Scottish Government Health Directorates Chief Scientist Office

CHIEF SCIENTIST OFFICE

FOCUS ON RESEARCH

Developing and evaluating novel gene therapy approaches in Rett syndrome.

Researchers

Dr Stuart Cobb, Dr Mark Bailey and Dr Ralph Hector

Aim

Rett syndrome (RTT) is a severe neurological disorder and leading cause of intellectual disability in females. The disorder results from mutations in the *MECP2* gene. In this study we aimed to develop a gene therapy approach in which a virus is engineered to reintroduce a healthy copy of the gene in cells and thus restore normal function. The project aimed to test the effectiveness and safety of this approach in genetically modified mice modelling human Rett syndrome.

Project Outline/Methodology

Mouse models which recapitulate the main neurological features seen in RTT were established. These included a knockout mouse model (in which the gene is silenced) and knock-in mice in which the most common *MECP2* mutation affecting patients was introduced. Gene therapy molecules were designed, packaged into a therapeutic carrier virus (known as adeno-associated virus 9; AAV9) and used for treatment. Mice were scored for a range of neurological features to assess the therapeutic potential of the therapeutic molecules as well as their safety profile and the spread of the virus.

Key Results

Our initial experiments established that MeCP2 protein is found at the highest levels in the brain and that the vast majority features that characterise RTT are due specifically to loss of functional MeCP2 within nervous system. These results confirmed that the brain should be the primary target for RTT therapies.

Our first generation gene therapy molecules produced modest therapeutic actions but had unacceptably narrow therapeutic windows when tested at higher doses. Our second generation design incorporated additional elements (DNA sequence which normally regulates levels of MeCP2) and when tested in mice, resulted in a much improved safety profile including reduced liver toxicity.

Peripheral administration of the second generation design (via the bloodstream) was safe but had very modest therapeutic action due to low levels of the gene reaching the brain. In contrast, direct brain delivery produced a profound amelioration of neurological signs suggesting that this route of delivery is likely to be required for human translation.

Finally, we showed that the presence of existing but abnormal MeCP2 protein (resulting from the gene mutation) does not impede the therapeutic potential of gene therapy. This was a hypothetical problem for gene therapy but our results suggest that this is not a concern.

Conclusions

We have developed a gene therapy molecule that can ameliorate neurological features seen in mice modelling RTT and which has an improved safety profile over first generation molecules.

What does this study add to the field?

This study provides proof-of-concept for the application of gene therapy in RTT. It shows that predicted concerns over the therapeutic effectiveness of gene replacement can be disregarded. However, it also highlights the need to optimise the gene therapy molecule to extend the therapeutic window and improve safety.

Implications for Practice or Policy

The results of the study are driving discussions (pre-IND) with regulators to identify preclinical safety and efficacy data that will be necessary for clinical development of gene therapy for RTT.

Where to next?

We are seeking translational funding to develop the gene therapy so that it will be attractive to commercial partners.

Further details from: Dr Stuart Cobb, stuart.cobb@glasgow.ac.uk