

SUMMARY OF FINAL REPORT

Degenerative ataxias and the potential for stem cell neuroprotection

Principal researchers: Dr Wilkins and Professor Scolding, Institute of Clinical Neurosciences, University of Bristol

Dates of project: August 2007 – July 2008

Background and aims:

This research project explores the potential for bone marrow-derived stem cells as a therapy for degenerative cerebellar ataxias and similar diseases. There has been much emphasis in the last few years on the potential for using stem cells to treat many different medical conditions, and this has been reflected in many advances in our understanding of the behaviour of stem cells. However, to date there has been limited research in this field relating to the ataxias. This project studied the potential for using adult-derived human bone marrow stem cells as a future treatment for degenerative ataxias. One of the advantages of using bone marrow stem cells is that bone marrow transplants have been used for many years for the treatment of leukaemia and there is therefore much information available on the safety of their use in humans.

This project represents the first step in a long-term strategy, the aim of which is to eventually bring stem cell-based therapies to clinical trials for the ataxias. For these experiments, cerebellar cells were taken from rodents and grown in culture. These nerve cells were subjected to toxins which cause damage (trying to replicate damage seen in the ataxias) and the effect of the presence of stem cells on survival of the nerve cells was tested.

Results:

We investigated mechanisms by which bone marrow-derived stem cells promote cerebellar neuronal and Purkinje cell survival *in vitro*, either through the process of replacement of damaged nervous tissue; or the expression of neurotrophic growth factors/cytokines. We established embryonic rat cerebellar neuronal cultures using serum-free culture conditions (with >95% of cells positive for β III-tubulin at 5 days *in vitro*). Using this model we have consequently studied the effect of nitric oxide and withdrawal of trophic factors on cerebellar neuronal/Purkinje cell survival and demonstrated significant cell loss after these insults. (*Neuronal survival was determined by immunohistochemistry using antibodies against β III-tubulin and Hoescht nuclear marker. Purkinje cell survival was assessed using antibodies directed against calbindin-1 (Calbindin-D28K).* Furthermore we have also

demonstrated that the addition of human bone marrow-derived stem cells during toxic insults, either in direct co-culture or through the production of soluble factors/cytokines with no cell-cell contact, can provide a significant level of neuroprotection to both cerebellar neurons as a population and Purkinje cells specifically, by improving cell survival. Thus, preliminary data has shown that both contact with stem cells and soluble factors produced by cells improve cerebellar neuronal survival (and Purkinje cell survival) when neurons are exposed to nitric oxide (simulating oxidative stress and mitochondrial energy depletion) and trophic factor withdrawal.

Subsequently, using the MEK/ERK and PI₃kinase/Akt pathway inhibitors, PD 98059 & LY294002 respectively, the effects of soluble factors, produced by human bone marrow-derived stem cells, on neuronal cell survival have been shown to require intact PI₃kinase/Akt intracellular signaling pathways, and not MEK/ERK pathways. This determines a specific cellular pathway by which bone marrow-derived stem cells promote cerebellar neuroprotection. PI₃kinase/Akt is of importance in growth factor mediated cerebellar survival and defects in these signalling pathways may underlie the process of neurodegeneration in a number of ataxic conditions.

The integration of human bone marrow-derived stem cells directly added to cerebellar culture has been assessed immunocytochemically using human-specific markers (Human Golgi Zone) and a panel of neuronal markers. Long-term (eight week) cultures have shown high levels of integration between both bone marrow derived stem cells and cerebellar neuronal cells *in vitro*. In addition, we have demonstrated that when human bone marrow-derived stem cells are co-cultured with rodent cerebellar neuronal cultures, these stem cells can be induced to express the neuronal marker β III-tubulin and the intermediate filament nestin. We are currently performing experiments to determine whether the process underlying this effect is fusion of nuclei (heterokaryon formation) or transdifferentiation of stem cells into neurons. We have also shown that stressed neurons, which have had trophic factor withdrawal for 24hours, significantly increase the number of human bone marrow-derived stem cells expressing β III-tubulin when the two cell populations are co-cultured, suggesting human bone marrow-derived stem cells may be more likely to differentiate into neurons or fuse with neurons when presented with an environment of stressed neurons. This is potentially important in disease models and in ataxia itself, in which the environment may induce repair through neuronal differentiation of bone marrow-derived stem cells.

To date we have carried out vital initial research demonstrating that in cell culture human bone marrow-derived stem cells are able to improve the survival of cells derived from the cerebellum. We have also shown that these stem cells can be induced to express neuronal markers, thus establishing that bone marrow derived cells may be able to replace lost brain cells or integrate with existing neurons. Stem cell therapies hold much therapeutic promise in a variety of neurodegenerative diseases and these studies are now at a critical stage. We therefore wish to investigate this further by developing human bone marrow-derived stem cell transplantation in a mouse model of Friedrich's ataxia, and hope this will ultimately lead to the establishment of clinical trials of stem cell therapies.

Lay summary:

For these experiments, nerve cells were taken from the cerebella of rodent brains and grown in culture. These nerve cells were subjected to damaging toxins and the effect of stem cells on the survival of the nerve cells was tested.

We found that after exposure to damaging toxins, nerve cells that were grown in the presence of stem cells were less likely to die than those that were grown in their absence. The results show that human bone marrow-derived stem cells are able to protect nerve cells that have been exposed to damaging toxins. Our investigations also revealed the nature of protective mechanism that is relayed within the nerve cells, by identifying parts of the signalling pathways involved.

The results demonstrate that bone marrow-derived stem cells are potential therapeutic agents for cerebellar ataxia and other ataxic conditions. This provides the necessary first step in designing stem cell-based therapies for the treatment of degenerative ataxias and, in time, we hope that the transplantation of bone marrow-derived stem cells may offer an effective therapy for neurological injury.

Benefits to people with ataxia arisen/likely to arise from this research:

These results are promising because they suggest that stem cell therapies may be beneficial for the treatment of the ataxias. This study is being followed up by further investigations in a mouse model of the disease. In time, we hope that the transplantation of bone-marrow derived stem cells may reach clinical trials and offer an effective therapy for progressive ataxias as well as other neurological injuries.

Publications arisen from this project:

A draft manuscript has been prepared ready for submission.

Conferences/ meetings where this research has been presented:

Ataxia UK/FASI conference Dublin 2008

For more support or information please contact: Ataxia UK, Lincoln House, Kennington Park, 1 – 3 Brixton Road. London SW9 6DE

Website: www.ataxia.org.uk.

Helpline: 0845 644 0606 **Tel:** +44 (0)20 7582 1444 **Fax:** +44 (0)20 7582 9444

Email: helpline@ataxia.org.uk