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NON-TECHNICAL SUMMARY

The mechanisms underpinning ‘steroid’ (glucocorticoid) development of obesity and diabetes

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

Steroids, Glucocorticoids, Diabetes, Obesity, Brain

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

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?

Synthetic glucocorticoids, commonly called steroids, are used as medicines to treat a wide range of illnesses such as asthma, rheumatoid arthritis, and multiple sclerosis. Although well tolerated, long term, high dose use can lead to side effects including obesity and diabetes. Therefore, the aim of this study is to identify how longer term use of glucocorticoids acts in specific parts of the brain to increase food intake, and to alter processes in the body leading to high glucose and diabetes. Our long term aim is to find alternative synthetic glucocorticoids or alternative ways of administering them to reduce the associated obesity and diabetes.

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?

Glucocorticoids (steroids) are a widely used class of medicines. They are anti-inflammatory and used to treat diseases such as asthma, arthritis and autoimmune conditions such as multiple sclerosis as well as in the treatment of cancer. With long term, high dose use, patients can develop side effects similar to those seen in the metabolic syndrome, a cluster of illnesses associated with obesity and diabetes. These include weight gain, especially around the waist, type 2 diabetes, and fatty liver disease. These side effects can limit the use of an otherwise good and inexpensive medication. In this body of work, we want to understand how the glucocorticoids cause the side effects. By understanding this, it might be possible to find other glucocorticoids that don’t cause the side-effects or medicines to be given with the glucocorticoids that decrease the side effects. This would mean that more patients could be treated with these medicines.

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

At the end of the project we will have gained new information about how glucocorticoid treatment causes side effects such as obesity and diabetes. Diabetes is a serious, longterm illness, that often requires daily medication. Furthermore, when not well controlled, diabetes can increase the risk of kidney disease, blindness and cardiovascular disease, including heart attacks and strokes. Obesity is a condition, which itself can lead to diabetes, but can also increase the risk of certain cancers. All of these are life limiting for the patient and the complexity of the treatment decreases their quality of life and makes it very expensive for the NHS.

The information generated from this proposal will be shared in research papers and with other scientists and doctors at conferences. If we can understand how the side effects develop then we, with

doctors and the pharmaceutical industry, will be able to design new types of glucocorticoids which don't cause the problems or different treatments given with the glucocorticoids to reduce the side effects.

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

The main beneficiary of this work will be patients treated with glucocorticoids and their doctors who are able to prescribe them with reduced worries about side effects. The NHS will also benefit, as glucocorticoids are cheap medicines and will be able to be used more widely. Additionally, the costs of treating the side effects such as diabetes are also high, so these may be reduced if the side effects of glucocorticoids are reduced. The scientific community will also benefit from the outputs generated in this project, as we will be increasing the knowledge of how these steroids work.

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

Previously, we have collaborated with groups at other universities, with pharmaceutical companies, and with clinical endocrinologists who see patients with these side effects. Therefore, we are well placed to contact those groups of people who can use the outputs of our studies most effectively. These collaborations have not only been for exchange of knowledge, but also to learn the most refined way of carrying out the experiments. We will present our early data at conferences, so fellow scientists and doctors can comment on the experiments while they are progressing. This will ensure that the correct studies are being carried out. We will also publish our data, whether successful or not, in open access scientific journals as well as sharing our new publications on social media platforms to increase the visibility. All raw data will be made available upon request.

Species and numbers of animals expected to be used

- Mice: 10500

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.

For our research we use an adult mouse model, which is treated with glucocorticoids (steroids). This model is able to mimic the side effects of the treatment that are seen in humans. Also, the pathways in the development of these side effects of obesity, diabetes and fatty liver disease are thought to be similar between humans and mice. In addition, we are looking at actions in the brain so we need to collect brain tissue and we look in mice bred with certain pathways removed, making these animals the best model for our investigations.

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

Most mice in this project will be treated with glucocorticoids to look at the side effects of diabetes and obesity. Typically, we give this treatment in their drinking water to reduce the stress of handling and the short term pain of an injection. They will be weighed frequently and depending on what we are looking at they may have a glucose tolerance test, where they will be fasted before they are injected with glucose and small blood samples taken from the tail. Alternatively, they may have their metabolic rate measured by placing them in special cages that can measure this. We also may look at how much fat they have using a type of MRI scan, but as it doesn't give an image, they don't need to stay as still. Before the end of the study, we take blood samples from the tail to measure things such as stress hormones and insulin. Many of our protocols look at changes in factors associated with appetite and feeding in the brain, but these measurements are made after the study finishes.

Some studies will be carried out where we need to give drugs directly into the brain. For these studies, mice will undergo a short surgery with a quick recovery anaesthetic and a device will be inserted, so these drugs can be directly administered to the brain when the mice are awake and freely running around. During and after surgery, mice will be given appropriate pain relief, kept warm and given soft food to help them recover as quickly as possible.

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

With glucocorticoid treatment the mice should gain weight and become overweight or obese. The treatment period for these studies are 4 weeks at their longest, so the animals are just at the start of developing diabetes. Some will develop diabetes and therefore drink more and urinate more, but we will change their bedding more frequently to account for this. As the measurement of food intake is important for our research, animals will often be in a cage on their own, which can disrupt their normal behaviour, but we provide them with tubes to play and hide in and additional bedding and chew sticks to reduce the impact of this. The total length of time where the animals would be housed alone is generally 7 weeks (1 week to acclimatise, 2 weeks to take normal measurement and then 4 weeks on treatment). A very few studies may want to investigate genetic changes related to glucocorticoids in mice and then mice may need to be housed alone for up to 6 months.

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)?

Most animals will have a mild experience. They will eat more and will gain weight, but not to the point that it impacts their welfare. There could be transient pain from injections or blood sampling. There may be a mild stress effect of social isolation.

The animal having surgery will have a moderate severity experience. They will be given quick recovery anaesthetics to reduce the impact of the anaesthetic, painkillers to reduce any pain and will be kept warm until fully recovered. Mice tend to recover quickly from this surgery.

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 look at the effects of glucocorticoids (steroids) on the whole of the body. We mainly give these to the animals in their drinking water, which is similar to patients swallowing tablets, and we look at their effects on multiple organs in the body, such as fat, liver and muscle. It is not possible to mimic these effects in dishes of cells as you cannot look at the overall effect on the body.

Additionally, we look at how glucocorticoids acting in the brain then signal to the other organs in the body. As we are looking at how one organ in the body controls others, a whole body system is needed for this.

We have found from our previous studies that mice respond similarly to patients when given these steroids, so they are used in our studies.

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

A potential alternative is to work on neurons growing in culture. However, these neurons do not survive and do not develop into cell lines. There is a technique for adapting them to grow continuously which is complex but possible, and this offers a way of addressing specific questions about the neurons that regulate food intake. Several years ago, we obtained immortalised hypothalamic neuronal cell lines from a collaborator to determine if they produced a key neuropeptide regulating food intake. Despite a lot of effort in culturing the cells, we were unable to get these neuronal cell lines to synthesise this neuropeptide.

We subsequently worked with another collaborator who uses embryonic stem (ES) cells differentiated into neurons and studied the production of these neuropeptides. As the neuropeptides are a glucocorticoid (steroid) target in the brain, we will be able to assess the effects of chronic glucocorticoid treatment on these peptides in the ES cells. This would complement the *in vivo* studies where we consider the effects of glucocorticoids on food intake.

Why were they not suitable?

As stated above, neurons which were adapted to grow continuously did not produce the neuropeptides, but we have subsequently collaborated with a research group using ES cells which could provide key information on the effects of chronic glucocorticoid treatment. However, there is growing evidence that these neurons are quite heterogeneous in the brain and they act at several different target neuronal populations which would be impossible to mimic *in vitro*.

In addition, to prove that glucocorticoids are acting via these specific neuropeptides to modify food intake, body weight and peripheral metabolism, we will still need to do these studies *in vivo*. We also need to study the effects of glucocorticoids on multiple body tissues such as liver, adipose tissue and

muscle at the same time, to examine the molecular changes underpinning the side effects in patients treated with these medicines. Therefore, single cell types will not inform us of the overall effects on the body.

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?

Generally, we use the data from previous studies to help us estimate the number of animals required for an experiment. This allows us to use the minimum number of animals to give us a reliable answer to the question. Where it is a completely new experiment, we will use a small group size at the start, so we can monitor how many animals we will need.

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

To design the experiments we use the NC3Rs experimental design assistant to get the best study design and to use the correct number of animals. At the end of the study we take organs from the animals that we might not necessarily need at the moment, but so we wouldn’t need to run another study in the future if we need to look at the effects of treatment that organ.

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

We breed the minimum number of animals possible for our projects that will give us the best experimental results. When we start new studies, we carry out pilot studies to understand how the animals will react and to help us estimate the number of animals we will need. At the end of the study, we take as many tissues and organs as possible, to minimise the need to run later studies, we also share the tissues from some studies with other groups, so they don’t need to run their own experiments.

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.

This licence application only uses mice, the least sentient animal possible for our studies. Mice are used as they give similar responses to glucocorticoids (steroids) as is seen in patients treated with these medicines. We mostly give the glucocorticoids in the drinking water, so the animals don't need to be handled and stressed as much and this approach reduces the need for multiple injections. None of the mice with genetic changes show any side effects to these gene changes.

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

We have investigated zebrafish models and there are some good examples of novel data describing how glucocorticoids act within the cells. There has also been information on how glucocorticoids control development of the zebrafish. However, the systems are too limited to be used to study whether the glucocorticoids are acting in the brain or directly in organs in the body that regulate metabolism. In addition, there seem to be key differences in zebrafish in the expression of enzymes that activate/inactivate glucocorticoids. We have also investigated what is known in zebrafish about the melanocortin system, which is a target of glucocorticoids that this project addresses. Unfortunately, previous work has only studied a rudimentary peptide system in another organ and there is no obvious melanocortin system in the brain to work on.

Therefore, mice are the least sentient animals that mimic the glucocorticoid induced changes that lead to obesity and diabetes as seen in human patients. As the glucocorticoid effects which lead to obesity and diabetes develop over time, we cannot do these studies in terminally anaesthetised animals.

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

The animals in our studies are already closely monitored to ensure they suffer the minimum pain and harm. The small number of animals that undergo surgery get pain medication, and are fed soft food and kept warm after the operation to minimise any pain and suffering. Where animals need injections, the mice will be handled to get them used to this prior to the experiments. We also keep ourselves updated so we are informed of any improvements that we can implement to minimise any harm to the animals.

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

We will follow the PREPARE guidelines as well as any advice we receive from the statisticians, vets and NACWOs.

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

To keep informed of advances in the 3Rs, we regularly check the NC3Rs website. We also receive updates from our animal unit and our NTCO about any changes. We also attend the workshops

organised by the NC3Rs and our university to keep informed of new advances in animal welfare and the 3Rs.