



RESEARCH ARTICLE

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 fluorescence. 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 fluorescence 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 contralateral 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:

P luripotent 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 heterogeneous 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 10 μ l of the adenovirus infected cell suspension (1x10⁵ cells) was slowly injected over 30min into the lateral ventricle at a depth 3.5mm from the surface of the brain by using a 10 μ l 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

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In all animal behavioral tests were assessed before MCAO and 7, 14 days after MCAO 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 \pm 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

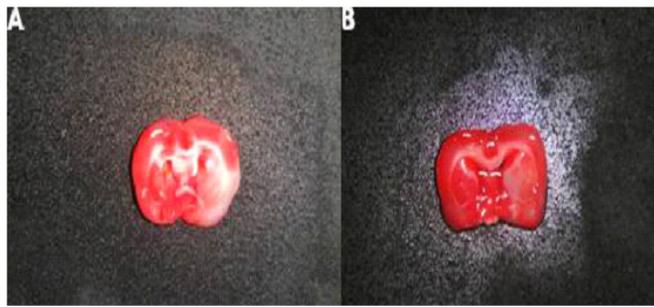


FIGURE 1: TTC-staining from PBS treated group(A) and hATSC treated group(B) are shown.



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.

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.

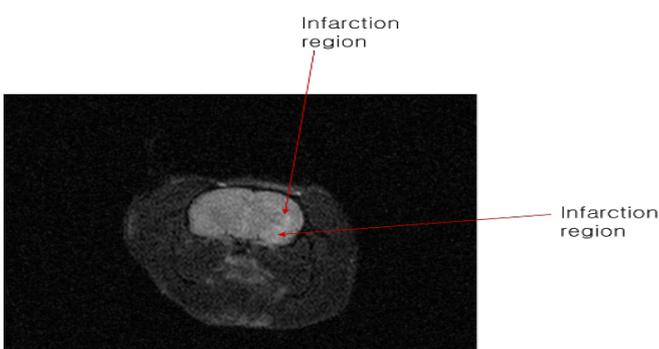


FIGURE 3: fMR imaging of infarction region showed in more white site in vitro.

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.

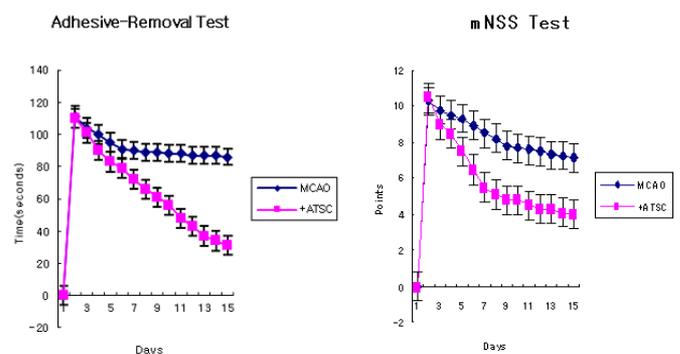


FIGURE 4: Behavioral Adhesive-Removal Test & mNSS test are shown between control group and transplantation of hATSCs group in vivo.

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 autoplasmic therapy for human neurological degeneration disorders and not only stroke.

6 | REFERENCES :

- Borlongan CV, Tajima Y, Trojanowski JQ, Lee VM, Sanberg PR: Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats, *Exp Neurol* 1998; 149 (2): 310-321.
- Weissman IL: Translating stem and progenitor cell biology to the clinic; Barriers and opportunities, *Sciences* 2000; 287: 1442-1446.
- Bjorklund LM, Sanchez-Pernaute R, Chung S, et al: Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model, *Proc Natl Acad Sci USA* 99:2344-2349.
- McDonald JW, Liu XZ, Qu Y, et al: Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord, *Nat Med* 1999; 5: 1410-1412.
- Sanchez-Ramos J, Song S, Cardozo-Pelaez F, et al: Adult bone marrow stromal cells differentiate into neural cells in vitro, *Exp Neurol* 2000; 164: 247-256.
- Woodbury D, Schwarz EJ, Prockop DJ, Black IB: Adult rat and human bone marrow stromal cells differentiate into neurons, *J Neurosci Res* 2000; 61:364-370.
- Deng W, Obrocka M, Fischer I, Prockop DJ: In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP, *Biochem Biophys Res Commun* 2001; 282: 148-152.
- Open GC, Prockop DJ, Phinney DG: Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains, *Proc Natl Acad Sci USA* 1999; 19: 10711-10716.
- Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats-similarities to astrocyte grafts, *Proc Natl Acad Sci* 1998; 95: 3908-3913.
- Pereira RF, Halford W, O'Hara MD, et al: Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice, *Proc Natl Acad Sci USA* 1995; 92: 4857-4861.
- Prockop DJ: Marrow stromal cell as stem cells for nonhematopoietic tissues, *Science* 1997; 276: 71-74.
- Hickey WF, Imura H: Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo, *Science* 1988; 239: 290-292.
- Mezey E, Chandross J, Harta G, Maki RA, Mcerche SR: Turning blood into brain: Cells bearing neuronal antigens generated in vivo from bone marrow, *Science* 2000; 290: 1779-1782.
- Brazelton TR, Rossi FM, Eshet GI, Blau HM: From marrow to brain; expression of neuronal phenotypes in adult, *Science* 2000; 209: 1775-1779.
- Li Y, Chopp M, Chen J, et al: Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice, *J Cereb Blood Flow Metab* 2000; 20: 1311-1319.
- Lee TH, Yoon JG. (2008) Intracerebral transplantation of human adipose tissue stromal cells after middle cerebral artery occlusion in rats. *J Clin Neuro*. 15:907-912.

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