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Clinical Effect through Histological Characteristics of Focal Ischemia Region

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뇌허혈성 부위의 조직학적 특성을 통한 임상적 영향

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요 약 마우스 배아 줄기 세포는 신경 세포 분화가 가능한 세포의 대안적인 공급원이 될 수 있으며 잠재적으로 신경계 질환의 치료에 유용하게 사용될 수있다. 우리는 배아 줄기 세포 (ESCs)가 신경 분화를 유도하도록 유도 될 수 있는지를 조사했다. 신경 세포 유도 후, mESC의 표현형이 뉴런의 형태학으로 변하였고, mESCs는 실험쥐 뇌의 측 뇌실로 주입되었다. 이식 된 세포는 뇌의 여러 부위로 이동하였고 중대뇌동맥 결찰에 의한 허혈성 뇌혈관 손상부위에 이식된 줄기세포군이 손상된 피질부위로 집중적으로 이동하여 손상복구 기전을 증가시켰다. mESCs의 뇌내 이식은 MCAO 쥐의 기능적 결손의 감각 및 운동 회복을 유의 적으로 향상시킨다. 이러한 데이터는 이식 된 mESC가 허혈성 미세 환경에서 생존, 이동 및 분화하고 쥐에서 뇌졸중 후 신경 기능 회복을 향상 시킨다는 것을 나타낸다. 따라서 우리는 mESC의 이식이 인간 신경계 손상 및 퇴행성 장애에 대한 강력한 이식 치료법을 제공 할 것으로 기대한다.

키워드 : 마우스배아줄기세포, 중대 뇌동맥 폐색, 이식, 허혈, 신경학적 손상

Abstract Mouse embryonic stem cell could show an substitutional materials of cells of neuron differentiation, positively increasing their effectiveness in the treatment of nervous symptom. We examined that mouse embryonic stem cells (mESCs) can be induced to undergo neuronal differentiation. After neuronal induction, the phenotype of mESCs changed towards neuronal morphology and mESCs were injected into the lateral ventricle of the experimental animal 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 mESCs mostly improve sensory and motor nervous system of neurological injury in focal cerebral rats.

Key Words : mESC (mouse embryonic stem cell), MCAO (middle cerebral artery occlusion), Grafting, Ischemia, Neurological injury

1. Introduction

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 symptom. The purpose of this study

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was to histologically stain the treatment effect of white blood cells in a cerebral ischemia model after middle cerebral artery ligation for the purpose of clinical treatment of stroke by using stromal cells isolated from human adipose tissue. Previous study has showed the results of changes in the actual tissue damage site using histological analysis[1,2]. Therefore, the mechanisms of various ischemic vascular diseases induced by the nervous system have been studied, and the importance of such neuronal diseases has been highlighted in relation to the neuronal cell death due to ischemic brain disease[3,4]. This research was carried out to prove the effectiveness of stem cells on cerebral infarction and various neurological diseases. In this study, stromal cells were transplanted into MCAO-induced brain tissues for TTC (2,3,5-triphenyltetrazolium chloride) staining. We report the results of histological brain injury sites.

2. Materials and Methods

2.1 Rat animal model

Rats were randomly divided into control group (n=5), rats were used in the experimental group (n=15) with grafted stem cell after reperfusion after the occlusion of the cerebral artery and the transplantation of stem cells. This experiment was approved by the animal committee with policies of Namseoul university. To prevent hypothermia during anesthesia, body temperature was maintained at 37 ° C using a thermostatically controlled plate. The animal surgery was treated under sterile conditions with safe experimental environment.

2.2 Implantation procedures

Cerebral ischemia rats (270-300g) were anesthetized in a sealed chamber using 5 % isoflurane. A 3-to 4-mm incision was made in the scalp 1.0mm lateral to the bregma. Administration of 10μl solution by the adenovirus infected cell suspension ($1x10^6$ cells) was slowly inserted over 20min into the lateral ventrical at a depth 3.0mm by using a 10μl Hamilton microsyringe (Hamilton, Reno, NV).

2.3 Histological and Immunohistochemial staining

For LacZ detection of injected human cells, serial 5㎛ thick frozen sections of brain were adhered to slides, and fixed in 4 % paraformaldehyde. The tissue sections were washed three times with HBSS(Hanks' Balanced Salt Solution) and stained in a solution of 1mg/ml X-gal substrate, 5mM 3Fe(CN)6, and 2mM MgCl2 in HBSS. The preparations were incubated for 8h. Immunocytochemical staining was used for characterization of differentiated mESCs. mESCs were cultured on cover slips, and induced to neural differentiation.

2.4 Statistical analysis

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

3. Results

3.1 Histological injury region

The infarct region were mostly occurred at the area between damaged brain tissue and in other sections within the infarct cavity. TTC staining was performed to determine the degree of injury to normal tissue sections and ischemia. In the control group, the entire cross section of the brain was reddish by TTC and the brain cells were normal. In the control group, it was not stained by TTC, and the area where neuronal death was presumed to occur was all or part of

the striatum supplying the middle cerebral artery, and it was also found in many parts of the cerebral neocortex. In the staining pattern, the core discoloration, which is an injury to the ischemia, was extended widely on the ischemic side and the penumbra discoloration area appeared on the periphery. In the experimental group, the range of decolorization was much smaller than that of the control group, and some injuries were observed in the cortex and striatum[Fig. 1]. The central decolorization area was confined to the cerebral cortex of the ischemic trigeminal ipsilateral side, and surrounding discoloration areas were widely scattered around it.

[Fig. 1] Brain tissue of the coronal plane

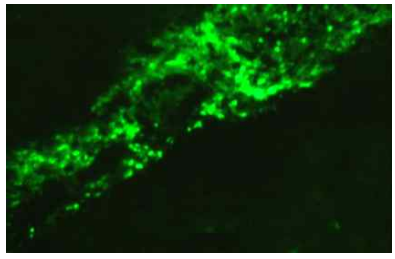
3.2 mESC treated injury region

In the experimental group, the range of decolorization was much smaller than that of the control group, and some injuries were observed in the cortex and striatum[Fig. 2]. The central decolorization area was confined to the cerebral cortex of the ischemic trigeminal ipsilateral side, and surrounding discoloration areas were widely scattered around it.

[Fig. 2] mESC treated-tissue of the coronal plane

3.3 Dectection of GFAP in the grafted area of the ischemic boundary zone

Examination of sections stained with GFAP(Glial Fibrillary Acidic Protein) indicated that there was significant gliosis or infiltration of leukocytes around the implantation site of mESCs[Fig. 3]. Implanted mESCs inter-grafted and migrated to several regions of the brain including the contralateral cortex. The cells grafted in the injured region to which they migrated during 15 days after implantation.



[Fig. 3] Immunohistochemical responses of GFAP protein characteristics(X-400 Magnification)

4. Discussion

In this study, we could not observe any evidence of an inflammatory response or rejection of mESCs during experimental periods. This data may be explained by the brain being a partially privileged site for transplantation and by the partially impaired immune status of albino rats[5,6]. The application by which grafted mESCs developing central nervous system after focal ischemia are proved in previous studies. In this study, the histological status of implanted cells was still needed to research and the infarction size in mESC-transplanted rats was significantly different from that in control ischemic rats at the time that motor and sensory recovery was not observed without transplantation. We postulated

that grafted stem cells integrate into the cerebral damaged tissue and make appropriate connections within days after transplantation in a various type of neurons like glial cells[7,8]. Neurotrophic factors could participate in mESC-mediated functional improvement[9,10]. Growth factors and neurotrophic factors 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[11-13]. Treatment of mESCs grafting may contribute to the clinical application which may approach to helpful method in a repair of severe brain ischemic disease[14,15].

5. Conclusion

Potentially, embryonic stem cell may be suggested to apply to treatement in a connection with pharmacology, chemistry, biomedical industry[16,17]. We concluded that stem cell therapy like mESCs is one of the most effective way to deal with various neurologic diseases. We also need to be further investigated in other types of central nervous system symptom.

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