Home Office

NON-TECHNICAL SUMMARY

Preservation of Pig Liver and Kidney for Transplantation, Using a Novel Solution

Project duration

3 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

Transplantation, Preservation, Kidney, Liver, Novel Solution

Animal types	Life stages
Pigs	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 determine whether LS-A preserves abdominal organs better than in use UW solution, in static cold storage preservation, using a large animal model (the pig).

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?

Organ transplantation not only saves lives but enhances the quality of the life of recipients allowing them to live long healthy and fulfilled lives. This costs society and in particular the NHS far less than having a critically ill person who takes up hospital time, treatment (such as dialysis prior to kidney transplant) and money; indeed many patients return to full time careers after successful transplantation so pay tax and put money back into the country. Children who have organ transplants return to school and many go on to further education, have lifelong careers, get married and have children of their own. One thing that is clear is that the better the organ preservation solution the higher the chances of the organ working and the patient surviving.

There are currently around 6,500 people on the UK transplant waiting list and for the last year that there is data for, nearly 500 people died whilst waiting for a transplant (NHS Blood and Transplant data 2017), and in the USA about 20% of people will die waiting for a transplant. Transplants often do not go ahead due to the organ being not suitable and decisions about their use having to be taken quickly, largely due to limitations related to the length of time that organs can be safely stored before transplantation. This is because the length of time an organ can be out of the body prior to being transplanted is dependent on the effectiveness of solution which has been used to perfuse and preserve the organ. For example, at present the maximum storage time for liver using the current “gold standard”, UW solution, is less than about 12 hours and for kidney only up to about 24 hours, even when the organs are considered to be in ideal condition before retrieval.

LS-A has been developed by a group of researchers for use in abdominal organ preservation and experimental data has shown that LS-A can extend the static cold storage period successfully for organs for liver and kidney transplantation.

Older, or less fit donors who are referred to as extended criteria or marginal donors are being used much more frequently now, as due to better medical and surgical care, people are surviving longer and not dying after such things as road accidents and brain haemorrhages. It is believed that using machine perfusion is one way to enable organs from these types of donors to be checked in a more effective way to see if they are suitable for transplantation and gives time to manipulate the organ and or the recipient if needed.

In order to combat the general shortage of organs available for life-saving transplants, older, or less fit donors who are referred to as extended criteria or marginal donors are being used more, including organs retrieved from DCD (donation after cardiac death) donors, and donors with fatty liver disease for example. These “extended criteria donors” are being used much more frequently now and it is clear that they need improved methods of organ storage to ensure function after transplantation. One method is machine perfusion after initial static cold storage, in particular a method known as Hypothermic Oxygenated PErfusion (HOPE). It is believed that using machine perfusion is one way to enable organs from these types of donors to be checked in a more effective way to see if they are suitable for transplantation and gives time to manipulate the organ and or the recipient if needed. Currently UW-MP (UW for machine perfusion), or an identical formulation sold under different trade names, is the only clinically available solution for HOPE. Paradoxically, one major failing of both UW and UW-MP is that they contain a high concentration of hydroxy ethyl starch (HES), which is known to be highly toxic. LS-A does not contain any HES.

Currently there is a need to optimise not only the solution used for static cold storage but also for HOPE as in clinical practice UW needs to be used for the initial flush and cold storage period and then exchanged for UW-MP for HOPE. A single improved formulation able to work effectively in both situations is needed. LS-A may be this solution so it needs to be tested for HOPE after static cold storage using both ideal and marginal organs.

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

At the end of this work, we should be in a position to do a clinical trial, using LS-A for the preservation of kidneys and livers to keep them in a better condition than the preservation solutions on the market at the moment can do. It will also allow organs to be kept for longer periods than is possible at present, and this in turn will give time for the manipulation of factors in both the organ and the recipient, which will mean that the organs will have a better chance of lasting in the recipient for longer. Organs from extended criteria donors will be kept in a better condition and will be able to be manipulated so that the recipient will recover quicker and have a better quality of life for a longer period.

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

Long term the patients who will benefit will be patients in chronic organ failure waiting for a kidney and/or liver transplant. By being able to preserve organs for longer and having time to manipulate the organ or the patient will mean there are more organs of better quality for transplantation, so hopefully more patients will get transplants which will last longer. This in turn will be better for the health service, as the patient will require less care, and society in general as many of the recipients will be able to return to work, have less time off work so help the economy of the country.

Medics and scientists will also benefit as it will allow time for the organs or patients to be treated in different ways so that the transplants will be successful. Many of these treatments have yet to be discovered but it is believed that stimulation of certain cells and suppression of others may help in this process.

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

It is intended that all the data from this work will be published in peer reviewed journals and presented at conference both nationally and internationally to disseminate the results.

Once clinical trials start, it is expected that other groups around the country and abroad will also be involved in these trials so as the largest cohort of patients and organs will be clinically tested.

Species and numbers of animals expected to be used

- Pigs: 12

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 female Landrace-Great White Hybrids Pig are used as these are the type which are used at our University, so no new breeds will need to be introduced. Adult pigs are used as they are the nearest in size to those found in human organ donors and will weigh about 30 – 40 kg.

Pigs are used in this experiment because the anatomy of the internal organs of the pig is similar to that of a human, making the surgery and procedure as near to what would be seen in human transplantation as is possible.

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

This process will kill the pig while still under anaesthesia. We plan to harvest kidneys and liver. Organs will be kept cool and will be flushed with a appropriate solution to keep them in optimum condition for storage and later in vitro studies. Each pig will be sedated All surgical procedures on pigs under this project license will be carried out while animal will be terminally anaesthetised. Each pig will be sedated and subsequently anaesthetised by a competent personal licence holder with expertise in pig anaesthesia. A human surgeon with a personal licence to carry out regulated procedures on pigs will then perform surgical procedures that include laparotomy, cannulation of peripheral and deep blood vessels, perfusion of organs with preservatives collection of large blood sample for later use, and removal of organs for subsequent in vitro studies on harvested organs.

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

The pig will be brought to from the establishment approximately 18 -24 hours before the induction of anaesthesia to enable it to acclimatise to new surroundings. It will be sedated prior to surgery and anaesthetised so should suffer no abnormal behaviour or tension.

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)?

All animals will be killed during the operation. The pigs will be sedated and all procedures will be carried out under anaesthesia from which the pigs will not recover.

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?

To show that any organ preservation has been successful whole organs need to be used as when an organ transplant takes place the majority of the time it is a full organ which is used, the only exception being when lobes of liver or lung are used on tiny children. The only way that this can be done is by using whole animals. Looking and testing the effect of the solution on the whole organ rather than just certain cells is required.

Earlier work was done on rats but pigs are to be used in this research as pig kidney is multilobular and multipapillar, with a complex network of blood vessels, also found in humans but not in mouse or rats, so they are the closest there is to the human model.

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

Some of the elements of the solutions have been tested initially on cell lines prior to their addition to the solution. If there were adverse events seen with the cell lines then these were not added to the solution and were replaced without testing on whole organs or animals. We have now come to the stage when we need to use whole organs.

Why were they not suitable?

After preservation of the organs they will be reperfused with blood to mimic what happens following organ transplantation. Blood used in the reperfusion so that different biochemical perimeters specific to the organ transplanted can be tested after different lengths of time to ascertain how well the organs are functioning, for which we require the full organ. For liver we will be also collecting bile and for the kidney we will be collecting urine both of which require the full organ as more than one cell type is involved in their production.

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?

These are preliminary studies to test viability and/or difference between two preservative solution being widely used in the preservation of organ before transplantation. We think 12 animals will give us statically viable information to see the differences in two solutions.

Many different types of tests on the organs, tissue and blood means plenty of results can be obtained despite the low number of animals used.

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

To reduce the number of animals used several strategies have been used. Firstly, as much as possible of each animal will be used, so both the liver and kidneys will be retrieved. Secondly blood will be taken for use in reperfusion parts of the experiments rather than being bought in which would entail more animals being killed. Thirdly each time when a pig is going to be used, other researchers who can use other parts and tissues will be informed so that they can take what they can use.

If a pig dies unexpectedly, the organs will still be taken and used, as this situation mimics another way in which organs are retrieved for transplantation in humans, called extended criteria donors. This means that if other researchers are euthanizing a pig for any reason, we may be able to retrieve and use the organs, again soon after death.

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

Rather than transplant the organs into other animals, to reduce the number of animals used it is intended that we use an isolated perfusion rig, to mimic transplantation. This equipment allows us to reperfuse both kidneys and livers at the same time, outside of the pig, so each pig will have the liver, both kidneys and blood removed from it. The rest of the pig will be offered to other groups to see if further use can be made of it.

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.

The type of animal used will be Landrace-Great White Hybrids as these were used in earlier in the development of the solution and are the breed of pig which is kept at the University farm; therefore no new species will be introduced and there will be the minimal of movement of the animals which will cause them less stress.

Pigs are social animals and should not be kept alone for prolonged periods of time, therefore the pigs will be brought from the establishment where they are kept to where the surgery will take place, only 24 hours prior to it being required for surgery. This gives it time to acclimatize after the move but not long enough for it to get lonely.

All work on the pig will be done under anaesthetic so it will not feel any pain and will be euthanized whilst still under anaesthetic. All work on the pig will be done under anaesthetic so it will not feel any pain and will be euthanized whilst still under anaesthetic. Anaesthesia on the pig will be carried out by a competent personal licence holder with expertise in pig anaesthesia. Surgical procedures will be carried out under aseptic conditions by a competent transplant surgeon with a personal licence to work on pigs. No animal will recover from anaesthesia. This will mean that the pig will be caused the least pain and suffering possible for these procedures.

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

Earlier work was done on rats but pigs are to be used in this research as pig kidney is anatomically more complex, with a lobular structure and a complex network of blood vessels, which are similar to those found in humans but not in mouse or rats, so they are the closest there is to the human model.

The pig liver is closer to human liver anatomy than rat or mouse liver and it is widely accepted that the porcine hepatocyte is the best non-human model to use as an alternative to human hepatocytes.

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

Each pig will be brought to the University only 24 hours in advance of their use. Where the pigs are reared they are in a group as they are not solitary animals. They will only be at the site for a short period and will be sedated prior to anaesthetic so that it will not suffer many adverse effects. The pigs will be monitored by the University Veterinary Surgeon at all time from the point of sedation, through the general anaesthetic and surgery until the point where it is killed. The monitoring will be the same as would be done during a routine operation on an animal, and if at anytime the pig is thought to be suffering initially we will try to alleviate it but if this is not possible it will be killed by a humane method so that it does not suffer any longer.

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

Animals (Scientific Procedures) Act 1986 (ASPA)

EU Directive 2010/663/EU

UK Research Integrity Office Code of Practice guidelines.

ARRIVE guidelines.

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

A computer search will be carried out prior to each period of work with the pigs to see if there are any changes or advances in the 3Rs which need to be accounted for with the work. This will include checking on the Home Office site, NC3Rs site and also the Royal Veterinary site We will also check with the University Veterinary Surgeon to see if they have any knowledge of any changes which would impact on this work.

I already receive updates from NC3R and any changes regarding the use of the animals will be done in accordance to their advice.