Administration of hATSCs results in recovery of cerebral infarction animal model

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Abstract
To examine pathway of stem cell transplanted to the brain, stem cells were infected with flourescence. hATSCs in the infarct region were mostly located at the border between intact brain tissue and the area of the infarction and in other sections within the infarct cavity. Examination of section with flourescence indicated that there was significant gliosis or infiltration of leukocytes around the implantation site of the stem cell. Implanted stem cell integrated and migrated to multiple areas of the brain including the contrallateral cortex. The cells persisted in the sites to which they migrated at 30 days after implantation. The heaviest concentrations of cells were transplanted into rats at 24hr after MCAO, more cells were migrated into injured area of brain cortex. Stem cell in the infarct region were found at the border between intact brain tissue and the area of infarction and within the infarct cavity.

Keywords: hATSC (human adipose tissue stromal cells), MCAO (Middle cerebral artery occlusion), stem cell, Implantation, Infarction.

1 | INTRODUCTION:

Pluripotent mesenchymal stem and progenitor cells have been detected in multiple tissues, including bone marrow, and umbilical cord blood. Under appropriate conditions, bone marrow stromal cells selectively differentiate into mesenchymal lineages. Recent studies have shown that bone marrow stromal cells can be induced to neuronal differentiation in vitro and in vivo. Neural tissue has long been regarded as incapable of regeneration and the identification of cell populations capable of neuronal differentiation has generated intense interest. Stem cells from embryonic tissue as well as adult brain are capable of undergoing expansion and neuronal differentiation in vitro and in vivo. Adipose tissue may represent an alternative source of cells capable of neuronal differentiation, potentially enhancing their usefulness in the treatment of neurological disease[1,2]. Adipose tissue, like bone marrow, is derived from the embryonic mesoderm and contains a heterogenous stromal cell population[3]. These similarities, together with the identification of MSCs in several tissues, make plausible the concept that a stem cell population can be isolated from human adipose tissue. Recently, MSCs isolated from adipose tissue has shown to be differentiated into multiple mesodermal tissues, including bone,
fat and muscle[4-7]. Therefore, adipose tissue has been identified as an alternative source of pluripotent stromal cells[8].

2 MATERIALS AND METHODS:

2.1 Focal MCAO injury model
Adult male Wistar rats weighing 250-300g were used in our experiments. The right common carotid artery, external carotid artery (ECA) and internal carotid artery (ICA) was exposed. A length of 4-0 monofilament nylon suture (18.5-19.5mm), determined by the animal weight, with its tip rounded by heating near a flame, was advanced from the ECA into the lumen of the ICA until it blocked the origin of the MCA. Two hours after MCAO, reperfusion was performed by withdrawal of the suture until the tip cleared the lumen of the ECA.

2.2 Intracerebral transplantation procedures
Cerebral ischemia rat (250-300g) were anesthetized in a sealed chamber using 5 % enflurane in oxygen. Anaesthesia was maintained by face mask of 2% enflurane. The animals were transferred to a stereotaxic apparatus in a clean field. A 2-to 5-mm incision was made in the scalp 1.5mm lateral to the bregma. A burr hole was made in the bone 3mm lateral to bregma with a dental drill, and about 10μl of the adenovirus infected cell suspension (1x10^5 cells) was slowly injected over 30min into the lateral ventrical at a depth 3.5mm from the surface of the brain by using a 10μl Hamilton microsyringe (Hamilton, Reno, NV). After injection, Hamilton syringe was left in place for an additional 5min before retraction. The wound was closed with interrupted surgical sutures.

2.3 Behavioral improvement tests

In all animal behavioral tests were assessed before MCAO and 7, 14 days after MACO with and without cell injection. For the measurement of somatosensory deficit, the adhesive-removal somatosensory test was measured both before and after. All rats were evaluated using a modified neurological severity score (mNSS). The mNSS is a composite of motor (muscle status, abnormal movement), sensory (visual, tactile, proprioceptive), reflex, and balance tests.

2.4 Statistical analysis
All data are expressed as the mean ± SD. Behavioral data were analyzed using repeated multiple ANOVA. P< 0.05 was considered statistically significant.

3 RESULTS:
Infarction localization of ischemic cerebral lesion
Normal brain (gray matter) tissue typically stains with TTC, but infarcted lesions show no or reduced staining. TTC staining obtained 4 weeks after MCAO without cell transplantation is shown in Figure 1 B. Note the reduced staining on the lesion side primarily in the corpus striatum. There was a progressive reduction in infarction size with hATSC treatment. Intracerebral delivery of hATSCs resulted in very substantial reduction in lesion volume as estimated from TTC staining. Cell treatment reduces MCAO-induced brain infarction. Representative TTC stained brain sections are shown where rats were injected with PBS or hATSC before MCAO. Infarct volumes in brains from PBS and hATSC treated animals are shown in the graph Figure 1 A,B).

Ischemic regions and migration of the cells after injection in the lateral ventricle
To examine pathway of hATSCs transplanted to the brain, hATSCs were infected with adenovirus containing LacZ. The migratory process, from the graft sites two migratory pathways were noted, on extending toward the midline of the brain and another extending toward or into the infarct area Figure 2. hATSCs in the infarct region were mostly located at the border between intact brain tissue and the area of the infarction and in other sections within the infarct cavity.
ADMINISTRATION OF hATSCS RESULTS IN RECOVERY OF CEREBRAL INFARCTION ANIMAL MODEL

**FIGURE 1:** TTC-staining from PBS treated group(A) and hATSC treated group(B) are shown.

**FIGURE 2:** A standard coronal section identified at the level of the anterior commissure of rat brain that hATSCs migrated to the right hemisphere into each subregion.

**FIGURE 3:** fMR imaging of infarction region showed in more white site in vitro.

**FIGURE 4:** Behavioral Adhesive-Removal Test & mNSS test are shown between control group and transplantation of hATSCs group in vivo.

**fMRI characteristics of infarction region**

White color of infarction region was shown mainly in corpus callosum & striatum. Figure 3 shows severe inflammation of infarction site with cytotoxic edema by diffusion coefficient in day 5 after MCAO. fMR imaging technique can be recognized specific infarction region in vitro.

**Analysis of hATSCs engraftment in ischemic rat brain after intraventricular injection**

Evaluation of mNSS demonstrated that motor and sensory behavior was impaired by the MCAO ischemic insult. Significant recovery of motor and somatosensory behavior was found in animals transplanted with hATSCs at 7 and 14 days after ischemia, compared with control ischemic animals Figure 4. There was no significant difference in mNSS tests between control and hATSCs groups.

**4 | DISCUSSION:**

It has been reported that transplantation of mouse ES cells into rat brain following experimental stroke reduced infarct volume and improved behavioral outcome. In the present study, it is reported that transplantation also stimulated neurogenesis in the SVZ ipsilateral to stroke[9]. The magnitude of the inflammatory response and its harmful effects as well as the types of released cytokines change with time after ischemia. The most important finding of this study is that hATSCs delivered to ischemic tissue through an intravenous route provide therapeutic benefit. This simple approach for cell therapy which does not necessitate invasive stereotaxic operations, could potentially target pathological sites in a number of brain disorders[10]. The main findings of the present study are that the transplanted hATSCs survived and migrated in the rodent brain without immunosuppression and that ischemic rats showed
improved neurological function after transplantation. Similar effects of hATSCs transplantation on functional deficits induced by ischemic brain injury have been reported[11-13].

5 | CONCLUSION:

Therefore, it is highly unlikely that transplanted cells integrate into the cerebral tissue and make appropriate connections within days after transplantation[14-15]. Cell transplantation may induce certain functional recovery of the brain tissue by endogenous cell mediated effect[16]. Our study suggested that intracerebrally hATSCs survived, migrated into the infarct area from inoculation site and neuroglially differentiated in the ischemic brain of adult rats. Potentially, adipose tissue may provide a powerful autoplastic therapy for human neurological degeneration disorders and not only stroke.

6 | REFERENCES:

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ADMINISTRATION OF hATSCs RESULTS IN RECOVERY OF CEREBRAL INFARCTION ANIMAL MODEL

How to cite this article: Lee T.H. Administration of hATSCs results in recovery of cerebral infarction animal model. Journal of Medical Care Research and Review. 2020;420–424. https://doi.org/10.15520/mcrr.v3i9.139