

Motor Function Recovery after Adipose Tissue Derived Mesenchymal Stem Cell Therapy in Rats with Cerebral Infarction

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Objective : There have been recent reports that mesenchymal stromal cells that are harvested from adipose tissue are able to differentiate into neurons. In the present study, we administered adipose tissue derived stem cells in rats with cerebral infarction in order to determine whether those stem cells could enhance the recovery of motor function.

Methods : Cerebral infarction was induced by intraluminal occlusion of middle cerebral artery in rats. The adipose tissue-derived mesenchymal stem cells were harvested from inguinal fat pad and proliferated for 2 weeks in DMEM media. Approximately 1×10^6 cells were injected intravenously or into subdural space of the peri-lesional area. The rotor rod test was performed at preoperative state (before MCA occlusion), and 1, 2, 3, 4, 6, 8 and 10 weeks after the cell therapy.

Results : The motor functions that were assessed by rotor rod test at 1 week of the cell therapy were nearly zero among the experimental groups. However, there was apparent motor function recovery after 2 weeks and 4 weeks of cell injection in intravenously treated rats and peri-lesionally treated rats, respectively, while there was no significant improvement till 8 weeks in vehicle treated rats.

Conclusion : These results demonstrate that the adipose derived stem cell treatment improves motor function recovery in rats with cerebral infarction.

KEY WORDS : Cerebral infarction · Adipose tissue derived mesenchymal stem cell · Motor Function recovery.

Introduction

Mature nerve cell has a limited capacity for self repair. Thus, various stem cell transplantations, such as embryonic stem cells, neural stem cells, and bone marrow stromal cells, have been applied to experimental animals with cerebral ischemia, because stem cells have the capacity for self-renewal and differentiation into various cell types. There have been recent reports that mesenchymal stromal cells that were harvested from adipose tissue were able to differentiate into neurons. Thus, adipose tissue derived stem cells (ADSC) therapy has emerged as an excellent candidate for stem cell therapy in ischemic brain disease because ADSCs have some advantages, such as their abundance and less effort required for tissue harvesting, compared with bone marrow derived stem cell

(BDSC). Kang et al.¹⁰⁾ reported that intracerebral transplantation of human ADSC improved the neurological deficits in the cerebral ischemia in rats. However, there are no research whether intravenous and peri-ischemic regional injection of autologous ADSC improve the motor function recovery to cerebral infarction. In this study, we evaluated the effect of autologous ADSC treatment improves the motor function in rats with cerebral infarction.

Materials and Methods

Animal care

Adult male Sprague-Dawley rats (250~300g) were used in this study. Animals were divided randomly into three groups : middle cerebral artery (MCA) occlusion + intrave-

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nous buffer treatment (n=9), MCA occlusion + intravenous ADSC treatment (n=7), MCA occlusion + peri-lesional ADSC treatment (n=8). The cerebral infarction was induced by MCA occlusion. The left common carotid artery, external carotid artery(ECA), and internal carotid artery(ICA) were exposed under anesthesia with xylazine hydrochloride (8mg/kg) and ketamine (90mg/kg, i.p). A length of 4-0 monofilament nylon suture (18.5~19.5mm) with its tip rounded by heating, was advanced from the ECA into the lumen of the ICA until it blocked the origin of the MCA. The nylon suture was then tied with the stump of ECA.

Isolation and culture of adipose tissue derived stem cells(ADSC)

Adipose tissue was excised from inguinal area at the same time as the MCA occlusion surgery for isolation of ADSC in the rats. Adipose tissue was washed extensively with equal volumes of phosphate-buffered saline to remove contaminating red blood cells and debris. Subsequently, the extracellular matrix was digested with 0.075% collagenase for 30 minutes at 37°C. Enzyme activity was neutralized with 10% fetal bovine serum (Hyclone, Logan, Utah), and the cells were centrifuged at 1200 × g for 5 minutes. The resulting pellet, which contained ADSC cells, was resuspended in complete culture medium that consisted of Dulbecco's Modified Eagle Medium (Gibco BRL, Rockville, MD, USA.), 10% fetal bovine serum, and 1% antibiotic/antimycotic. All the cells were distributed in 100 × 20mm tissue culture dishes and incubated at 37°C with 5% humidified carbon dioxide. The cells were washed thoroughly with phosphate-buffered saline after 24 hours of incubation, and all the non-adherent cells were discarded. Fresh complete culture medium was added every 3 days.

Treatment of the ADSCs

The proliferated ADSCs (approximately 1 × 10⁶ cells) were injected intravenously or perilesionally into the MCA occluded rats. It was 2-weeks after MCA occlusion. For perilesional treatment, the rats were anesthetized with xylazine hydrochloride (8mg/kg) and ketamine (90mg/kg, i.p). The rats were then transferred to a stereotaxic instrument in a clean field. A small incision (1cm) was made over the midline of the skull to expose the landmarks of the cranium (bregma

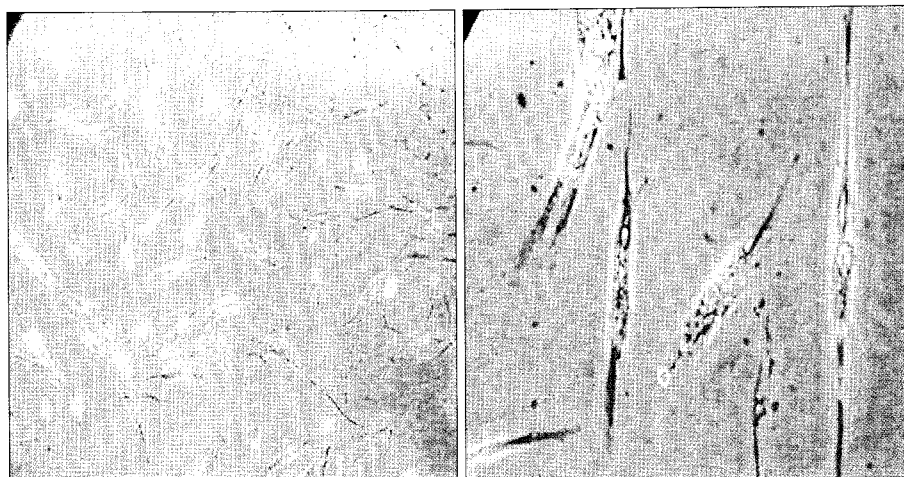


Fig. 1. Photomicrograph showing the adipose tissue-derived stem cell. (Prussian blue stain, X400).

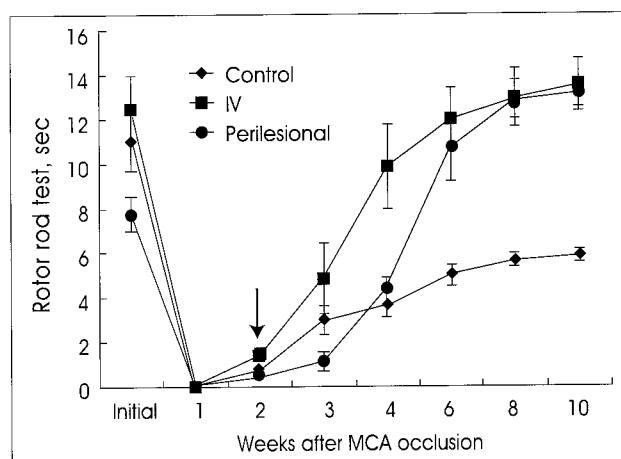


Fig. 2. Functional recovery after treatment of adipose-derived stem cells in the rats with cerebral infarction. Values are means ± S.E.. Arrow means the time of cell treatment.

and lambda). A burr hole was made in the bone 6mm posterior to bregma and 3mm lateral to midline with a dental drill. An incision was made over the dura, and then approximately 100ul of ADSC suspension was loaded slowly into the hole.

Rotor rod test

In all animals rotor rod tests were assessed at preoperative state, and 1, 2, 3, 4, 6, 8, and 10 weeks after MCA occlusion, with and without cell treatment. In order to measure the motor function, the rats were placed at a rotating rod (frequency, 6 per minute; rotor diameter, 20cm). The time, in second, that the rat was stayed there was recorded.

Statistical analysis

The data were presented as means + SE, and were analyzed by a T-test between the individual groups. A value of *p* < 0.05 was considered significant.

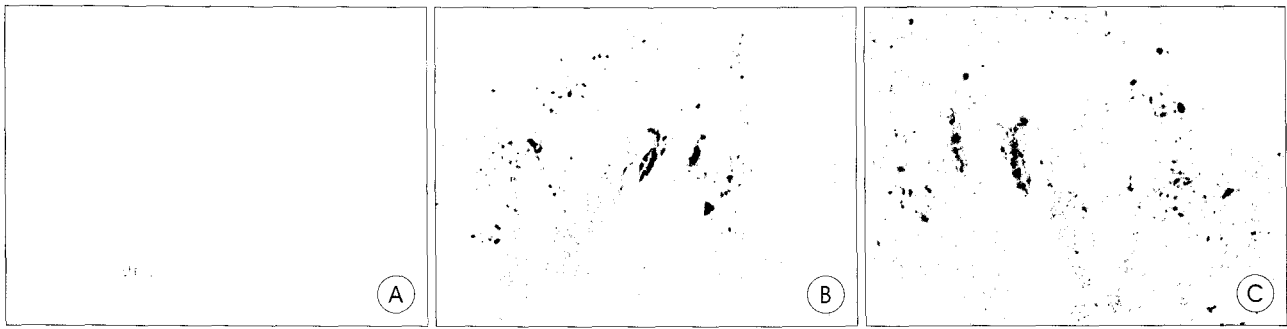


Fig. 3. Microphotograph taken from the rat's excised brain tissue after adipose-derived stem cell treatment. The blue spots are stained adipose-derived stem cell and they are seen at the basal area of the brain. (Prussian blue stain, A : X10, B : X40, C : X100).

Results

The features of cultured ADSCs demonstrated a spindle-like shape (Fig. 1). The results of the preoperative motor function of the three groups was 8 to 13 seconds. This variation in the results can be considered as the difference in basal motor function. After the 1 week of the occlusion of left middle cerebral artery, most rats were not tolerated on the Rota-Rod machine. A majority had minimal recovery of motor function.

The experimental groups had a fast recovery after treatment of stem cells. In particular, the first experimental group had the fastest recovery of results, which was statistically significant compared with the control group (Table 1, Fig. 2, $P=0.0029$).

The results of the second experimental group, which received injections into the peri-ischemic area after the burr-hole operation, recovered more slowly recovery than the first experimental group; however, 4 weeks later after operation, motor function recovery was faster than the previous time. That also was statistically significant compared with the control group (Table 1, Fig. 2, $P=0.0064$).

The control group had a natural recovery of the motor function, but there was difference in the results compared with the experimental groups, especially after 4 weeks after operation, and there was no recovery of motor function at 6 weeks later after operation (Table 1, Fig. 2).

And ADSCs were performed by the rat's brain histology (Fig. 3). ADSCs were detected at the basal area of the brain and were taken by the prussian blue stain as the morphology of blue spot. By the these images, we can confirm the living of the ADSCs in brain tissue of the ischemic rats.

Discussion

Stem cells are premordial, self-renewal and potentially can differentiate into multi-lineage cells. Numerous studies on stem cell treatment have been reported for cerebral infarction^{4,8}, myocardial infarction¹⁵, type 1 diabetes³, renal disease¹⁷, and spinal cord disease⁵. Gorio et al.⁶ reported about the fate of autologous dermal stem cells that were transplanted into the spinal cord after traumatic injury.

There are two types of stem cells : embryonal stem cells and adult stem cells. Embryonal stem cells are derived from the one embryo, so these present the ethical dilemma with respect to when they actually differentiate into a human life. Adult stem cells present fewer ethical problems compared with embryonal stem cells. However, adult stem cells do not have a precise mechanism for differentiation and can be differentiate to cancer cells, so there are more problems with respect their clinical application².

The majority of studies on cerebral infarction have proposed the intravenous transplantation of human BDSC into rats with cerebral infarctions. Chu et al.⁴ performed research about the distribution and in situ proliferation patterns of intravenously injected immortalized human neural stem-like cells in rats with focal cerebral ischemia. And Honma et al.⁸ estimated the reduced cerebral infarction volume in a cerebral ischemia model in adult rat using magnetic resonance imaging, spectroscopy, and histological

Table 1. Means of motor function according to the groups

Week	Preoperative state	AT#1	AT#2	AT#3	AT#4	AT#6	AT#8	AT#10
Group I (n=7)	12.43±1.58	0±0	1.43±0.35	4.86±1.06	9.86±1.87	12.00±1.45	13.00±1.29	13.57*±1.20
Group II (n=8)	7.75±0.73	0±0	0.50±0.26	1.13±0.39	4.38±0.52	10.75±1.48	12.87±0.83	13.25†±0.66
Control (n=9)	11.00±1.40	0±0	0.78±0.36	3.00±0.65	3.67±0.53	5.00±0.47	5.67±0.29	5.89±0.26

Values are means ± S.E. of the number of cases. Group I : stem cell treatment group through the intravenous route (tail vein). Group II : stem cell treatment group through the perilesional route (cerebral cortex). Preoperative state : before MCA occlusion. AT: After ADSC treatment. Stem cells treatment is performed at 2 week after MCA occlusion. * P value = 0.0029 (<0.05), † P value = 0.0064 (<0.05)

method after intravenous infusion of immortalized human mesenchymal stem cells.

Many studies used the BDSC and intrastriatal cord blood derived stem cells (CDSC)¹⁷⁾, but ADSC could be also used in order to isolate and incubate. Kokai et al.¹¹⁾ reported that ADSC could differentiate into skeletal muscle cells and chondroblasts. The ADSC have several benefits, which include rapid growth of ADSC from cultures that are initiated at a considerably lower cell density than cells that are typically used with marrow, and the yield of ADSC are several times the amount of BDSC¹⁸⁾. And ADSC can harvest faster than BDSC, so rapid treatment with stem cells could be performed faster than bone marrow stem cells after an injury to the tissues. So if there is the same result with use of ADSC compared with BDSC or CDSC¹⁹⁾, ADSC could be clinically useful. In our study, autologous ADSC of rats were used in the treatment of cerebral infarction, and motor function was recovered. Especially, ADSC treatment was applied in stroke patient, and this adipose tissue can be obtained by the lipo-suctions which helps the weight reduction.

The routes of treatment have been controversies according to various studies. Jin et al.⁹⁾ administered neuronal precursors from mouse embryonal cerebral cortex by different routes; for example, intrastriatal, intraventricular, and intravenous routes. Intrastriatal transplanted cells survived and migrated into the ischemic striatum, intraventricular transplanted cells were detected in the ventricular wall, and intravenous transplanted cells were also present in the penumbra regions of striatum and cerebral cortex.

However, there are no studies of autologous ADSC transplanted into the rats with cerebral infarction. In our study, ADSC were isolated and cultured in rats with cerebral infarction, and they were transplanted through the intravenous route and scattered into the peri-ischemic lesion. The result of recovery of the motor function has the statistical significance. Because there were no variation in the results of motor function, we could take the appropriate routes based on the clinical condition in stroke patients.

Many studies were performed on the mechanism of motor function recovery after stem cell treatment. Modo et al.¹⁴⁾ evaluated the magnetic resonance study images of migration of neural stem cells that were attracted to the lesion site in vivo. Specially, neural stem cells migrated from the injection tract mainly along the corpus callosum within 7 days of transplantation and extensively re-populated the peri-lesional area by 14 days following implantation. In contrast, neural stem cells transplanted into sham controls did not show any substantial migration outside of the injection tract, which suggesting that the trans-callosal migration observed in the stroke-lesioned animals was due to neural stem cells that were

attracted by the lesion site. And Jin et al.⁹⁾ thought that the migration of stem cells affect the recovery of motor function through the immunohistochemistry. There are new, previously unknown neuromodulative factors that are proposed to produce the adrenomedullin enhanced therapeutic potency of mesenchymal stem cells after experimental stroke in rats, through the induction of angiogenesis and inhibition of stem cell apoptosis⁷⁾. Many hypotheses have been proposed, such as neurogenesis¹²⁾, neuroprotection, and neuronal replacement from endogenous precursors¹⁾; and additional studies are requested for many of these situations. With these various studies, understanding the precise mechanism of stem cell therapy are necessary in clinical situations in order for stem cell therapy to be applied in the treatment of many intractable disease¹³⁾.

There are many hypotheses about the process of incubation and treatment with stem cells and their various side effects. Rubio et al.¹⁶⁾ proposed that after long-term in vitro expansion, ADSC populations can immortalize and transform spontaneously, and that transformed mesenchymal cells can become tumorigenic. Their study shows that all mesenchymal stem cell samples entered a senescence phase during 1 to 8 weeks, and post-senescence mesenchymal stem cells underwent the cell cycle crisis that produced chromosomal instability and altered phenotype. In particular, *c-myc*, which is the chromosome 8 oncogene that affects cell cycle progression and gene expression modifications, was affected in at least 30% of post-senescence mesenchymal stem cells and in some transformed mesenchymal stem cells. After this process, stem cells can be transformed into cancer cells. And Burns et al.²⁾ proposed that long-term cultures of telomerase-transduced adult human mesenchymal stem cells may evolve due to spontaneous genetic changes, which can lead to tumorigenicity in immunodeficient mice.

Our study was performed for the fundamental study of the application in the clinical state. The treatment of autologous ADSC can enhance the motor function of rats with cerebral infarction and have no difference of the result through the various routes of treatment. However, the application of the stem cell treatment in vivo must be studied further with respect to the times, methods of treatment, and side effects, with long-term monitoring.

Conclusion

ADSC therapy improved motor function deficits in the rat with cerebral infarction. The routes of cell treatment did not affect the final outcome of the motor function in the rats with cerebral infarction in this study. However, we believe that the treatment with ADSC is the procedure to expect the recovery of the motor function in many clinical cases.

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Commentary

The nervous system, unlike many other tissues, has a limited capacity for self repair. Mature nerve cells lack the ability to regenerate, and neural stem cells, although they

exist even in the adult brain, have a limited ability to generate new functional neurons in response to injury. For this reason, there is great interest in the possibility of repairing the nervous system by transplanting cells that can replace those lost through damage or disease.

Stem cells have capacity for self-renewal and differentiation into diverse cell types. Mesenchymal stem cells(MSCs) found in many adult tissues are an attractive stem cell source for the regeneration of damaged tissues in clinical applications because they are characterized as undifferentiated cells, able to self-renew with a high proliferate capacity, and possess a mesodermal (bone, cartilage, tendon, muscle and adipose), endodermal (hepatocyte) and ectodermal (neurons) differentiation potential³⁾. Although bone marrow(BM) has been the main source for the isolation of multipotent MSCs, the harvest of BM is an invasive procedure and the number, differentiation potential, and maximal life span of MSCs from BM decline with increasing age⁴⁾. Therefore, alternative sources from which to isolate MSCs are subject to intensive investigation.

One alternative source is umbilical cord blood(UCB), which can be obtained by a less invasive method, without harm for the mother or the infant. Human UCB is used as alternative source of stem cells, hematopoietic stem cells and MSCs¹⁾. Adipose tissue is another alternative source that can be obtained by a less invasive method and in larger quantities than BM. However, the fate of adipose tissue derived stem cells (ATSCs) and functional outcome after in vivo transplantation have not been widely determined, particularly, in neuronal disease.

The authors evaluated the effect of autologous adipose tissue derived mesenchymal stem cells(ADMSCs) treatment improves the motor function in rats with cerebral infarction. It is of great interest that autologous ADMSCs were used in the treatment of cerebral infarction, and motor function was recovered. But in this study, there are insufficient characterization of mesenchymal stem cells(MSCs) from adipose tissue like morphology, expansion characteristics, multilineage differentiation capacity, and immunophenotype etc.

Recent studies suggest intravenously transplanted MSCs can migrate and differentiate in the rat brain with focal ischemia and improve recovery. But, when Chen and associates injected hUCB into the tail vein, they identified approximately 1% of the injected cells in the side of the brain ipsilateral to the infarct. Among these cells only a small proportion became neurons (2~3%) or astrocytes (6%)²⁾.

Given this, it is reasonable to expect that the cells could be even more effective if the same numbers of cells were administered directed to the site of injury. The particularly interesting observation in this study is that there was similar degree of improvement in that the authors administered the

same numbers of cells by the two routes. This result suggest that although ADMSCs can express neural markers in vitro and vivo, it is likely that the behavioral improvements in function are the result of some other mechanism, such as the modulation of the immune/inflammatory response to the infarction, which requires further investigation. Certainly, if these results are shown to be consistent in the future studies, the potential therapeutic implications are enormous.

Even though this study provides that the treatment of autologous ADMSCs can enhance the motor function of rats with cerebral infarction, we have to study further about adequate cell numbers, therapeutic time window after stroke and the administering route for the application of the ADMSCs in the clinical state.

I appreciate the author's effort about the stem cell research for cerebral infarction.

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