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Human Dental Pulp-Derived Stem Cells Protect Against Hypoxic-Ischemic Brain Injury in Neonatal Mice

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Background and Purpose—Perinatal hypoxia-ischemia (HI) has high rates of neurological deficits and mortality. So far, no effective treatment for HI brain injury has been developed. In this study, we investigated the therapeutic effects of stem cells from human exfoliated deciduous teeth (SHED) for the treatment of neonatal HI brain injury.

Methods—Unilateral HI was induced in postnatal day 5 (P5) mice. Twenty-four hours later, SHED, human skin fibroblasts, or serum-free conditioned medium derived from these cells was injected into the injured brain. The effects of cell transplantation or conditioned medium injection on the animals’ neurological and pathophysiological recovery were evaluated.

Results—Transplanted SHED, but not fibroblasts, significantly reduced the HI-induced brain-tissue loss and improved neurological function. SHED also improved the survival of the HI mice. The engrafted SHED rarely differentiated into neural lineages; however, their transplantation inhibited the expression of proinflammatory cytokines, increased the expression of anti-inflammatory ones, and significantly reduced apoptosis. Notably, the intracerebral administration of SHED-conditioned medium also significantly improved the neurological outcome, inhibited apoptosis, and reduced tissue loss.

Conclusions—SHED transplantation into the HI-injured brain resulted in remarkable neurological and pathophysiological recovery. Our findings indicate that paracrine factors derived from SHED support a neuroprotective microenvironment in the HI brain. SHED graft and SHED-conditioned medium may provide a novel neuroprotective therapy for HI. (Stroke. 2013;44:551-554.)

Key Words: cell transplantation ■ functional recovery ■ inflammation ■ neonatal ischemia ■ stem cells ■ trophic factors

Materials and Methods

An expanded version of the Methods section is available in the online-only Data Supplement. SHED, human skin fibroblasts, and their serum-free conditioned medium (CM) were prepared as described. The SHED’s multi-differentiation potential and their expression of both mesenchymal stem cell and neural lineage markers were similar to those reported previously. HI brain injury was induced in postnatal day 5 (P5) mice as described. Cells (2×10^5) in 2 μL phosphate buffered saline or phosphate buffered saline alone (as a control) were transplanted into the ipsilateral hemisphere at 2.0 mm anterior and 2.0 mm lateral to bregma, and 2.0 mm deep to the dural surface, using a glass needle and a Kopf microstereotaxic injection system, 24 hours after HI (Figure 1A). These animals were given daily administration of cyclosporin A (Novartis, Nurnberg, Germany, 10 mg/kg, IP) throughout the experimental period, except when they were used for cytokine expression analysis. For the experiments using CM, mice were given a 2-μL injection of CM or Dulbecco’s modification of Eagle’s medium (as a control) without cyclosporin A treatment. The animals’ neurological recovery was examined by a foot-fault test in 4-, 6-, and 8-week-old HI mice. Tissue loss was examined by staining with hematoxylin and eosin, and brain injury was evaluated using a neuropathological scoring system, by an observer blinded to the identity of the animal group. The level of apoptosis was analyzed by staining with anti-caspase-3 (Cell Signaling). Real-time reverse transcription PCR was carried out as described. GAPDH cDNA was amplified as an internal control. Primer sequences are shown in the online-only Supplemental Table 1. Data were expressed as means±SEM. Survival data were analyzed by applying the Kaplan-Meier curve, followed by the Mental-Cox
log-rank test to identify differences between the curves. Behavioral data were analyzed by 2-way ANOVA. Comparisons of parameters among the groups were made by 1-way ANOVA. Post-hoc analyses were performed with Bonferroni test. All statistical analyses were performed with Stata version 11.0 (Stata Corp, College Station, TX). A value of $P<0.05$ was considered statistically significant.

Figure 1. Neurological outcomes and survival rate after HI. A, Experimental protocol. Mice underwent HI insult at P5, and then received cell transplantation (Cell) or CM injection (CM) at P6. In the analyses of cell transplantation’s effects on functional recovery and brain damage, the phosphate buffered saline-, Fb-, and SHED-treated groups received daily administrations of cyclosporin A (CsA). The cytokine assay and brain damage evaluation were performed at P7 and P8, respectively. Neurological recovery was examined at 4, 6, and 8 weeks. B, Survival curve (SHED n = 14; Fb n = 12; PBS n = 18; SHED-CM n = 16; Fb-CM n = 16; DMEM n = 16; Sham n = 5). C, Foot-fault test (SHED n = 12; Fb n = 9; PBS n = 9; SHED-CM n = 15; Fb-CM n = 10; DMEM n = 13; Sham n = 5). D, Statistic data. Values are means±SEM, *$P<0.05$, **$P<0.001$.

Figure 2. Histological evaluation. A, Representative HE-stained coronal brain sections. Scale bar: 1 mm. B, Cell-transplantation. C, Conditioned medium (CM)-injection. Tissue loss (each group, n=10–12); Caspase-3 (+) cells (each group, n=6); Pathological score (each group, n=5–8). D, Statistic data. Values are means±SEM, n=6 per group, *$P<0.05$, **$P<0.01$. 

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Results

The HI mice that underwent SHED transplantation exhibited significant neurological recovery compared with the fibroblasts- and phosphate buffered saline-treated groups (Figure 1C). The SHED-transplanted group also displayed better survival over time (Figure 1B). Histological examination revealed that the tissue loss, number of apoptotic cells, and neuropathological score in the SHED-transplanted group were significantly lower than in the other experimental groups (Figure 2A and B). Cell-type analysis showed that the apoptosis of neurons in the cortex, corpus callosum, and hippocampus and of oligodendrocytes in the corpus callosum was significantly reduced in the SHED-transplanted group (online-only Supplemental Figure 2).

The expression levels of proinflammatory cytokines interleukin-1β and tumor necrosis factor-α were upregulated in the phosphate buffered saline- and fibroblasts-transplanted groups 24 hours after HI, but those of anti-inflammatory cytokines interleukin-4 and interleukin-10 were downregulated. Notably, engrafted SHED significantly suppressed the expression of proinflammatory cytokines, whereas strongly upregulating anti-inflammatory cytokines (Figure 3).

Eight weeks after transplantation, little or no SHED had differentiated into neurons, oligodendrocytes, or astrocytes (online-only Supplemental Figure 3). Taken together, these results suggested that SHED promoted recovery after HI by paracrine mechanisms. In support of this idea, we found that mice receiving a 2-μL injection of SHED-CM in the HI-injured brain exhibited significant or better recovery in neurological function (Figure 1C), survival rate (Figure 1B), and neuropathological score (Figure 2C) than those receiving fibroblasts-CM or cell-culture medium (Dulbecco’s modification of Eagle’s medium) alone.

Discussion

Here we demonstrated that the transplantation of SHED into the HI-injured mouse brain improved the neurological outcome and survival rate. The engrafted SHED shifted the HI-induced proinflammatory state to an anti-inflammatory one and inhibited apoptosis and tissue loss. Importantly, mice receiving an injection of 2 μL SHED-CM 24 hours after HI exhibited significant recovery as assessed by both neurological and pathological examinations. These results suggest that most of the SHED-mediated therapeutic benefits were elicited by paracrine mechanisms. It was difficult to compare the level of therapeutic benefits between engrafted SHED and SHED-CM, because in the SHED experiments, the administration of cyclosporin A, which protects engrafted cells from the xenogeneic host immune response, significantly suppressed the HI-induced inflammatory response and apoptosis (online-only Supplemental Figure 4). Furthermore, cell transplantation may have an advantage in providing a prolonged delivery of paracrine factors, compared with the transient delivery by the CM treatment.

Previous reports indicate that the engraftment of various types of transplanted stem cells is a promising regenerative therapy for HI. However, for clinical use, mesenchymal stem cells must be expanded by a reliable cell-culture system that produces sufficient cell numbers to elicit clinical benefits, while also meeting safety requirements. These severe restrictions may impede the progress of regenerative therapy for HI. Our data suggest that the administration of SHED-CM provides a portion of the therapeutic benefit of SHED.
transplantation, and this finding may be useful in establishing a practical regeneration therapy for HI.

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Disclosures
None.

References