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Locomotive effect by stem cell after cerebral ischemia in rats

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Abstract
We examined that human adipose tissue stromal cells (ATSCs) can be induced to undergo neuronal differentiation. After neuronal induction, the phenotype of hATSCs changed towards neuronal morphology and hATSCs were injected into the lateral ventricle of the rat brain. Transplanted cells migrated to various parts of the brain and ischemic brain injury by middle cerebral artery occlusion (MCAO) increased their migration to the injured cortex. Intracerebral grafting of hATSCs significantly enhances sensory and motor recovery of functional deficits in MCAO rats. These data indicate that transplanted hATSCs survive, migrate and differentiate in the ischemic microenvironment and improve neurological function recovery after stroke in rats. Therefore, we anticipate that transplantation of hATSCs may provide a powerful autoplastic therapy for human neurological injury and degenerative disorders.

Keywords: HATSC (human adipose tissue stromal cells), MCAO (middle cerebral artery occlusion), Ishemic brain injury

Introduction
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*. However, the inaccessibility of these stem cells limits their clinical utility and has led to the search for alternative cells that are capable of neuronal differentiation. Adipose tissue has been identified as an alternative source of pluripotent stromal cells. To date, ATSC cells have not been demonstrated to be capable of differentiation towards non-mesenchymal lineages. We now report that human ATSC cells can be induced to undergo morphologic and phenotypic changes consistent with neuronal differentiation. Adipose tissue may represent an alternative source of cells capable of neuronal differentiation, potentially enhancing their usefulness in the treatment of neurological disease (Defer *et al.*, 1996; Lopez-Lozano *et al.*, 1997; Turner and earney, 1993).

Adipose tissue, like bone marrow, is derived from the embryonic mesoderm and contains a heterogenous stromal cell population (Hausman & Campion, 1982; Pettersson *et al.*, 1984) [20]. 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 (Halvorsen *et al.*, 2001; Zuk *et al.*, 2001; Erickson *et al.*, 2002) [22, 23]. In addition, differentiation into neuron-like cells expressing neuronal markers has been reported (Safford *et al.*, 2002) [25]. Therefore, adipose tissue has been identified as an alternative source of pluripotent stromal cells (Patrick, 2000; Halvorsen *et al.*, 2001; Zuk *et al.*, 2001) [22, 23]. However, the fate of hATSCs and functional outcome after *in vivo* transplantation has not been determined.

Materials and methods
1. Transient animal middle cerebral artery occlusion model
Adult male Wistar rats weighing 200-240g were used in our experiments. Briefly, rats were initially anesthetized with 5 % enflurane and maintained with 2 % enflurane in O_2 by a face mask. Rectal temperature was maintained at 37℃ throughout the surgical procedure using a feedback regulated heating system. We induced transient MCAO using a method of intraluminal vascular occlusion (Chen *et al.*, 1992) [15]. Two hours after MCAO, reperfusion was performed by withdrawal of the suture until the tip cleared the lumen of the ECA.

2. Implantation procedures
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$\mu\ell$ 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 10$\mu\ell$ 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. After 14 days, rats were sacrificed by under deep anesthesia with xylozine and ketamine. The brains were removed, and the samples were immediately frozen.

3. Histological and Immunohistochemical staining
Immunocyto chemical staining was used for characterization of differentiated hATSCs. hATSCs were cultured on cover slips, and induced to neural differentiation. After 10 days, hATSCs were fixed with 4 % paraformaldehyde fixative and subjected to immunocytochemistry. Cells were incubated with primary antibodies against anti-cow glial fibrillary acidic protein (GFAP) (DAO, Canprinteria, CA), anti-mouse microtubule associated protein 2 (MAP2)

(Sigma) and anti-rabbit BDNF followed by biotin-conjugated anti mouse or anti rabbit IgG (Vector Laboratories).

4. Functional tests

In all animals behavioral tests were assessed before MCAO and after MACO during 15 days with and without cell injection. For the measurement of somatosensory deficit, the adhesive-removal somatosensory test was measured both before and after surgery (Schallert *et al.*, 1986; Hernandez and Schallert, 1988). All rats were evaluated using a modified neurological severity of motor test (Chen *et al.*, 1996; Li *et al.*, 2000) [15].

5. Statistical analysis

All data are expressed as the mean ± SD. Statistical comparisons were determined using analysis of variance followed by Fisher's protected least significant difference post hoc analysis. Behavioral data were analysed using repeated multiple ANOVA. *P*< 0.05 was considered statistically significant.

Results

1. Detection of the cells after injection in the lateral ventricle 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 s stained with GFAP indicated that there was significant gliosis or infiltration of leukocytes around the implantation site of the human hATSCs (Fig 1).

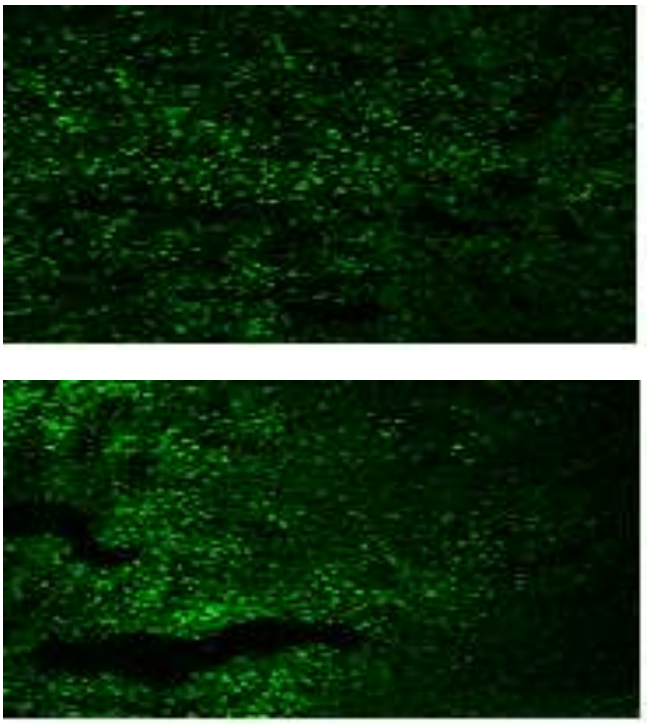

Fig 1: Immunohistochemical responses of Brdu-positive cells have shown in the near grafted area of the ischemic boundary zone after MCAO. Brdu-positive cells expressed the GFAP protein characteristics of astrocytes. X-400 Magnification.

2. Effect of hATSCs improves functional recovery after ischemia

The overall effects of hATSCs on functional recovery were significant for rotarod tests compared with MCAO alone (P<0.05). Treatment at 1 day after MCAO with hATSC significantly improved rotarod test scores during 15 days (P<0.05) compared with MCAO alone, respectively (Figure 2).

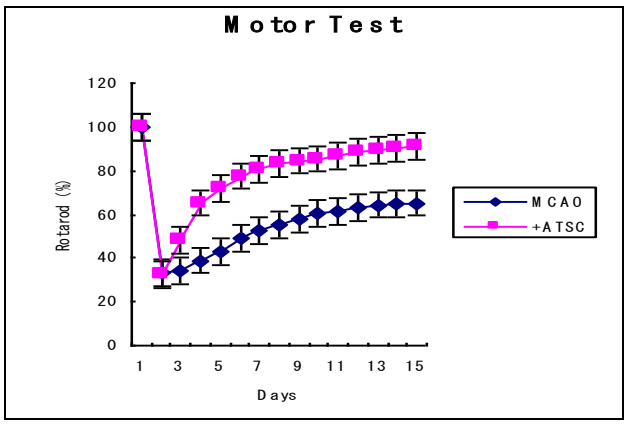

Fig 2: Behavioral functional tests before and after MCAO. Groups were as follows: group 1, MCAO alone (n=10); group 2, intracerebral infusion of ATSC ($1x10^5$ n=22) at 24 hours after MCAO.

Discussion

In our study, we transplanted hATSCs cells into ischemic brain and these cells migrate, differentiate, and reduce functional deficits after stroke. The most important finding of this study is that hATSCs delivered to ischemic tissue through an intravenous route provide therapeutic benefit. Similar effects of hATSCs transplantation on functional deficits induced by ischemic brain injury have been reported (Li *et al.*, 2000; Zhao *et al.*, 2002) [15]. The surface phenotype of ATSCs is similar to that of bone marrow-derived stromal cells (Barry *et al.*, 1999; Pittenger *et al.*, 1999; Gronthos *et al.*, 2001). Both ATSCs and marrow stromal cells share many of the same adhesion and receptor molecules (Bruder *et al.*, 1998; Hicok *et al.*, 1998; Pittenger *et al.*, 1999; Gronthos *et al.*, 2001). We also found that hATSCs has similar expression profile of protein and mRNA levels with hBMSCs. More hATSCs were found in the cortex in MCAO rats than in control rats, which suggest that ischemia-induced chemotatic factors facilitate hATSCs migration. These data suggest that hATSCs may have similar potentials with BMSCs in tissue engineering and regenerative medicine.

Neurotrophic factors could participate in hATSC-mediated functional improvement. Neurotrophic factors are survival and/or differentiation factors for neuronal progenitor cells (Cameron *et al.*, 1998), and they may reply an important role in proliferation or differentiation of neural tissue. A recent report showed that hMSCs from bone marrow express several neurotrophic factors (Tremain *et al.*, 2001). During development, different neurotrophic factors may be needed for optimal growth, differentiation and survival of neurons (Cameron *et al.*, 1998). BDNF regulates and differentiation in the developing nervous system and neural precursors cells (Ahmed *et al.*, 1995; Ang *et al.*, 2001; Trucker *et al.*, 2001). In the mature nervous system, BDNF is also involved in activity-dependent synaptic plasticity and appear to protect neurons against different types of brain insults (Guillin *et al.*, 2000; Perez-Navarro *et al.*, 2000). The administration of BDNF to the intact adult rat brain is associated with significant behavioral and neurochemical

alterations (Altar *et al.*, 1992; Martin-Iverson *et al.*, 1994). Intraventricular administration of BDNF itself (Beck *et al.*, 1994; Schabitz *et al.*, 1997) or continuous intrastrial BDNF delivery by adeno associated virus (Andsberg *et al.*, 2002) show protective effects to neuronal damage after focal cerebral ischemia in rats.

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