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NON-TECHNICAL SUMMARY

The impact of ageing and diet on connective tissue chemistry.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Mice	adult, aged, juvenile

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?

We aim to understand how the chemistry of connective tissue changes with age and also how diet might impact this process. We are particularly interested in the chemical changes which occur in collagen and elastin.

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?

It is unclear from the literature what the most important chemical and biological changes that occur in ageing tissue are (chemical changes are ones that occur spontaneously when two reactive molecules come into close proximity to each other, biological changes are ones driven by the biological conditions/environment, for example an enzymatically catalysed reaction which only occurs when a specific enzyme is produced). It is self-evident that changes occur with age which alter the characteristics of tissues, we can all see and feel these changes, but there is a surprising lack of clarity in the description of what chemical and biological changes are actually occurring at a molecular level.

We have shown in our own research (submitted for publication) that tendon collagen chemistry is much more dynamic than previously thought, changing in response to stretching, and that the chemistry that underlines this also changes with age. We have found that circulating glucose levels can impact the chemistry and physical properties of tendon which has led to an interest in whether diet also impacts the ageing process. Different tissues have different chemical profiles which reflect the different functions required from the tissue, for example tendon has a quite different function to bone or the wall of the aorta which is seen in the chemistry found in these tissues. In the work we plan to carry out under this licence we wish to continue to study connective tissues to understand how the changes in the chemical properties seen as the result of ageing and diet impact the normal functioning of the tissue. Understanding this may lead to improvements in treating diseases such as diabetes and cystic fibrosis, as well as potentially providing a rationale for strategies for healthy ageing or tissue repair.

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

We will publish the results of our animal work in respected peer reviewed journals.

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

A better understanding of the processes involved in ageing could ultimately help in both treatment and prophylaxis of diseases associated with normal ageing. It is expected that this project will lead to the development of methods to study ageing tissues and identify the important changes which occur as we age. Ultimately we aim to transfer the methods developed here to study the ageing of tissues in humans.

It is likely that changes in connective tissue chemistry impact many disease processes. For example, changes in crosslinking and glycation in the collagen of arterial walls are expected to lead to increased stiffening leading to increased blood pressure, an increased incidence of aneurisms and arterial valve stenosis. With respect to ageing, the stiffening of joints and tendons would also be expected as a consequence of increased collagen crosslinking and glycation. The treatment of aspects of diseases not necessarily liked to ageing, such as lung damage in cystic fibrosis and poor wound healing and tendon ruptures in diabetes may also benefit from this research.

The tissues generated in this project will be stored and made available to other researchers interested in the ageing of connective tissue, providing a valuable and unique resource of isotopically labelled and chemically profiled tissues of different ages. This will be done through personal contact with other researchers in this field of research. It is reasonable to expect that other researchers studying normal ageing processes would find this resource valuable. We also try and time our experiments so that other researchers within the organisation can make use of fresh tissues when we kill aged animals, such as lymph nodes, bone marrow etc.

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

We collaborate with other researchers on the ageing of connective tissue and give presentations on our latest results when appropriate. This is the best way to disseminate information on approaches that do not work.

Species and numbers of animals expected to be used

- Mice: 650

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 are studying the chemical changes which occur in connective tissues (primarily to collagen and elastin) with age. As such we require mice of all ages. Ageing mice are extensively used as a model for human ageing because they show the same attributes of ageing as seen in humans, but over a shorter time frame.

We have shown previously in tissue from db/db mice that higher levels of glycation are seen without increases in other crosslinking. This is required to understand the mechanism and impact of collagen glycation on function without the complication of other increases in crosslinking typically associated with changes in tissue function.

Nrf2-/- mice are required to test a hypothesis that glutathione levels play a role in crosslink reduction in elastin found within the extracellular matrix.

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

The mice will be kept for up to an age of 130 weeks and fed balanced diets. In some cases mice will be put under a light general anaesthesia so that ultrasound measurements can be made to determine the stiffness of the cardiovascular system. These cardiovascular measurements may be carried out up to 4 times with a minimum period of 4 weeks between each measurement.

Mice will be kept under standard animal house conditions up to an age of 130 weeks.

db/db mice and Nrf2-/- mice will be purchased at various ages and kept for the minimum period of time to allow a single cardiovascular ultrasound measurement to be obtained from each animal. This will be no longer than 1 month.

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

There are no specific impacts or adverse effects expected on wild type mice ageing healthily during this project. The majority of the animals used on this project will be wild type. Animals that are developing irreversible ill health conditions will be killed before they exceed a mild threshold because we are interested in healthy ageing and not the ageing of diseased or unhealthy animals.

We have kept mice routinely to 98 weeks with an upper limit of 104 weeks and found that these animals have been remarkably healthy and could have been kept longer without causing harm.

Two standard genetically altered strains from commercial sources will be used in addition to the wild type animals. One strain (db/db) is diabetic and the other (Nrf2-/-) has a poorer immune system linked to a mutation that causes reduced levels of glutathione. These will be monitored closely and kept for the minimum time required to made a single cardiovascular ultrasound measurement, and no longer than 1 month. These animals will only be fed a normal standard diet typically used in the animal unit.

The animal house staff are trained and experienced in the recognition of signs of ill health found in ageing animals.

Animals are inspected during routine cage cleaning with in hand inspection weekly for mice over 52 weeks old.

The staff will be looking particularly for eye abnormalities, malocclusion, over grooming, ulcerative dermatitis, abscesses, rectal prolapse, weight loss (a potential sign of other underlying problems), neurogenic symptoms (fits / seizures / spinning / head tilt), abdominal swelling. These are conditions that could develop as a result of old age and diseases expected to be found in old age.

Any animals showing signs of illness which, in the opinion of the NVS, cannot be ameliorated with minor interventions will be killed using a Schedule 1 method.

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)?

The mice are not expected to exceed a severity of Mild.

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?

The ageing of tissues is a complex process involving a multitude of cell types and factors. For example, a blood vessel like the aorta is a complex highly organised structure of cells, collagen, elastin and other connective tissue components. The blood vessel wall is not believed to have a fixed composition at a molecular level, changing with age and in response to factors such as blood pressure. Similarly, the structure of other tissues such as tendons and bone respond to stresses and age related factors which it is not yet possible to reproduce in culture.

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

There are no alternatives that I am aware of that could be used to study changes to the chemistry of connective tissue during ageing.

Why were they not suitable?

There is no alternative model that has the complexity of the interacting biological processes found in animals during ageing.

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 is based on the experience of running a project over the last 5 years and typical animal usage from our records. We involve a professional biostatistician in the design of experiments to minimise the

use of animals wherever possible. Many of the animals used are to provide fresh aged tissues for study after they have been killed.

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

We typically start experiments with a small number (5 to 10) animals in each group, collect the required data and then collaborate with a statistician to decide if we need to increase the number of animals used. We then collect data from additional animals and add this to the original data set, if appropriate.

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

Where possible we keep the carcasses of the animals that we use so that we can sample tissue from different organs. This helps to minimise animal usage and allows us to build up a picture of changes in the connective tissue across the whole body of individual animals. For example, this potentially will allow us to correlate changes seen in skin with changes in the aorta, tendon or bone.

Where old and young animals are killed by other researches for tissues and the carcasses are unwanted, we often take these and keep the remaining tissues that could be of use in our 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.

The mice are allowed to age normally under this project and so they should not experience anything that a normal healthy mouse would not.

Where we require cardiovascular ultrasound measurements the mice will be put under general anaesthesia.

We have limited the time that commercially available genetically modified animals can be kept to minimise the potential for suffering or distress. The genetically modified strains required are extensively used by others and the phenotypes characterised.

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

We are trying to understand why connective tissue ages and relate these changes to those seen in humans. The animals chosen are required to have a biology that is as close to human biology as possible. Mice fit this requirement.

The extracellular matrix found in non-mammalian species is chemically different to that of mammals which makes them unsuitable as an alternative. The physiological environment within the extracellular matrix, organ structure and diet also effect the chemistry of the matrix. These are too dissimilar in non-mammalian species.

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

The mice should not experience any harm.

In this project we use two strains of commercially available genetically modified animals. One strain is diabetic (db/db) and the other has reduced glutathione levels (Nrf2-/-) with increased risk of infection. These two strains of mice will be kept for less than 1 month to enable the required measurements to be made and minimise the potential for harm to the animals.

Neither the db/db mice nor the Nrf2-/- mice will be fed altered diets. The db/db mice will not be fed diets containing raised levels of sugars.

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

The NC3Rs website contains a wealth of information on best practices, experimental design and animal welfare.

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

Through reading of literature and listening to presentations on advances in the 3Rs.