International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 7, 2020
ISSN: 1475-7192

Pathological Differences through Stem Cell Therapy to the Infarction area by Cerebral Artery Hypoxia

TAE-HOON LEE

Professor, Emergency Medical Service Department, Namseoul University, 31020, Korea.
thlee@nsu.ac.kr
Corresponding author: 82-10-2045-2460

Abstract

Background: Mouse embryonic stem cell could show alternative materials of neuron cell differentiation, positively increasing their effectiveness in the treatment of nervous symptom. We examined which embryonic stem cells (ESCs) may be showed to undergo neuronal differentiation.

Methods: After neuronal induction, the phenotype of mESCs changed towards neuronal morphology and mESCs were inserted to the ventricle of the experimental animal brain. Intragraft cells translocated to several regions of the brain and cerebral infarction of brain artery occlusion showed their relocation to the damaged striatum and cortex.

Findings: Intracerebral grafting of mESCs mostly improve sensory and motor nervous system of physical default in focal cerebral model. This study proved that grafted mESCs dislocate and develop in several infarction condition and decrease central nervous system injury after focal ischemia in animals.

Improvements: We anticipate that transplantation of mESCs could suggest a strong grafting therapy for various neurological injury and degenerative diseases.

***Keywords*:** mESC (mouse embryonic stem cell), Artery occlusion, Grafting, Transplantation, Neurological injury.

1. Introduction

Embryonic stem cells selectively differentiate into mesenchymal lineages. Recent research has suggested that embryonic stem cells could be expressed to neuron differentiation in vitro and in vivo[1]. Cerebral tissue has long been mentioned as incapable of survival and physiological status of cell. Stem cell grafting therapy is one of generated intense interest in the area of various neurological symptoms. The characteristics of stem cells were reported differentiated function similar to specific types of cells expressing neurons[2]. Other studies have also shown that administration of stem cells would be applied as a powerful therapy method of multi-plastic substrate cells[3]. However, the developmental and functional consequences of stem cells after in vivo transplantation have been known through previous studies that stem cells may not differentiate into groups of cells predicted by experiments because of the inherent variety of variable factors[4,5]. In this study, stem cells were intended to identify whether various parts of the brain could transmit and integrate signals to each other through synaptic connections. In addition, neuronal differentiation is intended to confirm that the degree of neurological damage in animal model suffering from ischemic brain damage is improved. After the middle brain artery occlusion of the experimental animal model, current studies proved to extract the results by comparing them with experimental results from various previous studies[6]. Several experimental studies showed to prove that stem cell application contributes to improved portability and functional recovery to the damaged area[7]. In the present study, we determined that mouse embryonic stem cells reduce neurological deficits in rat model with ischemic brain damage if they could express to make new neuronal cells of the brain. The implantation of mESCs through neuronal differentiation has essential approach for clinical therapy[8].

2. Materials and Methods

2.1. *Control & grafted group animals*

Animals were separated into non transplant control group (n=8), rats were used in the experimental group (n=16) with grafted stem cell after reperfusion after the occlusion of the cerebral artery and the transplantation of stem cells. This experiment was proceeded by the animal department with polices of Namseoul university. To prevent hypothermia during anesthesia, animal temperature was sustained at 37 ° C using a thermostatically controlled plate.

2.2.*Animal middle cerebral artery occlusion model*

Animal model is followed with transient Middle Cerebral Artery Method(MCAO) method of intraluminal vascular blocking. The method of local cerebral ischemia was modified to ligate the middle cerebral artery (MCA). This method blocks the flow of blood from the ICA to the anterior cerebral artery (ACA) and the posterior cerebral artery (PCA) to the MCA. Thereafter, the MCA was closed for one hour to block blood supply. After reperfusion, the incision of the ECA was blocked with a 5-0 silicone coated suture and the incised skin was sutured.

2.3.*Implantation procedures of stem cells*

Cerebral ischemia rats (220-260g) were deeply anesthetized in a blocked chamber using 5 % isoflurane. A 3-mm incision was cut in the bone 1.0mm lateral to the bregma. Administration of 10 $\mu\ell$ solution by the adenovirus infected cell suspension ($1x10^6$ cells) was inserted over 20min into the ventricle at a depth 3.0mm by using a microsyringe.

2.4.*T2-weighted MRI*

T2-weighted images were taken 1 day after MCAO with the infarction area of cortex and striatum. The dark gray image surrounding the partial striatum shows normal region without injury. The white image of ischemic lesion express injury tissues in corpus callosum.

2.5.*TTC staining*

A brain slice 2 mm thick was prepared according to the internal distance of The Rat Brain using a tissue cutter (Stoeling co, USA). Each brain slice was reacted with 1% 2,3,5-triphenyl tetrazolium chloride (Tetrazolium Red: TTC staining) for 10 min for finding abnormal injury region.

2.6. *Cell Tracker™ CM-Dil & Fluorescent dye*

Transplanted ESC were labeled with Cell Tracker™ CM-Dil (Molecular Probes, Eugene, OR). CM-Dil is especially applying for long-term labeling and tracking of specific cells. For Fluorescent tracking of inserted embryonic cells, serial 5 μm thick frozen sections of brain were adhered to slides. The tissue slides were washed two times with HBSS and put into in a solution of 1mg/ml X-gal substrate in HBSS for a staining. The preparations were incubated for 8h. Histochemical staining was applied for characterization of developed mESCs. mESCs were cultured on cover slips, and induced to neuronal development.

2.7.*Behavioral Functional tests*

All rats were evaluated using motor test (rotarod test) & neurological severity score (mNSS). To estimate the motor function effectively, animal models were trained on the rotarod device 5 days prior to the injury surgery. The mNSS is the component of locomotor (muscle strength, movement status), sensory (tactile, proprioceptive). Neurological assessment was scored on a unit of 0 to 18 (normal status, 0; maximal severity status 18).

2.8.*Statistical Analysis*

All histological differences were evaluated the overall comparison between non transplant control group and cell transplant group at each point after injury.

3. Results and Discussion

Detection of brain ischemic region in MCAO model

Using imaging nuclear morphometry, we applied for specific and accurate parameters for use in expecting potential injury area of the infarction. T2 MR imaging shows that we can distinguish between the infarction region and normal region. The injury region can be detected precisely by in vivo T2 MRI. Without sacrificing the animal rats in vitro. T2 nuclear MR images could be simultaneously effective imaging method of detecting tissue edema and infracted areas through ischemic phenomenon. The white color imaging can express the injury site and the

gray color imaging shows the normal region [Fig. 1].

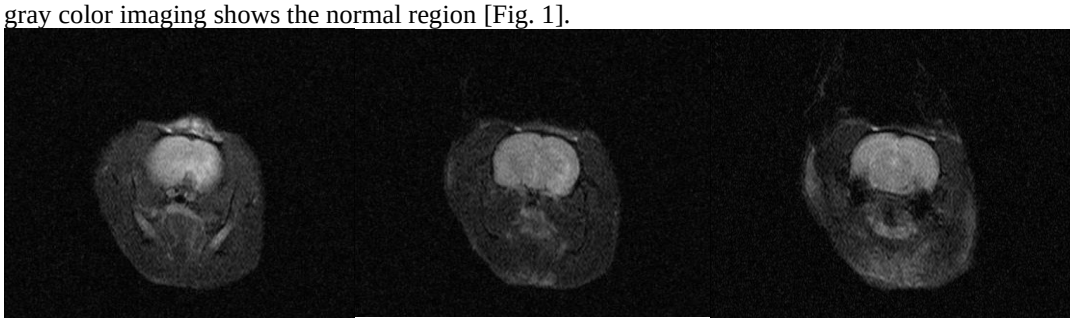

Figure 1. Morphological features of infarction region with white color was shown mainly in corpus callosum and striatum

Histological injury region

The infarcted regions were mainly occurred at the area between damaged brain tissue and in other sections within the infarct cavity [Fig. 2]. Volume of cerebral infarction through migration of transplanted cells was clearly smaller the control group animal. TTC staining of the experimental group was revealed that the degree of staining of the injured area was different from that of the control group. The core of the control group was almost discolored and no red light could be seen. The periphery of the injury related to the ischemia in the control group animals was widely spread compared to the periphery of the injury of the engrafted group animals. Transplanted engrafted cells migrate intensively to the infarct area and contribute to the reduction of white range which can suggest severe tissue necrosis. The decolorization of brain infarction region was shown to prove the severe damage of neuron cells in specific brain area.

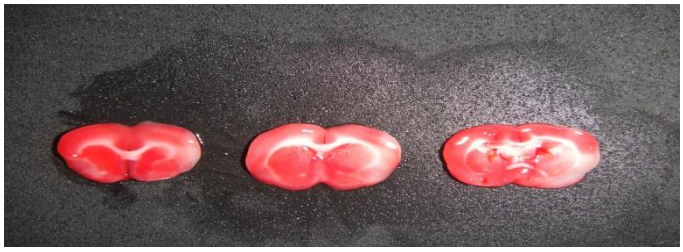

Figure 2. Cerebral tissue of the frontal plane. Non-Infarction region can be identified as a bright red area through TTC staining but infarction areas are showed white color characteristic.

mESC treated injury region

In the engrafted group, the region of white color was lesser than that of the control group, and infarction was showed in the cortex and striatum [Fig. 3]. The central white color region of the grafted group area was showed much smaller than the brain cortex and striatum of control group as well as showing partial decolorization of the ischemic trigeminal ipsilateral side

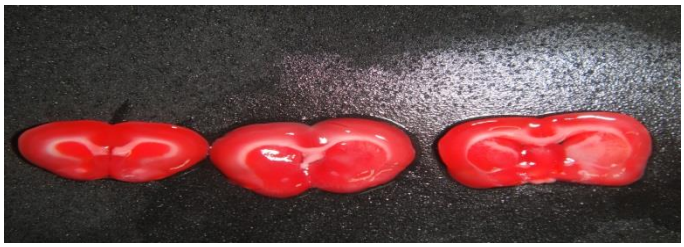

Figure 3. mESC treated-brain tissue of the coronal plane. The infarction area was showed the reduction in cortex and striatium.

Localization and survivals of transplanted cells in ischemic brain

To examine pathway of ESCs transplanted to the brain, ESCs were stained with Cell Tracker^{TM} CM-Dil. Labeled ESCs were administrated to the brain area of lateral ventricle and CM-Dil positive cells were broadly detected in the several injury regions. Implanted ESCs merged and moved to ischemic injury areas of the brain including the striatum and the cortex [Fig. 4].

Figure. 4. Implanted ESCs gradually moved to several regions of the brain injury and integrated to the injury site. Magnification, ×40

Tracking of GFAP in the implanted cell survival of the infarction region

Investigation of section s stained with GFAP suggested that showed manifest fibrosis in the early inflammation stage or survival of glial cells near the grafted site of mESCs [Fig. 5]. Implanted mESCs inter-grafted and migrated to several regions of the cerebral part including the central cortex. The cells of grafted in the injured region survived and majority of engrafted cells translocated to the inflammation site during 15 days after implantation procedure. Immunohistochemical responses of Brdu-positive cells have shown in the peripheral & central areas of the infarction region after focal ischemia. Brdu-positive cells including gliosis phenomenon showed the GFAP cell type as characteristics of astrocytes.

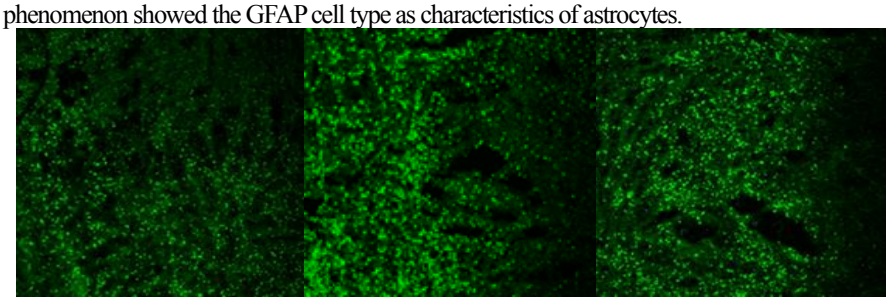

Figure 5. Immunohistochemical expressions of the GFAP characteristics. (X-400 Magnification)

Effect of ESCs improves functional recovery after ischemia

The overall effects of ESCs on locomotive difference were showed from motor and mNSS tests compared with PBS treated group ($P<0.05$). Similar ESCs group was shown compared with MCAO with PBS treated group. Rats treated PBS show a little behavioral enhancement, while implantation of mESCs treated displayed an early beneficial recovery on rotarod and NSS tests and significantly showed functional improvement better than the PBS treated group. Treatment at 2 or 3 days after MCAO with ESC group significantly distinguished in rotarod test and mNSS testing during 15 days ($P<0.05$) from PBS treated animal group, [Figure 6].

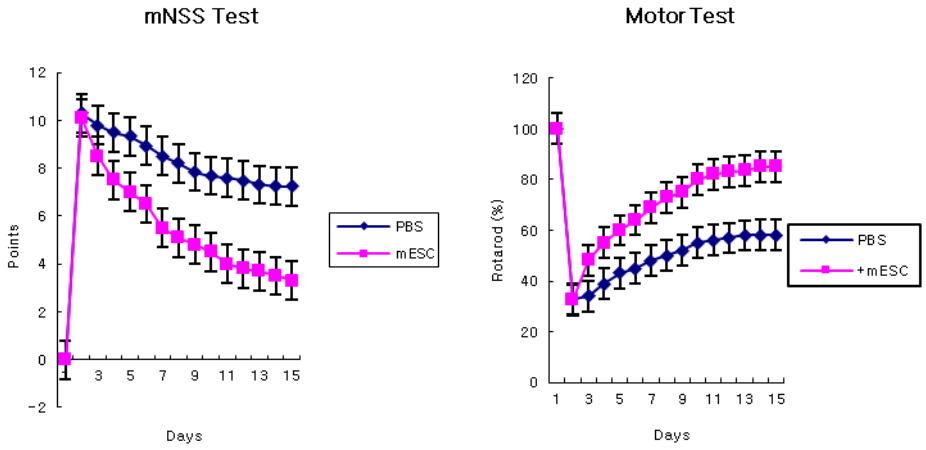

Figure 6. Groups were as follows: group 1, PBS treated; group 2, intracerebral infusion of ESC at 24 hours after MCAO.

In this experiment, we would investigate data of an early stage acute inflammation reaction or survival possibility of mESCs

during 4 weeks. This result may be postulated by the cerebral function a partially improved through grafted application and by the positively enhanced immunological environment of transplant experimental animals[9,10]. The application by which grafted mESCs developing central nervous system after focal ischemia are proved in previous studies[11]. In this case, the histological effectiveness of implanted cells was strongly suggested that the infarction range in mESC-transplanted rats was strongly lesser than from the injured size of control group at the time. Transplanted group was found the functional enhancement of motor and sensory recovery, but non transplant rats were not observed any improvement of motor and sensory status. We postulated that grafted stem cells merge into the focal damaged region and make synaptic function during experimental period after graft in some types of neurons like glial cells[12,13]. Growth trophic factors can contribute to mESC-related functional development[14]. Growth factors and neurotrophic materials play an important role for neuronal survival cells in an acute inflammation condition and they could support a favorable environment in proliferation or cellular differentiation of injured region[15,16]. Further research should specifically need to investigate that the synchronized interactions of various factors could be connected to the mechanism of the infarction processing[17,18]. Treatment of mESCs grafting may contribute to the clinical application which may approach to helpful method in a repair of severe brain ischemic disease. The research showed that implanted cell therapy could be strongly recommended to treat CNS diseases in the future[19]. This method will be broadly applied to clinical patient that has neurological function deficits[20].

4. Conclusion

We suggested that m-ESC transplant method contributed motor & sensory function recovery and also induced the reduction of morphological infarcted region. Potentially, embryonic stem cell technique may be suggested to apply for treatment in a connection with pharmacology, chemistry, biomedical industry. We concluded that stem cell therapy like mESC is one of the most powerful ways to treat neurological severe diseases. Our research suggests that grafted cells make a reduction of the several ranges in severe focal infarction and the protective effect of nerve cells was shown. Our study should to identify the most effective way of stem cell therapy in related to various conditions of central nervous system symptom. Conclusively, mESC transplant therapy can contribute to the effectiveness of neuronal regeneration for motor and sensory nervous system. Therefore, stem cell approach will be powerful clinic alternation for the treatment of severe nervous system injury.

5. Acknowledgment

Funding for this paper was provided by Namseoul University 2020.

6. References

1. Park W, Eglitis MA, Mouradian MM. Protection of nigral neurons by GDNF-engineered marrow cell transplantation. Neurosci Res. 2001;40:315-23.

2. Jin H, Carter JE, Huntley GW, Schuchman EH. Intracerebral transplantation of mesenchymal stem cells into acid sphinomyelinase-deficient mice delays the onset of neurological abnormalities and extends their life span. J Clin Invest. 2002;109:1183-91.

3. Lu L, Juan C, Yong M, Jianbin L, Biansheng J. Transplantation of human umbilical cord blood mononuclear cells attenuated ischemic injury in MCAO rats via inhibition of NF-kB and NLRP3 inflammasome. Neurosci. 2018;369(15):314-24.

4. Wang B, Tian S, Wang J, Han F, Qu Y. Intraperitoneal administration of thioredoxin decreases brain damage from ischemic stroke. Brain Res. 2015;1615:89-97.

5. Linden J, Beeck LV, Plumier JC, Ferrara A. Procedural learning as a measure of functional impairment in a mouse model of ischemic stroke. Behav Brain Res. 2016;307:35-45.

6. Zuk PA, Zuh M, Mizuno H. Multilineage cells from human adipose tissue: implication for cell-based therapies. Tissue Eng. 2001;7:209-26.

7. Erickson GR, Gimble JM, Franklin DM, Rice HE, Awad H, Guilak F. Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo. Biochem Biophys Res Commun. 2002; 290: 763-69.

8. Hou X, Cheng H. Long non-coding RNA RMST silencing protects against middle cerebral artery occlusion (MCAO)-

induced ischemic stroke. Biochem Biophys Res Commun. 2018;495:2602-8.

9. Patrick CW. Adipose tissue engineering: the future of breast and soft tissue reconstruction following tumor resection. Semin Surg Oncol. 2000:19: 302-11.

10. 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;290:1779-82.

11. Brazelton TR, Rossi FM, Eshet GI, Blau HM. From marrow to brain; expression of neuronal phenotypes in adult. Science. 2000;209:1775-79.

12. Behrouzifar S, Vakili A, Bandegi AR, Kokhaei P. Neuroprotective nature of adipokine resistin in the early stages of focal cerebral ischemia in a stroke mouse model. Neurochem Int. 2018;114:99-107.

13. Zong X, Wu S, Li F, Lv L, Xu T. Transplantation of VEGF-mediated bone marrow mesenchymal stem cells promotes functional improvement in a rat acute cerebral infarction model. Brain Res. 2017;1676:9-18.

14. He X, Cai Q, Li J, Guo W. Involvement of brain gut axis in treatment of cerebral infarction by β-asaron and paeonol. Neurosci Lett. 2018;666:78-84.

15. Morita T, Sasaki M, Sasaki YK, Nagazaki M, Honmou O. Intravenous infusion of mesenchymal stem cells promotes functional recovery in a model of chronic spinal cord injury. Neurosci. 2016;335:221-31.

16. Chang YP. Cellular and biochemical mechanism of perinatal hypoxic-ischemic brain injury. J Korean Pediatr Soc. 2002; 45:560-567.

17. Bursch W, Ellinger A. Autophagy-a basic mechanism and a potential role for neurodegeneration. Folia Neuropathol. 2004;43(4): 297-310.

18. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke. 1989;20:84-91.

19. Ohta Y, Hamaguchi A, Ootaki M, Watanabe M, Takenaga M. Intravenous infusion of adipose-derived stem/stromal cells improves functional recovery of rats with spinal cord injury. Cytotherapy. 2017;19(7):839-848.

20. Watanabe T, Nagai A, Sheikh AM, Mitaki S, Yamaguchi S. A human neural stem cell line provides neuroprotection and improves neurological performance by early intervention of neuroinflammatory system. Brain Res. 2016;1631:194-203.