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Research Report

The treatment of TBI with human marrow stromal cells impregnated into collagen scaffold: Functional outcome and gene expression profile

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ABSTRACT

We have previously demonstrated that human marrow stromal cells (hMSCs) embedded in collagen I scaffolds significantly enhance the restorative therapeutic effect of hMSCs after traumatic brain injury (TBI). In this study, we test the hypothesis that the collagen scaffold alters gene expression in hMSCs and that hMSCs impregnated into scaffolds increase the astrocytic expression of vascular endothelial growth factor (VEGF) in the injured brain. Following TBI induced by controlled cortical impact injury, scaffold with hMSCs (3.0×10^6), hMSCs-only and saline were implanted into the lesion cavity one week after brain injury (n=8/each group). Morris water maze and modified neurological severity scores were performed to evaluate the spatial learning and sensorimotor functions, respectively. Lesion volume and expression of VEGF were measured one week after different treatments. In vitro, total RNA from hMSCs was extracted one week after culture with or without collagen I scaffold for evaluation of gene microarrays. Furthermore, an RT-PCR study on a select subgroup of genes was performed to identify the changes of expression between the culturing hMSCs with collagen scaffolds and hMSCs only. The treatment of TBI with collagen scaffold impregnated with hMSCs significantly decreases the functional deficits from TBI within 7 days after treatment, and significantly enhances the VEGF expression of astrocytes in the injured brain compared to the hMSCs-only group. In vitro data indicate that collagen scaffolds stimulate hMSCs to express multiple factors which may contribute to hMSC survival, tissue repair and functional recovery after TBI.

1. Introduction

Traumatic brain injury (TBI) remains a major health problem worldwide. In the USA alone, the incidence of closed head injuries admitted annually to hospitals is 200 per 100,000 (Narayan et al., 2002). Despite extensive research, no effective clinical treatment has been found to repair the biostructural damage resulting from TBI. Neurorestorative treatments for

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neural injury have taken essentially two paths, cellular and pharmacological (Mahmood et al., 2005). Cellular therapy has advantages over pharmacotherapy in that the interaction between exogenous cells and endogenous cells is dynamic and sensitive to the microenvironment (Chen et al., 2002). Marrow stromal cells (MSCs) have shown efficacy in improving functional outcome after TBI by direct intracerebral as well as systemic administration (Lu et al., 2001; Mahmood et al., 2001a, 2002, 2003, 2005, 2006). We have employed collagen scaffolds populated with human marrow stromal cells (hMSCs) to treat rats subjected to TBI and found reduction of lesion volume and improvement of functional outcome (Lu et al., 2007). However, the mechanism by which the scaffold augments functional recovery has not been investigated.

One of the major mechanisms by which hMSCs promote neural function is by induction of growth factors such as nerve growth factor (NGF), vascular endothelial growth factors (VEGFs) and brain-derived neurotrophic growth factor (BDNF) (Lu et al., 2002, 2004). hMSCs produce these growth factors and more importantly induce growth factors within parenchymal cells (Mahmood et al., 2004, 2005). Growth factors influence different aspects of neurogenesis, synaptogenesis and angiogenesis (Bibel and Barde, 2000; Huang and Reichardt, 2001; Palmer et al., 2000; Silverman et al., 1999). VEGF is an angiogenic factor and has multiple restorative effects (e.g. neurogenesis and axonal outgrowth) (Silverman et al., 1999; Sun et al., 2003). VEGF is a potent mitogen for endothelial cells and astrocytes, and promotes growth and survival of neurons (Silverman et al., 1999). In addition VEGF enhances neurogenesis in the adult brain, possibly via the establishment of a "vascular niche" that favors the proliferation and differentiation of neuronal precursors (Palmer et al., 2000; Silverman et al., 1999; Skold et al., 2005; Sun et al., 2010).

In the present study, we initially tested the functional changes both with the modified neurological severity score (mNSS) and Morris water maze (MWM) test. We then used immunohistochemistry to measure the expression of VEGF at one week after transplantation of hMSCs and scaffold+hMSCs in a rat TBI model. To test whether the gene expression profile is altered between hMSCs seeded into the scaffold and hMSCs-only in culture, we probed the interaction between hMSCs and scaffold in vitro using microarrays and real time polymerase chain reaction (RT-PCR).

2. Results

2.1. Neurological and sensorimotor functional responses

Injury in the left hemisphere cortex in rats caused neurological functional deficits as measured by mNSS. The higher the modified neurological severity score (mNSS), the worse the sensorimotor function. Fig. 1 shows the changes of sensorimotor function in injured rats after different treatments. There was no significant difference in the mNSS scores among scaffold+hMSCs, hMSCs alone and the saline group on days 1, 4, and 7 after TBI. However, treatment with scaffold+hMSCs significantly deceased the mNSS score on day 14 (7 days after transplantation, $P<0.0001$) compared to the hMSCs-alone and saline groups.

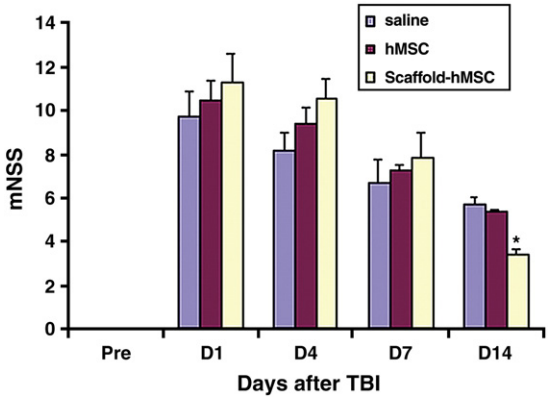

Fig. 1 – Functional improvement detected on the modified neurological severity scores (mNSS). The scaffold+hMSCs group showed a significant functional improvement on day 14 after TBI, compared with the saline (*$P<0.0001$) and the hMSCs-treated (*$P<0.0001$) groups. Data are presented as the mean±SD ($n=8$/group).

2.2. Spatial learning function changes

Spatial learning was tested during the last five days (days 10–14 post injury) using the MWM test without prior training before injury. TBI rats treated with scaffold+hMSCs spent significantly more time in the correct quadrant than those treated with saline or hMSCs only on days 12 ($P=0.016$ vs saline, $P=0.02$ vs hMSC), 13 ($P=0.04$ vs saline, $P=0.004$ vs hMSC) and 14 ($P=0.036$ vs saline, $P=0.018$ vs hMSC) after TBI (Fig. 2). These data demonstrate that scaffold+hMSCs improve spatial learning function after TBI more effectively than do hMSCs-alone or saline.

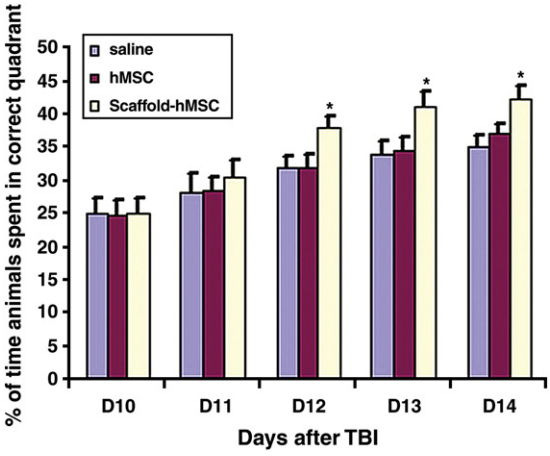

Fig. 2 – This figure shows the spatial learning function after different treatments. The scaffold+hMSCs treated group had a significant functional improvement from day 12 to day 14 after TBI, compared to saline and hMSCs-treated groups (day 12, $P=0.016$ vs saline, $P=0.02$ vs hMSC; day 13, $P=0.04$ vs saline, $P=0.004$ vs hMSC; day 14, $P=0.036$ vs saline, $P=0.018$ vs hMSC). Data are presented as the mean±SD ($n=8$/group).