Final Report

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Title of Project: **Using cells derived from teeth to promote functional recovery following a stroke**

Summary:
Stroke most commonly results from blockage of an artery in the brain. The resultant reductions in blood flow cause the localised death of cells within the affected tissue (generating an infarct) that leads to the impairment of functions normally controlled by that part of the brain. Many people affected by stroke show improvements in function within the first few weeks to months after onset. Nonetheless, recovery is usually incomplete and major disabilities often persist. Studies in animal models of stroke during the last decade indicate that the spontaneous recovery is at least partly due to changes in growth and connectivity of nerve cells in the "peri-infarct tissue" immediately surrounding the region of damage. These changes allow the surviving nerve cells to take on some of the functions of cells that have been lost.

There is currently considerable interest in developing treatments that can promote these adaptive responses and further improve recovery. A particularly promising approach involves the administration of multipotent or pluripotent stem cells either directly into the brain or via the circulation. Some of these cells migrate to the region of damage and can contribute to improved recovery. The improvements do not primarily involve a direct replacement of lost nerve cells by progeny of the stem cells but rather are due to the release of molecules or other signalling mechanisms induced by these cells that promote adaptive changes in the peri-infarct tissue.

Dental pulp from human teeth provides a source of stem cells with properties that make them potentially very attractive for future treatment of stroke. These cells are relatively easy to access in adult humans and have a high proliferative capacity allowing rapid generation of sufficient cells for treatment. Furthermore, the research team led by Professor Simon Koblar (South Australian Health and Medical Research Institute), a collaborator on this project, has shown that these stem cells can generate multiple types of cells including neural cells under selected growth conditions and has provided initial evidence that these cells can improve recovery in an animal model of stroke.

The main aim of the present project was to test the hypothesis that human dental pulp stem cells can improve functional recovery in a rat model of stroke that mimics

several key features of stroke in humans. We further hypothesised that improvement would be associated with early modifications to the responses of the astrocytes in the peri-infarct tissue. Astrocytes play key support roles in the brain and are important contributors in the response of brain tissue to injury. Although the responses of these cells are helpful in limiting the spread of damage, there is evidence to suggest that they also restrict potentially useful adaptive responses of neighbouring neurons in disorders involving extensive tissue damage including stroke.

We used a photothrombotic model of stroke in which local disruption of flow in blood vessels was induced non-invasively using a light sensitive chemical to produce a well-defined infarct within the region of sensorimotor cortex that controls forepaw function. The lesion occupied a small proportion of the brain, mimicking those strokes that are more likely to be associated with restoration of some function in humans. The technique also produced a permanent loss of blood flow and thus effectively mimicked the permanent disruption or markedly delayed restoration of blood flow that occurs in a majority of human strokes. Stem cells in culture medium or medium alone were injected into the infarct and into brain tissue immediately caudal to the infarct at three days after stroke induction (9 rats per treatment group).

Forepaw function was assessed over four weeks using three tests: (i) a skilled reaching test in which animals were pre-trained to collect food pellets through a narrow slit using the forelimb that was subsequently affected by the stroke; (ii) a placing test in which whisker stimulation was used to stimulate placement of a forelimb on an adjacent ledge; (iii) a cylinder test which assessed spontaneous forelimb use during rearing in a Perspex cylinder.

All functions were greatly impaired within the first 24 hours of stroke induction. At this time, there was almost complete loss of successful skilled reaching and of the placing response with a lesser reduction in use of the affected limb in the cylinder test. Over the next four weeks, substantial recovery was seen for all three tests in both groups. However, recovery of forelimb placing was significantly faster in animals treated with the stem cells compared to the medium-treated control rats ($p < 0.05$). This difference was largest at 14 days after stroke induction and then diminished as the performance of rats in both groups approached that seen prior to the stroke. There was no statistically significant difference in recovery on the placement test or in spontaneous forepaw use assessed in the cylinder test. The spontaneous paw use recovered more rapidly and completely than the other two measures and thus was felt not to be a useful discriminator for the purposes of this study.

The responses of astrocytes to the treatment was evaluated in peri-infarct tissue from rat brains removed at seven days after stroke (and four days after stem cell treatment) based on the content of selected proteins (vimentin, glial fibrillary acidic

protein and neurocan) that are markedly upregulated as part of the astrocytic response to injury. There was no significant difference in the content of these proteins assessed from Western blots between rats treated with stem cells and the control rats. Thus, the improvements in recovery were apparently not associated with early changes in the astrocytic response to tissue damage, at least as assessed from these widely-used marker proteins.

In summary, the findings demonstrate that delayed treatment with dental pulp stem cells improves recovery of a complex learned function in a rat stroke model that has features relevant to many human strokes. They substantially strengthen the case that dental pulp stem cells could provide an effective treatment for stroke. The results provide a sound basis for further investigations to optimise treatment conditions, understand the mechanism of damage and define the types of stroke-related impairments that might be particularly responsive to this treatment.

The findings suggest that the stem cell treatment was more effective in promoting recovery of a complex learned task than a behaviour that involved a direct motor response to tactile stimulation and did not require training. In other unpublished studies involving drug treatments directed at improving recovery from stroke, we have also seen differential consequences for performance on these two tests. In particular one drug also substantially improved skilled reaching following stroke but significantly impaired performance in the placing test. Together these results point to differences in sensitivity of even closely related functions to changes induced in the brain by treatments. Further studies are needed to better understand the cellular and molecular basis of these differential responses and to assess their implications for treating human stroke.

Hypothesis vs Findings

The findings supported the primary hypothesis that treatment with dental pulp stem cells can improve recovery following stroke. However, the second hypothesis that this would involve modifications of the responses of astrocytes, within the first few days of treatment was not supported. The analysis of astrocytic responses was restricted to the initial four days after injection of the stem cells. Thus, we cannot rule out the possibility that the responses of these cells might have been modified at later times.

Unanswered Questions

This study did not provide insights into the mechanism underlying the better recovery following stem cell treatment. Brains removed from rats at the conclusion of the behavioural analysis (28 days) will be evaluated, as part of ongoing studies, to identify possible differences in protein expression or other cellular responses that could have contributed to the improved rate of recovery in the stem-cell treated rats. This information will guide further studies of the underlying mechanisms.

What these research outcomes mean

As discussed in the summary, these investigations demonstrate that delayed treatment with dental pulp stem cells improves recovery of a complex learned function in a model of stroke exhibiting features that are relevant to many strokes in humans. The findings also add to evidence that neurological functions affected by stroke may be differentially responsive to treatments.