Transplantation of Adipose Stromal Cells after Stroke

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Abstract

Recovery in central nervous system (CNS) disorders is hindered by the limited ability of the system to regenerate lost cells, replace damaged myelin, and re-establish functional neural connections. Cell transplants are being actively researched for treatment of CNS injuries. This study was undertaken to examine the effects of transplanted human adipose-derived stromal cells (hADSC) on cerebral injuries in rats. The cerebral injuries were produced by middle cerebral artery occlusion (MCAO). The transplants were made on the seventh day after the injuries. The injury areas of cavity volumes were smaller in the transplant group than they were for the non-transplant group. Immunohistochemical study revealed that the transplanted hADSC infiltrated into the injured areas of the brain, but the transplanted hADSC were not differentiated into glial or neuronal cells. Considering that hADSC can be used for autologous transplant, the results of the present study suggest that the transplant of hADSC may be used for the treatment of brain injuries.

Keywords

CNS (Central Nervous System); HADSC (Human Adipose-derived Stromal Cell); MCAO (Middle Cerebral Artery Occlusion); Transplant; Treatment; Brain Injury

Introduction

Cerebral ischemia via middle cerebral artery ligation is a disease that can be caused by cardiac arrest or cardiopulmonary bypass, which has been known to cause nerve cell damage in certain areas in humans and laboratory animals. Cerebral ischemia leads to decreased motor function and decreased sensory function. The purpose of this study was to histologically stain the treatment effect of white blood cells in a cerebral ischemia model reperfused after middle cerebral artery ligation for the purpose of clinical treatment of stroke by using stromal cells isolated from human adipose tissue. It is reported that the results of changes in the actual tissue showed damage site using histological analysis. The major mechanism of apoptosis by brain ischemia-hypoxic injury is mainly caused by ischemia around the middle cerebral artery [1]. 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 [2].

This study was carried out to investigate the effect of stromal cells on cerebral infarction and various neurological diseases. In this study, stromal cells were transplanted into MCAO-induced brain tissues for TTC staining. We report the results of histopathological comparison of brain injury sites.
Materials and Methods

1. Experimental Animals

Twenty four transient ischemic middle cerebral artery occlusion (MCAO) rat models were prepared. After the MCAO procedures, all of the rats were randomly assigned to one of two groups (n=24): infarct with PBS-only injection (group A, n=10), infarct with hADSC transplantation (group B, n=14).

2. Reperfusion after Middle Cerebral Artery Occlusion

The method of local cerebral ischemia [3] was modified to ligate the middle cerebral artery (MCA). First, the animals were anesthetized with sodium pentobarbital, and the median line of the neck was dissected to separate the external carotid artery (ECA) and the only branch of the internal carotid artery (ICA). The pterygoid artery (PPA) was isolated. The beginning of the PPA was ligated with a micro vascular clip to prevent the thread in the blood vessel from entering the PPA. The branches of the ECA were then cauterized with electrical pharyngeal and the distal portion of the common carotid artery (CCA) was ligated and severed 1 cm above the base. A 6-0 silk suture was used to loosely tie the ECA proximal portion and temporarily place the micro vascular clip on the ECA stump. The proximal portion of the ECA was punctured using micro scissors and a single 20 mm 3-0 Nylon yarn was pushed into the base of the ECA. The end of the thread going into the blood vessel was rounded with fine sandpaper. In order to prevent hemorrhage, ECA containing 3-0 nylon thread was gently tied with a 6-0 silk suture, the micro vascular clip was removed, and the nylon thread was gently pushed into the ICA. The resistance of the nylon thread is about 17.5 mm, which indicates that the end of the thread has reached the base of the ICA branch and MCA in the two courses. 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 closed with a 6-0 silk suture and the incised skin was sutured.

3. Transplantation of Human Fat Stromal Cells into Brain Tissue

On the first day after MCAO treatment, brain injured rats are anesthetized by mixing 5% enflurane anesthesia with an oxygenated respiratory system, and then using an operating table for transplantation into brain tissue. Bore a hole 3 mm laterally from the Bregma and insert the injection needle slowly into the 3.5 mm depth from the surface of the brain using a 10 μl Hamilton micro syringe. After transplantation, keep the needle in place for 5mins before removing the Hamilton micro syringe.

4. TTC Staining

Seven days after the cerebral ischemia, the animals were sacrificed by sacrificing the brains and the brain was excised. A brain slice 2 mm thick was prepared according to the internal distance of The Rat Brain [4] using a tissue cutter (Stoeling co, USA) Were stained with 1% 2,3,5-triphenyl tetrazolium chloride (Tetrazolium Red: TTC) for 30 min. After the reaction, the brain slice was frozen at 4°C in a formalinsolution.

5. Statistical Analysis

Statistical analysis and significance of the test results were processed by Sigma Plot Program 4.0 (Sigma,USA).

Results

1. TTC Findings

TTC staining was performed to determine the degree of injury to normal tissue sections and ischemia. In the normal 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 neo cortex. It was shattered and severely discolored and red. 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, and the degree of decolorization was not significantly higher than that of the control group (Figure 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.

2. Volume of Cerebral Infarction and Migration of Transplanted Cells

The TTC staining of the experimental group revealed that the degree of staining of the injured area was...
different from that of the control group. The core of the control was almost discolored and no red light could be seen. The periphery of the injury was narrowed around the periphery of the injury. Thus, transplanted cells migrate intensively to the infarct area and contribute to the regeneration of new neurons, which can lead to recovery of motor function and sensory function (Figure 2).

**Figure 1:** Brain Tissue of the Coronal Plane. It Is A Tissue Fragment of The Brain Where The White Infarction Is Caused By the Infarct Area. The Area That Does Not Become An Infarction Can be Identified as A Bright Red Area Through TTC Staining And Induce A White Infarction of A Specific Infarct Area

![Brain Tissue Image](image1.png)

Figure 2: The Ischemic Injury of Transplanted Adipose Stem Cells (A:PBS only, B:hADSC)

![Transplanted Cells Image](image2.png)

**Discussion**

It is important to prevent damage to the brain or other life-critical organs through prevention of complications such as respiration, circulatory failure, and so on. These measures include the management of respiratory and circulatory systems, the treatment of body temperature abnormalities, the treatment of urination and defecation disorders, and the treatment of cerebral. The mechanism of neuronal damage after cerebral ischemia can be classified into two groups according to the timing. The initial neuronal cell damage is due to energy deficiency due to depletion of oxygen and glucose in blood flow restricted tissue, mass inflow of cations such as calcium due to changes in cell membrane permeability. It is caused by acidification, cell edema, protein denaturation, and accumulation of glutamate at neuronal junctions, and cells...
eventually become necrotic [5]. Late neuronal cell death is a delayed neuronal death in the CA1 region and basal ganglia of the hippocampus, which is transient ischemic 3-4 days. It is characterized by glutamate excitotoxicity [6], protein synthesis disorder [7], heat shock protein gene expression disorder [8], free oxygen, and cell death [9]. This study was conducted to examine the effect of transplantation through human adipose tissue stromal cells on cerebral ischemia. After induction of cerebral ischemia in rat, TTC staining showed that most of the putamen in the ischemic- Although a large part of the neo cortex was not stained with TTC, it appeared white. In the experimental group, however, the extent of decolorization of the injured area was much reduced compared to the control group. As a result, in the control group, injuries occurred in almost all areas where the middle cerebral artery is responsible for blood supply, whereas the injured areas in the experimental group were reduced. In comparison of the intensity of staining, it was found that the degree of whiteness in the experimental group was weaker than that of the control group. These results suggest that human adipose stromal cells reduce the volume of the central region of injury in cerebral infarction induced by reversible cerebral ischemia in rats and have a protective effect against neuronal injury at the periphery of injury.

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References