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The neuroprotective effect of bone marrow stem cells is not dependent on direct cell contact with hypoxic injured tissue

Anna Sarnowska,[a,c] Holger Braun,[b] Steven Sauerzweig,[b] Klaus G. Reymann[a,b]

[a]Research Institute for Applied Neuroscience, (FAN) GmbH, Leipziger Str., 44, D-39120 Magdeburg, Germany, [b]Leibniz Institute for Neurobiology, Brenneckestrasse, 6, D-39118 Magdeburg, Germany, [c]NeuroRepair Department, Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Correspondence : Anna Sarnowska, Leibniz Institute for Neurobiology, Project Group of Neuropharmacology, Brenneckestrasse, 6, D-39118 Magdeburg, Germany, E-mail: a_sarnowska@tlen.pl

Gillipsie Minhas

Neuroscience Research Lab, Department of Neurology, Post Graduate Institute of Medical Education and Research, Chandigarh, INDIA

Background

Numerous studies have shown that the pharmacological neuroprotection has failed to prevent damage following stroke in clinical trials.[1] The focus has shifted to new strategies to involving replacement of dead cells after injury. Stem-cell based therapy can be an important supportive strategy in brain protection and repair. The bone-marrow derived stem cells have been shown to confer beneficial effects after transplantation into the animals with ischemic brain injuries. Animal studies have shown that transplanted stem cells improve functional deficits after stroke.[2,3] However, it is unclear whether the beneficial effect is associated with stem-cell differentiation into neural cells or is induced by the release of secreted growth factors, which are capable of stimulating endogenous repair mechanisms and improving functional deficits.[4-6]

Study Design

The study was designed to determine whether the neuroprotective effects of BMSCs in ischemic brain injuries are dependent on direct cell-cell contacts.

Sarnowska *et al* used an *in-vitro* model of hippocampal organotypic slice cultures (OHCs). These OHCs were subjected to oxygen-glucose deprivation (OGD) to mimic the ischemic injury *in-vitro*. OHCs were prepared from hippocampus slices of 7-9 day old rats. The OHC slices were maintained in serum-based medium for 2-3 days and thereafter cultivated in serum-free medium for 14 days. To mimic the ischemic injury, the authors subjected the culture to oxygen-glucose deprivation by setting up culture in mannitol saturated medium under anaerobic conditions. BMSCs were collected from the femurs of 5-week old rats and were cultured to 80% confluence. The authors designed two paradigms to evaluate the neuroprotective effects of BMSCs. In one design, approximately 10^5 CFDA labeled BMSCs were transplanted into one-week-old, OGD-treated OHCs, while in the other paradigm, OGD treated OHCs and cells were cultivated in same medium, separated through a membrane but without direct cell-cell contact. Both populations of BMSCs were compared with a mixed cortical primary culture prepared from embryonic rats.

The cell death was quantified by adding fluorescent cell death marker, PI 24h prior to experiment and measuring the intensity. The cell viability was also characterised by immunochemical analysis for cytochrome c and caspase 3. The authors also analysed cells and hippocampal slices immunocytochemically for GFAP, Nestin, MAP2, NG2, TUJ1. The mRNA expression level for various growth factors such as NGF beta, IGF-1, bFGF, was analysed using PCR.

Implications

Sarnowska *et al* have elucidated the potential for BMSCs to confer neuroprotection within a short period of time (24h), and thus excluding any chance of differentiation into neural cells. Thus, the authors concluded that the neuroprotective effect evoked shortly after the ischemia injury is not dependent on the cell-cell contacts and involves the secretion of growth factors like NGF, bFGF and IGF. The ischemic tissue is also involved in induction of growth factor production, thus demonstrating a bilateral interaction between transplanted BMSCs and ischemic injured tissue. This implied that in clinical practice bone marrow stem cells could deploy beneficial effects even when applied intravenously after stroke. Even if the BMSCs do not reach the infarcted area, therapeutic effects can still be expected. This information is of clinical relevance because the most appropriate route for cell delivery is actively debated.

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