Magnetic resonance imaging in studying the therapeutic effect of iPSCs transplantation for experimental ICH

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Abstract: Intracerebral haemorrhage (ICH) is usually along with high mortality and disability, and lacking of effective treatment. Transplantation of induced pluripotent stem cells (iPSCs) has shown promising effects on the recovery of neural disfunction in animal ICH models. However, there is little evidence about the application of magnetic resonance imaging (MRI) in studying the therapeutic effect of iPSCs transplantation for ICH. In this study, MRI was used to observe the effect of iPSCs transplantation on the intraparenchymal blood clot volume and apparent diffusion coefficien (ADC) value, which is the indirect evidence of vasogenic edema after ICH. iPSCs were delivered intracerebrally 6 hours after collagenase-induced ICH in a rat model. Three days later, MRI showed that iPSCs transplanted group resulted in fewer perihematoma ADC value than control group, while contralateral ADC values and blood clot volume between two groups didn't show any difference. In summary, MRI technology can be used in the study of iPSCs transplantation for ICH.
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1. Introduction

Intracerebral haemorrhage (ICH) is a serious stroke subtype which is associated with high morbidity, mortality, recurrence rate and usually leaves severe neurologic dysfunction even after recovery. (1, 2) The primary injury of hematoma formation and its expansion within brain parenchyma (3) and the secondary injury of three intertwined degenerative cascades including inflammation, red cell lysis and thrombin production are the major mechanisms of ICH's injury. (1,2,4) The injury can induce severe neural dysfunctions following the formation of cerebral edema, which is one of the main cause as well as ending of secondary damage, even one of causes of death by cerebral hernia.(1)

Cell therapy for ICH is a promising therapeutics.(5) In recent years, the stem cell therapy in animal models of cerebral hemorrhage had received many researches, in which there are sporadic reports of reduced cerebral edema with traditional methods.(6,7,8) Induced pluripotent stem cells are new kind of stem cell population converted from somatic cells through reprogramming by definite transcription factors.(9,10) Over the past few years, some studies have suggested that transplantation of iPSCs has great potential treatment of ischemic stroke (8) and ICH (9,11) using immunofluorescence or immunohistochemical methods or other invasive methods. However, there was little evidence about the application of magnetic resonance imaging (MRI) in studying the therapeutic effect of iPSCs transplantation for ICH, which is a kind of in vivo research technology without invasive procedures. Thus, in this study, we observed whether the transplantation of iPSCs can reduce intraparenchymal blood clot volume and ADC value in rat ICH model induced by VII collagenase with MRI, and discussed the possible applyment of MRI technology in the study of iPSCs transplantation for ICH.

2. Materials and methods

2.1 Cell culture

IPSCs were provided by our laboratory, which were authorized by Ethoric Committe of Zhengzhou University. Mouse embryonic fibroblasts (MEFs) feeder was prepared two days before iPSCs recovery in fibroblasts medium(Dulbecco's modified Eagle's medium (DMEM), then iPSCs were resuscitated on well-grown MEF feeder with hESC medium just as previous reports.(9,11,12) 4-5days later, iPSCs colonies were seen on MEF feeder and

subcultured every 6-7days as previous reports.(9,11,12) Medium was changed every day.

2.2 Establishment of rat ICH model and iPSCs transplantation

Male Sprague-Dawley (SD) rats, each weighing 250-270g, purchased from Animal Center of Henan (Henan province, China) were raised in independent ventilation isolation cages. All experimental procedures and protocols were carried out under the Institutional Animal Care and Use approved by Ethics Committee of Zhengzhou University, Zhengzhou, China and complied with the "Guide for the Care and Use of Laboratory Animals". Each rat was localized pronely in the stereotactic frame (Narishige SN-3, Tokyo, Japan) after anesthetized with chloral hydrate (300mg/kg, intraperitoneally). The procedure of ICH was as same as previous reports. (9, 11, 12) Of course, sham ICH was induced with a stereotaxic needle insertion and an injection of equal volume of sterile saline. The rats recovered from surgery in a cage containing food and water and were kept warm by an incandescent light bulb. Six hours later, rats were randomized into controls versus iPSCs treatment. IPSCs (1×10^6 cells in 5μl phosphate buffer saline (PBS)/rat) (referred to as iPSCs group) or PBS 5μl/rat (referred to as PBS group) was intracerebrally via original needle tract with depth of 3.5mm as previous reports. (9,11,12)

2.3 MRI scanning

At 3 days post-ICH, the rats of iPSCs group and PBS group were scanned by 3.0T GE 750 imager with rat dedicated coil. Following were scanning parameters and sequence: T2-weighted MR images (T2WI) (T2WI: TR/TE = 3340/110.0ms, FOV=50×50mm, NEX=1.00, matrix=160×160, section thickness=2 mm), diffusion-weighted images (DWI) (DWI: TR/TE=3340/110.0ms, FOV=110×110mm, NEX=1.00, matrix=96×96, section thickness=2 mm,interlayer spacing=0.5mm). The MR images of T2MRI and DWI were processed with GE 750 3.0T system to obtain hematoma volume and ADC values of iPSCs group and PBS group. For hematoma volume, the boundaries of the hematoma were traced by hand on each section at T2MRI, then area of each section was added up to get hematoma volume, ADC values were obtained with GE workstation system on DWI by two radiologists who were blined to groups.

2.4 Statistical analysis

All results were expressed as means ± standard deviation. ($\overline{X}$ ±SD) The collections of all experimental data were performed by experimenters who were blind to the group identity. Data were analyzed by two-tailed Student's *t* test. Two-tailed $P<0.05$ was considered statistically significant.

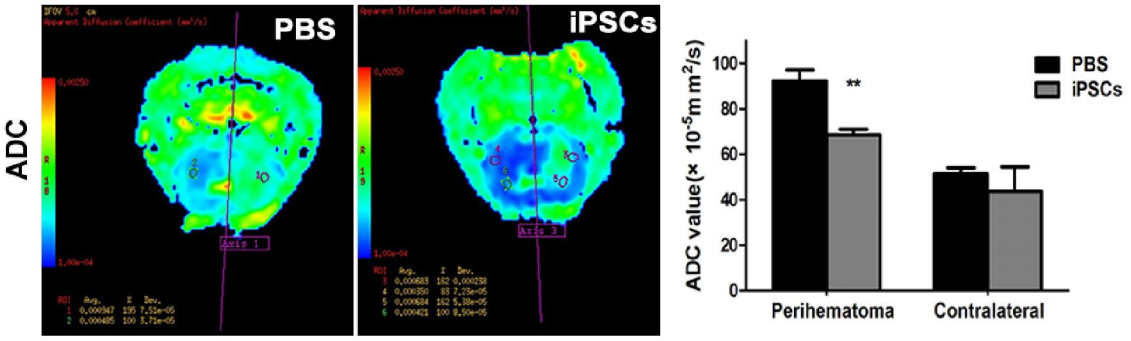
3. Results

3.1 IPSCs transplantation reduced perihematomal edema

Brain edema peaked around the 3 or 4 day post-ICH, then it declind slowly (4). So perihematomal edema was measured at 3 days after ICH using MRI. According to the MR images scanned at 3 days post-ICH, perihematomal ADC value, which was an indirecet evidence of perihematomal edema in iPSCs group ($68.46\pm2.47\times10^{-5}mm^2/s$) was lower than PBS group ($92.2\pm4.79\times10^{-5}mm^2/s$, $P<0.01$). The ADC values of two groups in contralateral hemisphere didn't show any significal difference. (PBS: $51.38\pm2.67\times10^{-5}mm^2/s$; iPSCs: $43.8\pm10.58\times10^{-5}mm^2/s$, $P=0.07$) (Figure 1).

3.2 IPSCs transplantation did not alter hematoma volume

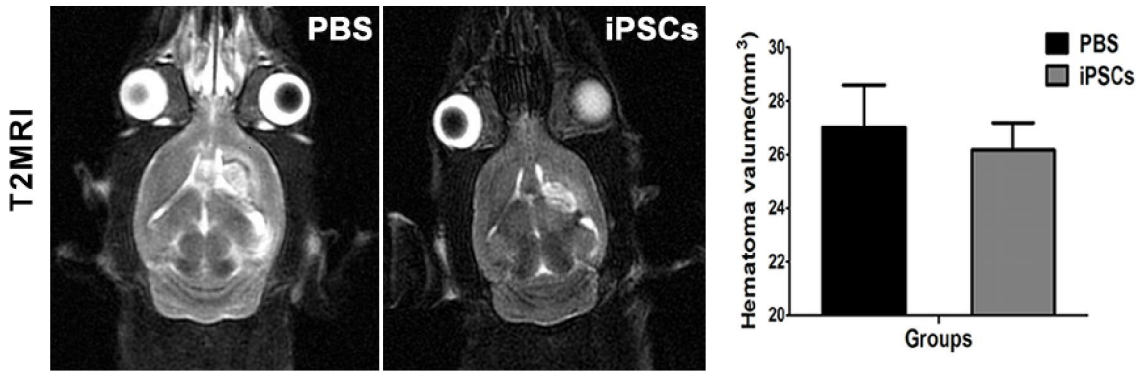
On T2MRI, we obtained the hematoma volume of two groups. The volume in iPSCs group was 26.17±1 mm^3 and 27±1.6 mm^3 in PBS group, which showed no significant difference ($P=0.31$). (Figure 2).



The ADC values (n=8, $\overline{X}$ ±SD, $\times10^{-5}mm^2/s$)

groups	perihematoma	contralateral
IPSCs	68.46±2.47**	43.8±10.58
PBS	92.2±4.79	51.38±2.67

Figure 1. Diffusion-weighted images of rats of PBS group and iPSCs group .The perihematomal ADC value of iPSCs group was lower than PBS group (**$P<0.01$), not contralateral hemisphere.



The hematoma volume (n=8, $\overline{X}$±SD)

volume	iPSCs	PBS
Hematoma volume	26.17±1 mm^3	27±1.6 mm^3

Figure 2. T2MR images of two rats in PBS group and iPSCs group. Hyperintense halo was found around the hematoma. Hematoma volumes of two groups showed no difference.

4. Discussion

Currently, the experimental animal models of hemorrhagic stroke include two most commonly methods, induced by autologous blood or bacterial collagenase injection into different brain areas.(13,14) Intracranial injection of autologous blood (arterial or venous) can mimic the effects of cerebral hematoma. However, clinical ICH is mostly due to arterial bleeding, so arterial blood has better potential for hematoma mimic. But scientific researchers can also choose venous blood according to their own research needs, but this model cannot represent vascular structures rupture of blood vessels, which is different with clinical ICH. In the models induced by intracranial stereotactic injection of collagenase, collagenase extracted from bacterial may dissolve the extravascular protein component of connective tissue and blood vessels, resulting in disruption vasculature rupture, which is preferable to simulate the clinical cerebral hemorrhage. However, it can simultaneously damage vein wall, even capillaries, resulting in an uneven blood components, which is contrary to clinical cerebral hemorrhage. In this study, left striatum VII colagenase injection was adopted to observed the area of brain edema and hemorrhagic lesions around the hematoma better with MR.

ICH brain damage can be considered to be divided into primary injury and secondary injury. The initial bleeding will lead to brain damage with structure damage of brain cells and changes of intracranial pressure.(3) Severity of primary injury is mostly based on the amount of bleeding or hematoma volume and bleeding site.(1) According to the results of T2MRI, the hematoma volume of two groups didn't exist difference, which was consistent with other article.(6) However, when Lee et al. (6) measured hematoma volume using a spectrophotometric assay, they had to kill the rats, unlike MRI scanning. The secondary damage is caused by the release of blood clots component, cascade reaction of thrombin, activation of the complement system and inflammation.(1) Cerebral edema is the most serious secondary injury, showing abnormal accumulation of water in the brain, which can be embodied hyperintense halo around blood clot on T2MR images, exceeding the original hematoma volume and leading rise of intracranial pressure and threaing life.(15) Almost each part of primary damage and secondary damage are involved in cerebral edema formation.(1) For example, Clot retraction leaves a large number of proteins in the brain tissue retention gap, which rise the osmotic pressure and form interstitial edema.(16) Even though thrombin activation can prevent further bleeding, thrombin can also affect the brain endothelial cells, leading to damage of the blood-brain barrier to form brain edema directly.(1) Moreover, activation of the complement system and the hemoglobin and iron released from red blood cell lysis can aggravate cerebral edema as well.(17,18,19) The cerebral edema, perihematomal tissue water content, whose boundary exceed the hematoma volume is accompanied by elevated ADC values on DWI. Apparent diffusion coefficient (ADC) values elevation can be indirect evidence of vasogenic edema.(20) In this study, we found the average ADC value of perihematomal edema in iPSCs group was lower than PBS group, which indicated iPSCs transplantation reduced perihematomal edema.

Hematoma and edema can increase intracranial pressure, leading to the decline of cerebral blood flow. However, because pathophysiology of ICH is very complex, and the cerebral tissue metabolism also declines in perihaematomal zone, which means that metabolic rate for oxygen or oxygen extraction fraction declines. (21) Therefore, ischemia of brain tissue surrounding hematoma occurs after ICH is still controversial. Ischemia can be reflected as the ischemic penumbra on MR images, which was first put forward for ischemic stroke, for the presence of ischemic brain tissue surrounding hematoma, and was still possible to save.(22) But we didn't found typical ischemic penumbra next to cerebral hematoma in the MRI images of rats' brains. In this study, MRI technology was used in the study of iPSCs transplantation for ICH, and showed iPSCs resulted in lewer perihematoma ADC value, which meaned lower cerebral edama. In summary, MRI technology can be new non-invasive method to atudy the treatment of iPSCs transplantation for ICH.

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