pancreatitis.

2-Secretion of antimicrobial agents from pancreatic acinar cells into the gut where they maintain a healthy balance of good vs bad bacteria and protect the gut lining that prevent bacteria from “leaking” into the blood.

This means that loss of insulin secretion (diabetes) or loss of insulin action on pancreatic acinar cells leads to worse pancreatic injury and a leaky gut which make pancreatitis more severe.

We aim to compare the severity of experimentally-induced acute pancreatitis in normal mice vs genetically altered mice. These include mice lacking insulin secretion (diabetic mice) and mice lacking insulin receptors that respond to insulin specifically in pancreatic acinar cells (PACIRKO mice).

We also aim to test the effects of therapeutic substances in reducing the severity of acute pancreatitis which includes drugs that mimic the effect of insulin on pancreatic acinar cells and antibacterial substances that repair the leaky gut.

A retrospective assessment of these aims will be due by 02 February 2028

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?

Unmet clinical need—Acute pancreatitis (AP) is a serious and sometimes fatal inflammatory disease affecting 45 per 100,000 with a 30-day case-fatality rate of 8 %. In England alone more than 1000 people die each year. One-third of episodes are severe, characterised by pancreatic injury, sepsis and multiple organ failure. This increases death rate and patients spend prolonged periods in critical care, at an annual cost of £200 million in the UK and $2.6 billion in the USA. Treatment is supportive and restricted to pain control and organ support. There is a pressing need for an effective therapy to reduce disease severity, deaths and critical care occupancy.

Potential solution—We aim to test new therapies that will “heal” the underlying pancreatic injury and “repair” the leaky gut. This will halt the escalation from mild to severe acute pancreatitis, responsible for prolonged critical care and deaths.

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

Short-term Outputs Within the lifetime of this project, we aim to determine:

1-The relative contribution of direct insulin-mediated protection of pancreatic injury and secretion of antibacterial agents, that prevent “leaky” gut, during pancreatitis.

2-The therapeutic benefits of “insulin-mimetics” and “agents that repair the leaky gut” on the severity of pancreatitis in diabetic and PACIRKO mice.

Longer-term Outputs Following the success of the above outputs, we aim to immediately progress the most promising therapies to the treatment of severe acute pancreatitis patients. These treatments are likely safer and more amenable to use in patients and will not require the usual and prolonged clinical approvals that new drugs require.

For example, the insulin-mimetic, metformin, is currently licensed and in widespread use for the treatment of type-2 diabetes and if proven to be effective would simply require "repurposing" for use as a treatment of acute pancreatitis and thus would not require the lengthy toxicity studies and approvals of a new drug.

The administration of depleted antimicrobial peptides via a nasojejunal feeding tube to acute pancreatitis patients designed to "repair the leaky gut" would be classed as a "novel treatment" and would thus require clinical approval. However, these are essentially natural products, normally secreted by the pancreas, that would be supplementing the nutritional support that is already being administered to these patients through a feeding tube into the gut and would not enter the general circulation. Therefore, clinical approval for such a treatment would likely to be much shorter than a novel synthetic drug that enters the blood stream with potentially unknown toxicity to other organs.

Similarly, the use of the natural plant-derived sprouted multigrain nutraceutical, SPROTONE, may have "insulin mimetic" activity and/or may contain "agents that repair the leaky gut", however, we do not yet

know the identity of any active ingredients, which will be the focus of parallel non-animal laboratory studies. Nevertheless, the successful outcome of these studies would add SPROTONE, or its active ingredients to the pipeline of drug discovery for the treatment of acute pancreatitis.

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

Short term (scientific)—These include the scientific and clinical research communities in the field of Pancreatology. We aim to publish our findings, both positive and negative in high impact journals that will reach as wide an audience as possible. This means that other scientists will benefit from any positive findings that will help to move the field forward, but also learn from any negative findings, thereby avoiding any unavoidable repetition and thus animal suffering, with the benefit of taking the field into new directions.

Longer-term (Clinical)—Although any successful treatments will be in animals, these can be easily progressed to clinical studies and are within touching distance of reaching patient benefit with genuine potential to make a real-world clinical impact.

Therefore, the major beneficiaries are the patients that suffer from acute pancreatitis and the healthcare professionals that care for these patients. Specifically, severe acute pancreatitis patients who are critically ill and at very high risk of death will benefit the most. The NHS and UK tax payers, and other national healthcare systems across the world, will also benefit from these discoveries. Acute pancreatitis represents the majority of gastrointestinal-related emergency hospital admissions, putting a huge burden on health care systems. If these treatments reduce the time patients spend in critical care by half, this will save the NHS £100M.

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

All outputs from this project (both positive and negative observations) will be published in high impact scientific journals and communicated at major international conferences. These include the annual American Pancreatic Association (APA), European Pancreatic Club (EPC) and Union of European Gastroenterologist (UEG) conferences, which are the major international fora for pancreatitis research.

We have established strong collaborations with key clinical researchers within critical care and Hepato-Pancreatico-Biliary (HPB) surgery who jointly manage acute pancreatitis patients admitted to hospital. This will facilitate the seamless clinical translation of any positive outputs of this project to early phase clinical studies. Any commercial exploitation from our discoveries will be managed by the Establishment's Innovation Factory. This will help to speed up the clinical translation and ensure therapies reach patient benefit without unnecessary bottlenecks.

There is also a strong culture throughout the Establishment that promotes public and patient involvement in research. Our clinical collaborators regularly liaise with patient groups and both clinicians and basic scientists from within the research group regularly present our research to patients and family members at annual Supporters Conferences.

Species and numbers of animals expected to be used

- Mice: Total = 11,616 (2904 each for Ins2Akita, WT, PACIRKO and IRlox/lox)

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.

Why choose mice?—Mice represent important models for the characterization of complex cellular processes underlying disease, how these cellular processes interact within a whole organ context and how multiple organs interact with each other to produce a “whole body” response to disease. One advantage relies on the targeted deletion of multiple genes to identify the precise mechanisms underlying disease, which has been particularly successful in mice.

Why choose Diabetic mice?— These mice carry a mutation in the insulin gene that leads to the accumulation of faulty insulin protein within the pancreatic beta-cell. This leads to the selective loss of these cells and the gradual loss of insulin secretion and therefore the mice develop diabetes. This makes this mouse strain a good model for studying the effects of impaired insulin secretion during acute pancreatitis.

Why choose PACIRKO mice?— The PACIRIKO mice represent a drug-inducible ***P***ancreatic ***A***cinar ***C***ell-specific ***I***nsulin ***R***eceptor ***K***nock ***O***ut (***PACIRKO***) mouse. This means that the receptor that normally responds to this drug has been genetically engineered into the mice so that administering the drug over 5 days causes the insulin receptor gene to be permanently cut out of the DNA of the pancreatic acinar cells (gene deletion). Permanent deletion of the insulin receptor in pancreatic acinar cells means that these cells no longer respond to circulating insulin, whereas all other tissues around the body respond to insulin normally.

PACIRKO mice are essential because mice lacking insulin receptors in every tissue throughout the body develop diabetes as soon as they are born and die young due to severe ketoacidosis (a dangerous condition caused by a toxic build-up of ketones and acid). Furthermore, insulin is required for normal development of the pancreas which is why it is necessary to use a drug inducible insulin receptor deletion in adult mice (when they reach 6-9 weeks old) to allow the pancreas to fully develop.

Choice of life stages—The use of diabetic mice at 6-9 weeks old was chosen as this is the youngest age in which consistent high blood glucose and thus loss of insulin secretion is achieved. This prevents any long-term adverse effects of chronic high blood glucose produced in older mice. Although there are no adverse effects the use of PACIRKO mice at a comparable age removes any confounding effects of age.

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

1-Breeding of genetically altered mice (diabetic and PACIRKO mice) by conventional methods and the administration of drugs to induce gene deletion (PACIRKO mice), either using an oral feeding tube or using food containing the inducing drug.

2-Acute pancreatitis is induced by repeated abdominal injections of caerulein for up to 2 days. Caerulein is an analogue of the naturally occurring hormone, cholecystokinin, which normally stimulates the pancreas to secrete digestive enzymes into the gut. However, at high doses caerulein over-stimulates the pancreas and mimics pancreatic inflammation similar to the disease in humans. At the end of the experiment mice are killed painlessly and humanely and the pancreas, gut, faeces and blood collected at various time points after the last injection (2 hours to 14 days) to determine severity and recovery of pancreatitis. The extent of pancreatic injury, impaired gut function, altered gut bacteria in the faeces and the infection of gut bacteria in the blood and pancreas will all be used to determine disease severity and recovery.

3-Administration of therapeutic substances, either before or during pancreatitis. These include substances that either mimic the protective effects of insulin on pancreatic acinar cells or antibacterial substances that repair the leaky gut. These will be administered either using oral dosing, added to drinking water or by abdominal injection.

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

Breeding and maintenance of genetically altered mice—The breeding of diabetic mice can produce harm in some of the offspring. Male mice gradually develop high blood sugar causing excessive drinking and urination at 6-9 weeks old. The breeding of PACIRKO mice produces no harm to the offspring, until the mice are administered the drug that induces gene deletion.

Experimentally-induced acute pancreatitis— Mice receiving repeated injections of caerulein experience abdominal pain associated with acute pancreatitis. This pain gradually accumulates due to inflammation of the pancreas and persists for up to 48 hours after which the pain progressively declines as the pancreas recovers. In normal mice the pancreas completely recovers to normal within 5-7 days although the pain may be worse and persist longer in diabetic and PACIRKO mice.

Administration of substances—Whether inducing pancreatitis or administering therapeutic substances to reduce the severity of pancreatitis the route of administration of substances (abdominal injections or oral dosing) may cause some discomfort or pain. There may also be a small chance that the oral dosing of therapeutic substances may cause diarrhoea.

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)?

Breeding and maintenance of diabetic mice – Moderate in 25% of offspring

Breeding and maintenance of PACIRKO mice – Mild in all mice

Treatment with tamoxifen to induce gene deletion – Moderate in all mice treated

Insulin receptor deletion in PACIRKO mice – Moderate in all PACIRKO mice

Experimental induction of acute pancreatitis – Severe in 50 % of the total number of mice used in this study, which represents those mice that receive caerulein. The remaining 50 % of mice will receive

innocuous salt solution

Administration of therapeutic substances – Moderate severity in all mice treated

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

- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 02 February 2028

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?

Mice represent the least sentient species that have been used extensively for genetic deletion and manipulation and in the study of pancreatitis. This is because they have a well characterized biology that allows sensible comparison to human biology and disease. One of the major strengths of this project is the use of PACIRKO mice, in which the insulin receptors are specifically deleted in acinar cells of adult mice. The major benefit of using both PACIRKO and diabetic mice is that we can distinguish between effects of reduced insulin secretion and high blood glucose (which occurs in diabetic mice) from loss of direct action of insulin on pancreatic acinar cells (which occurs in PACIRKO mice). This will reduce any ambiguity when interpreting our results and provide a very precise and holistic understanding of severity of acute pancreatitis, which in the long-term will reduce the number of animals required.

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

Cultured cells and acutely isolated mouse pancreatic acinar cells that represent “cellular models” for studying pancreatitis which in theory reduces the need for and are a convenient alternative to animal experiments.

Why were they not suitable?

1-Cultured cells—unfortunately their underlying biological function is just far too different and therefore unreliable.

2-Acutely isolated pancreatic acinar cells—Although these cells represent an excellent model for studying the mechanisms of cellular injury and insulin-mediated protection, these cell very rapidly die following isolation from their natural tissue environment.

3-Understanding severity of acute pancreatitis—A major problem with using “cellular models” of acute pancreatitis is understanding how cellular injury relates to whole organ pancreatic injury and in turn, how other organs respond to pancreatic injury to influence disease severity. Specifically, this project investigates how the pancreas and gut “communicate” with each other and how the injured pancreas might release toxic enzymes into the blood that lead to injury of other organs, such as the lungs. This could never be achieved using the “cellular model” of acute pancreatitis.

A retrospective assessment of replacement will be due by 02 February 2028

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?

This was achieved by measuring blood amylase during intravenous caerulein infusion-induced pancreatitis combined with insulin infusion as the therapeutic intervention. Amylase is a highly abundant pancreatic digestive enzyme secreted into the gut where it digests starch. However, it leaks out of the injured pancreas into the blood during pancreatitis and therefore is an accurate and easily quantifiable measure of disease severity. The magnitude of these responses and how they vary between animals can be used to estimate the minimum numbers of animals.

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

We will always aim to maximise the amount of data we get from each mouse by collecting as much tissue, blood and faeces as possible from each mouse. This guarantees that as many experimental measures of pancreatitis and gut function can be assessed as possible, rather than having to repeat experiments to capture additional responses. We may also employ national on-line design resources, e.g. the Experimental Design Assistant (EDA) tool created by the NC3Rs, and consult with the NC3R team who provide periodic onsite help, to improve our approach.

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

Breeding mice—Breeding will be optimized by replacing breeders before their reproductive performance declines (15 months). We will also adopt staggered breeding cycles to ensure optimum production of litters at appropriate times when it's logistically feasible to perform experiments.

Parallel in vitro experiments—The protective effects of "insulin-mimetics" will be tested on acutely isolated pancreatic acinar cells and any antibacterial substances will be tested on bacteria in a culture dish. Only those substances that show clear-cut therapeutic benefit will progress to pancreatitis experiments in mice. This will reduce negative responses and therefore avoid unnecessary suffering of animals.

A retrospective assessment of reduction will be due by 02 February 2028

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.

Genetically altered mice— Diabetic mice will assess the effect of loss of insulin and high blood sugar on the severity of acute pancreatitis. PACIRKO mice will assess the effect of loss of insulin action of pancreatic acinar cells on the severity of acute pancreatitis. Using diabetic mice for experiments at 6-9 weeks old minimises any adverse of diabetes and using PACIRKO mice within 5 days of drug-induced gene deletion also minimises harm.

Why caerulein-induced acute pancreatitis?—Caerulein-induced acute pancreatitis in mice has been extensively characterized, is easy to execute, reliably reproducible and the specific dose and number of injections can be adjusted to control disease severity. Unfortunately, this produces characteristic abdominal pain that is comparable to the human disease. However, the administration of adequate pain relief that does not interfere with the pancreatitis responses will minimise suffering and will reduce the number of animals required as results will be more reliable and reproducible.

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

The mouse is the lowest vertebrate animal that shares the most common biological and disease mechanisms to humans which can be genetically manipulated so that we can understand these mechanisms.

Animals cannot be terminally anaesthetized as this cannot be maintained over the prolonged periods required for the manifestation of pancreatic injury, systemic inflammation and distal organ injury required to assess pancreatitis (2-3 days).

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

Breeding and maintenance of genetically altered mice—Any adverse and serious clinical symptoms of diabetes, which normally occurs in older diabetic mice (12 weeks and older) are minimised by using mice for experiments at 6-9 weeks old. Similarly, any adverse effects of drug-induced gene deletion in PACIRKO mice are minimised by using the mice for experiments within a few days of inducing gene deletion.

Experimentally-induced acute pancreatitis—Although all experimental models of acute pancreatitis cause characteristic abdominal pain, we will attempt to reduce pain, suffering or lasting harm to the animals as much as possible by the use of appropriate pain management. Pilot studies will determine the most appropriate and effective pain relief, using well-established pain measurements (facial grimace score), without affecting pancreatitis responses.

Administration of substances—For repeated injection in the same mouse, injection sites within the lower abdomen will be rotated to avoid excessive injury at the injection site. Substances will be administered at doses known to be tolerated and only those that are effective in in vitro experiments will be used in animals.

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

We will be guided and directed by the most up to date documents on the welfare and use of animals in pancreatitis research. Specifically, a recent paper has identified a specific pain relief that is not only as effective as some of the more commonly used pain relief drugs, such as morphine, but also has no impact on the measurement of pancreatitis responses. Additionally, we will follow relevant ARRIVE guidelines to ensure that our studies are reported in enough detail to add to the knowledge base.

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

We will frequently liaise with the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3R) team who provide very helpful advice and consult their website (https://www.nc3rs.org.uk/resource-hubs) for updates on advances in 3Rs. We will also refer to peer-reviewed scientific papers, oral communications/posters at conferences and engage in regular discussions with scientific colleagues on the 3Rs specifically related to models of acute pancreatitis.

A retrospective assessment of refinement will be due by 02 February 2028

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?