In vivo tracing of superparamagnetic iron oxide-labeled bone marrow mesenchymal stem cells transplanted for traumatic brain injury by susceptibility weighted imaging in a rat model

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【Abstract】Objective: To label rat bone marrow mesenchymal stem cells (BMSCs) with superparamagnetic iron oxide (SPIO) in vitro, and to monitor the survival and location of these labeled BMSCs in a rat model of traumatic brain injury (TBI) by susceptibility weighted imaging (SWI) sequence.

Methods: BMSCs were cultured in vitro and then labeled with SPIO. Totally 24 male Sprague Dawley (SD) rats weighing 200-250 g were randomly divided into 4 groups: Groups A-D (n=6 for each group). Moderate TBI models of all the rats were developed in the left hemisphere following Feeney’s method. Group A was the experimental group and stereotaxic transplantation of BMSCs labeled with SPIO into the region nearby the contusion was conducted in this group 24 hours after TBI modeling. The other three groups were control groups with transplantation of SPIO, unlabeled BMSCs and injection of nutrient solution respectively conducted in Groups B, C and D at the same time. Monitoring of these SPIO-labeled BMSCs by SWI was performed one day, one week and three weeks after implantation.

Results: Numerous BMSCs were successfully labeled with SPIO. They were positive for Prussian blue staining and intracytoplasm positive blue stained particles were found under a microscope (×200). Scattered little iron particles were observed in the vesicles by electron microscopy (×5000). MRI of the transplantation sites of the left hemisphere demonstrated a low signal intensity on magnitude images, phase images and SWI images for all the test rats in Group A, and the lesion in the left parietal cortex demonstrated a semicircular low intensity on SWI images, which clearly showed the distribution and migration of BMSCs in the first and third weeks. For Group B, a low signal intensity by MRI was only observed on the first day but undetected during the following examination. No signals were observed in Groups C and D at any time points.

Conclusion: SWI sequence in vivo can consecutively and noninvasively trace and demonstrate the status and distribution of BMSCs labeled with SPIO in the brain of TBI model rats.

Key words: Mesenchymal stem cells; Brain injuries; Echo-planar imaging

In recent years, a great progress has been made in the management of central nervous system disease such as brain trauma by transplantation of bone marrow stromal cells. Fluorescence microscopy of host brain sections can trace and show the proliferation, migration and differentiation of bone marrow mesenchymal stem cells (BMSCs) labeled with green fluorescent protein. However, in clinic we need a noninvasive approach. In vivo monitoring of magnetically labeled stem cells by routine MRI has been widely applied abroad, but according to our knowledge, there are no reports that have ever described the in vivo tracing of BMSCs labeled with superparamagnetic iron oxide (SPIO) and transplanted for brain injuries by susceptibility weighted imaging (SWI) at home or abroad.

METHODS

Modeling and grouping of traumatic brain injury (TBI)
Totally 24 male Sprague Dawley (SD) rats weight-
ing 200-250 g were randomly divided into 4 groups: Groups A-D (n=6 for each group). Moderate TBI models of all the rats were developed in the left hemisphere referred to Feeney et al’s and Wu et al’s methods. Group A was the experimental group and stereotaxic transplantation of BMSCs labeled with SPIO was conducted in this group 24 hours after TBI modeling. The other three groups were control groups with transplantation of SPIO, unlabeled BMSCs and injection of nutrient solution respectively conducted in Groups B, C and D at the same time.

BMSCs culture
SD rats aged 4-6 weeks and weighing about 120 g were killed by cervical dislocation. The bilateral tibia and femur were obtained under sterile condition and the medullary cavities were washed timely with Dulbecco minimum essential medium (DMEM) containing no serum. After centrifugation of the obtained cell suspension, low glucose DMEM supplemented with 10% fetal calf serum was added into the cell precipitation. Cells were incubated at 37°C in a humidified incubator containing 5% CO₂. The media were timely changed to purify BMSCs, and the sixth generation was obtained for experiment.

SPIO labeling
SPIO of 1 µl (trade name: Resovist, 0.5 mmol/ml, Schering Corporation, Berlin, Germany) was added into 2 ml culture medium and mixed sufficiently to gain a final concentration of 14 µg/ml. BMSCs of the sixth generation after digestion and centrifugation were added into the above medium and fully blended, and then incubated at 37°C in a humidified incubator containing 5% CO₂. The media were timely changed to purify BMSCs, and the sixth generation was obtained for experiment.

Identification of the intracellular iron particles

Prussian blue staining After elimination of the nutrient solution, adherent stem cells were washed with phosphate buffered saline (PBS), then fixed in 4% glutaraldehyde solution for 30 minutes and washed with distilled water for 3 times, then incubated in 2% potassium ferrocyanide and 6% HCl solution for 30 minutes, and finally re-stained with 1% fast red for 3 minutes. The additional fast red was washed away and BMSCs were observed under a microscope (×200).

Electron microscopy After digestion, BMSCs labeled with SHU555A were centrifugated for 5 minutes (1500 r/min) and fixed in 4% glutaraldehyde solution. Then they were washed in PBS, fixed in 1% osmic acid and washed again in PBS completely. Gradient dehydration by ethanol was conducted once and routine dehydration by 100% acetone solution twice. Then they were embedded in epoxide resin and cut into thin sections with the Leica ultra microtome (Leica, Solms, Germany). The sections were double electron stained with uranyl acetate and lead citrate, and observed under a Hitachi H-7500 transmission electron microscope (Hitachi Ltd, Tokyo, Japan, ×5000).

Stereotaxic transplantation of BMSCs after labeling
Stereotaxic transplantation of BMSCs was performed 24 hours after TBI modeling. The model rats were anaesthetized by 10% chloral hydrate injected intraperitoneally at a dose of 0.32 ml/100 g body weight. Rats were fixed on the stereotaxic bracket in a supine position, and then BMSCs were transplanted to the peripheral cortex of the left injured parietal cortex (3 mm behind and 2 mm beside the bregma, 5-mm deep). In Groups A-C, BMSCs labeled with magnetic SPIO, SPIO and unlabelled BMSCs of the same quantity and density were respectively transplanted, and in Group D, culture medium in the same volume was injected. Cell suspension (5 µl, about 0.5×10⁶ cells) or contrast solution was injected within 5 minutes and the needle was left there for 5 minutes. When the transplantation procedure had been completed, 2 000 U gentamicin was injected into the rat abdominal cavities. Then all the rats were put back and fed in cages.

Parameters of MRI
We adopted the SIEMENS 3.0 Trio Tim I-class MR scanner (Siemens, Munich, Germany) and LOOP 4 coils (Siemens, Munich, Germany) for SWI sequence in the transverse plane. Relative data of the scanning parameters were as follows: repetition time/echo time (TR/TE), 29/20 ms; slice thickness, 0.6 mm; inter-slice gap, 0.12 mm; spatial resolution, 0.2 mm×0.2 mm×0.6 mm; FOV, 6 cm×6 cm; flip angle, 15°; matrix, 256×256. The scanning range was the whole rat brain.

Prussian blue staining
After MRI scanning, 10% chloral hydrate (0.3 ml/100 g) was injected into the abdominal cavity and 4% paraformaldehyde was perfused in the ventriculus sinister. Then paraffin sections of the hindbrain were stained by Prussian blue.
RESULTS

BMSCs culture
On the second day of primary culture, many monocytes adhering to the wall were observed, but there were lots of red cells intermixed, which were partly removed during the medium change on Days 3-4. On Days 5-6, the adherent cells entered the logarithmic phases and increased rapidly. On Days 6-7, cells in the bottom began to grow and the cell fusion reached 70%. Cells appeared circular or spindle-shaped (Figure 1A). The culture medium was changed after the digestion by trypsin. Thereafter cells grew rapidly and covered the whole bottom about 2-3 days later to get a full fusion. The cell morphology gradually became uniform after several times of medium change and most cells were fibroblast-like cells after the third passage. BMSCs of the sixth generation were relatively pure (Figure 1B).

BMSCs labeling
Yellow iron particles were observed in the cytoplasm of SPIO-labeled BMSCs under an inverted microscope (×200). Intracytoplasmic blue stained positive particles were found after Prussian blue staining (Figure 2A). Under a transmission electron microscope (×5000), there were many vesicle-like inclusion bodies in the cytoplasm and concentrated little iron particles in the vesicles (Figure 2B).

MRI of SPIO-labeled BMSCs after transplantation
MRI scan was performed one day, one week and three weeks after transplantation in each group. Punctiform low signal intensity on magnitude images, phase images and SWI images was observed by MRI of the transplantation site of the left hemisphere in all the six test rats in Group A (Figures 3A, 3B and 3C). The lesion in the left parietal cortex demonstrated a semicircular low intensity on SWI images, which can clearly show the distribution and migration of BMSCs at the first and third weeks (Figures 3D and 3E). For Group B, the low signal intensity by MRI was only observed on the first day but undetected during the following examination. No signals were observed in Groups C and D at any time points.

Prussian blue staining of tissue sections
Blue stained positive cells for Prussian blue staining were observed in brain tissue sections at or around the injection site in Group A (Figure 4). A few diffused blue iron particles were found at the injection site in Group B, while no abnormal blue stained cells had been observed in Groups C and D.

DISCUSSION

Technical principles of SWI
The technique of SWI has been developed based on MRI T2-weight imaging (T2WI), which dose not use the 180° refocusing pulse to remove the inhomogeneities of magnetic fields that are able to cause phase loss of the proton. Thus T2WI is very sensitive to the inhomogeneities of magnetic fields and easy to show materials that can cause nonuniformity of magnetic fields because of their paramagnetism or diamagnetism. SWI succeeds to this characteristics of T2WI, meanwhile, different from it. SWI adopts the technique of three-dimensional image acquisition, fully flow compensation and thin slice reconstruction. The magnitude and phase images are combined digitally, simultaneously postprocessed by a complex program to highlight the different susceptibility among tissues. Until now SWI images cannot be obtained directly from MRI scanners but have to undergo a complex image processing. Raw images including the magnitude image and phase image are obtained from T2 weighted gradient echo sequence scan and then processed by a complex program. Both the magnitude and phase images are saved, and the phase images are high-pass-filtered to remove the unwanted artifacts and then transformed to a special phase mask that is later combined with the magnitude image to create a susceptibility weighted image. Either the paramagnetic or the diamagnetic materials can cause changes of the magnetic moment alignment and magnetic fields further, then lead to dephasing of the proton and loss of signals, thus detected by SWI. However, paramagnetic materials always demonstrate a low signal intensity on magnitude images, phase images, SWI images and MINP images obtained by minimum intensity projection. While diamagnetic materials, if calcified, will demonstrate a high signal intensity on phase images. The SPIO-labeled BMSCs in this experiment demonstrate a low signal intensity on all the SWI images.

Significance of SWI in tracing SPIO-labeled BMSCs
SPIO is a contrast agent applied to label stem cells utilizing the phagocytosis of stem cells in recent years in foreign countries and satisfactory results of in vivo tracing of these SPIO-labeled cells have been reported.
These intracellular SPIO can cause a proton spin and rapid phase loss on MRI, thus decreasing the values of T2 and T1, especially T2. SPIO used in this study is SHU555A (trade name: Resovist) produced by Schering Corporation (Berlin, German). It is a kind of superparamagnetic iron oxide composed of Fe₃O₄ and Fe₂O₃ and can be 100% labeled without transfection. Due to its special chemical structure, SPIO can produce great magnetism even at a weak external magnetic field and the magnetism will disappear rapidly after removal of the external magnetic field. This characteristic is defined as superparamagnetism. The superparamagnetism of SPIO can significantly reduce the signal intensity of every target region on MRI sequence and produce a negative effect of the contrast agent.

Figure 1. A: BMSCs primarily cultured for six days. They appear circular (×100). B: BMSCs of the sixth generation. They cluster together and appear spindle-shaped (×100). Figure 2. A: Prussian blue staining of rat SPIO-labeled BMSCs. There are many tiny blue stained particles that crowd in the cytoplasm. The cell colors range from light blue to dark blue, as the arrows show (×200). B: Observation of intracellular iron particles under a transmission electron microscope. There are a lot of vesicle-like inclusion bodies scattering in the cytoplasm and concentrated tiny iron particles in the vesicles, as the arrows show (×5000).

Figure 3. MRI sequence and SWI images of the SPIO-labeled BMSCs. The right arrows show the lesion area and the swallow arrows show the transplanted SPIO-labeled BMSCs. Magnitude image (A), phase image (B) and SWI image (C) one day after transplantation; SWI images one week (D) and three weeks (E) after transplantation, which show a migration tendency of the low signal intensity to the lesion area. Figure 4. Some blue stained positive brain tissue cells reveal a migration tendency under a microscope (Prussian blue staining ×200).

Ge et al and Wu et al have reported that T2WI has better sensitivity and contrast in tracing SPIO-labeled BMSCs compared with T1-weighted imaging (T1WI) in routine MRI. According to the principles of SWI and composition of SPIO, we can know that SWI is more sensitive to different susceptibility than T2WI and can cause a stronger reaction. Moreover, the fact that SPIO-labeled BMSCs can be clearly visualized on SWI sequence is partly related to the reason that the obtained image is 4-6 times of the volume of the real sample. Furthermore, the spatial resolution of SWI sequence is 0.2 mm×0.2 mm×0.6 mm, higher than that of routine MRI and T2WI. Because of its high sensitivity and resolution, SWI can well show the distribution and migration of SPIO-labeled BMSCs in the brain. As showed by the above figures, the phase image, magni-
tude image and SWI images after postprocessing can clearly show the location and morphology of SPIO-labeled BMSCs one day after transplantation. SWI performed one week and three weeks after BMSCs transplantation obviously reveal the morphology of the cell mass. SWI can well show the migration tendency of low intensity-labeled cells by its magnification principles.

Our experience reveals that SWI can clearly demonstrate the morphology and distribution of SPIO-labeled BMSCs at the transplantation site.

REFERENCES


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