G. NON TECHNICAL SUMMARY (NTS)

Project title: Investigation of anti-insulin receptor antibodies as a potential therapy for extreme insulin resistance
Duration of project - years: 3
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: YES
(b) translational or applied research with one of the following aims:
   (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants: YES
   (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants: NO
   (iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes: NO
   (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 aims mentioned in paragraph (b): NO
   (d) protection of the natural environment in the interests of the health or welfare of man or animals: NO
   (e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work: NO
   (f) higher education or training for the acquisition, maintenance or improvement of vocational skills: NO
   (g) forensic inquiries: NO

Keywords:
Insulin resistance, antibodies, Donohue Syndrome, Rabson Mendenhall Syndrome, Insulin receptor

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The hormone insulin is critical to life and works together with the insulin receptor on the surface of cells to drive energy storage after nutrient consumption. Rare people with genetically damaged or "mutant" insulin receptors have an uncommon form of diabetes with extreme resistance to insulin that is very difficult to treat using currently available therapy. A great deal of information is available about the structure of the insulin receptor, and the consequences of such mutations, but to date, there has been no reliable way to directly bypass the blocking effect of the receptor mutations in affected people. As a result, death occurs in infancy or in the second decade of life for less extreme cases. Our work aims to use the power of specially created antibodies that bind and activate the insulin receptor in a different way to insulin, to overcome the lethal consequences of insulin receptor mutations. By investigating how these antibodies are able to activate damaged receptors, and by testing whether they can lower blood glucose in mice with the same mutations that we see in human patients, we aim to take a major step towards developing these antibodies as potential life-saving new treatments for this group of conditions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?
This research aims to test mouse antibodies as a potential novel treatment of severe, life-threatening forms of diabetes caused by mutated forms of the insulin receptor. The results of these studies will be a key step in helping to decide whether the antibodies should be developed further and humanised to enable their use in people. If the antibodies show promise, a further potential benefit may even be that they are developed for use in a wider range of clinical settings and more common forms of diabetes. A secondary potential benefit will be increased knowledge of the molecular mechanisms by which some insulin receptor mutations that don’t have obvious functional defects when studied in vitro cell models cause severe disease in humans. Understanding of these mechanisms may potentially identify further ways to overcome the effects of these mutations leading to other therapeutic strategies.

What types and approximate numbers of animals do you expect to use and over what period of time?
Over 3 years, approximately 770 mice will be used.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?
These mice will be genetically altered such that the normal insulin receptor can be removed from their livers with a single injection. Mutant insulin receptors will then shortly afterwards be ‘added back’ to the liver with a second injection. Re-introduction of the mutant insulin receptors will permit the degree of impact that each mutation has on insulin receptor function and glucose metabolism to be tested, enabling a baseline to be established before the antibody treatments are tested. The most invasive procedure in living mice will be injections into veins, or blood sampling from veins. This will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. We are expecting to see high insulin and maybe high blood glucose levels once the insulin receptor has been knocked out of the liver, however prior studies of liver-specific insulin receptor knockout suggest that diabetes will not be a threat to wellbeing. Hypoglycaemia due to antibody administration is unlikely at low doses. At higher doses of antibody, sucrose can be included in the injection to avoid any transient hypoglycaemia associated with anti-insulin receptor antibody injection. At the end of the study, and to ensure the maximum information is gained from every mouse and each experiment, animals will be humanely sacrificed so their livers and other key tissues can be examined.

Application of the 3Rs
Replacement:

We have already conducted extensive in vitro studies in two separate cell line models to assess the ability of antibodies to activate insulin receptor mutations and have learnt as much as we can from observing the problems of humans with the same gene changes. Together with the observation that some mutant receptors don’t have obvious impairments when studied in cell in vitro models but do cause severe disease in humans, confirms that cellular studies are incomplete surrogates for assessing in vivo insulin action as they cannot represent a whole-body system which includes variable, pulsatile insulin exposure, and involves extensive receptor turnover and recycling. Thus we are at a point where studying the glucose-lowering effects of the antibodies in a living creature is an essential next step. This cannot be done in rare, very unwell babies without extensive prior in vivo
Testing, and because we require the study of specific mutant insulin receptors, the animals used have to be amenable to genetic manipulation. Thus, genetically modified mice represent the only practical in vivo model.

Reduction:

We shall minimise the number of animals first with good experimental design that relies on injections of genes rather than requiring extensive breeding of new animals. This approach substantially reduces the amount of breeding and numbers of animals required and avoids problems due to the primary genetic defect. We shall also rely heavily on the extensive experience in our animal centre with the measurements we shall be making. Because we have a good understanding of the variability in our measurements, we can be precise in using power analysis software in calculating exactly how many animals we need to show a clinically significant effect size. Together with the refinement of the experimental model, techniques and animal environment (outlined below) this will prevent unnecessary experiments and therefore reduce the number of animals required.

Refinement:

Along with the use of appropriate anaesthetics and analgesics, animal suffering will also be minimised by having tests performed by experienced staff familiar with the protocols which will further reduce pain and stress to the animals. Separate to this, as much as possible the cage environment will be enriched as to encourage normal mouse behaviour, thereby reducing stress of the housed animals. Our experimental design of injecting genes will avoid the liver pathologies reported previously with congenital insulin receptor knockout mouse models thereby minimising any potential adverse effects. Furthermore, utilising this approach, the time frame from inducing liver knockout of the insulin receptor and performing the experiments will be minimised thus reducing the time the mice will display a diabetic phenotype.