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Effect of transplantation route on stem cell migration to fibrotic liver of rats via cellular magnetic resonance imaging

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Abstract

Background aims. Assessing mesenchymal stromal cells (MSCs) after grafting is essential for understanding their migration and differentiation processes. The present study sought to evaluate via cellular magnetic resonance imaging (MRI) if transplantation route may have an effect on MSCs engrafting to fibrotic liver of rats. *Methods.* Rat MSCs were prepared, labeled with superparamagnetic iron oxide and scanned with MRI. Labeled MSCs were transplanted via the portal vein or vena caudalis to rats with hepatic fibrosis. MRI was performed *in vitro* before and after transplantation. Histologic examination was performed. MRI scan and imaging parameter optimization *in vitro* and migration under *in vivo* conditions were demonstrated. *Results.* Strong MRI susceptibility effects could be found on gradient echo-weighted, or T2*-weighted, imaging sequences from 24 h after labeling to passage 4 of labeled MSCs *in vitro*. *In vivo*, MRI findings of the portal vein group indicated lower signal in liver on single shot fast spin echo-weighted, or T2-weighted, imaging and T2*-weighted imaging sequences. The low liver MRI signal increased gradually from 0–3 h and decreased gradually from 3 h to 14 days post-transplantation. The distribution pattern of labeled MSCs in liver histologic sections was identical to that of MRI signal. It was difficult to find MSCs in tissues near the portal area on day 14 after transplantation; labeled MSCs appeared in fibrous tuberculum at the edge of the liver. No MRI signal change and a positive histologic examination were observed in the vena caudalis group. *Conclusions.* The portal vein route seemed to be more beneficial than the vena caudalis on MSC migration to fibrotic liver of rats via MRI.

Key Words: *cell labeling, magnetic resonance imaging, mesenchymal stromal cells, transplantation*

Introduction

Hepatic fibrosis is overly exuberant wound healing in which excessive connective tissue builds up in the liver and has been recognized as the final common pathway for a multitude of liver injuries (1). Research in the pathogenesis of hepatic fibrosis suggests the possibility of method to correct its underlying dynamic process. Studies have demonstrated the migrational capacity of endogenous stem or progenitor cells in rat and mouse livers during normal and pathophysiologic conditions (2–5). Terai *et al.* (6) induced a mouse hepatic cirrhosis model by carbon tetrachloride (CCl_4), infused 1×10^5 mesenchymal stromal cells (MSCs) labeled with green fluorescent protein (GFP) through the vena caudalis and observed that these labeled MSCs were present in hepatic lobules surrounding the portal vein 1 day later. Additionally, 25% of cells in liver were MSCs 4 weeks later; they differentiated into mature hepatic cells and secreted albumin. However, a conflicting report by Kanazawa and Verma (7) indicated that labeled bone marrow cells were noted in liver among only 5 of 18 mice with (i) CCl_4-induced hepatic lesion, (ii) albumin-urokinase transgenic model and (iii) hepatitis B virus transgenic model after vena caudalis transplantation.

The key issue is how stem cell migration may be improved. It may be argued that transplantation through the portal vein or hepatic artery seemed more superior to transplantation through other pathways. If there is no difference between stem cell migrational effect via a pathway of the portal vein versus the vena caudalis, it would be a natural option not to use the more traumatic procedure (i.e., to infuse transplanted cells through the portal vein). In the present investigation, we sought to assess the

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potential differential effect of MSC transplantation pathway on migrational dynamics and to evaluate if there is any benefit associated with the invasive strategy of transplantation through the portal vein. Standard stem cell labeling technique, validated magnetic resonance imaging (MRI) analysis *in vivo* and *in vitro* and histologic section methodology (8–11) were used to enable objective temporal analysis of MSC migrational potential and engrafting at different checkpoints in the liver of rat model.

Methods

Preparation of rats and model of hepatic fibrosis

Experiments were performed in accordance with the Sun Yat-sen University animal protection guidelines and approved by the Animal Ethics Committee of the Guandong province. The animal experiment center of Sun Yat-sen University provided 39 male Wistar rats (body weight, 200–250 g) for the experiments.

CCl_4 (Hangzhou Biology Technology Company, Zhejiang, China) was used to induce hepatic fibrosis in 36 rats (12). Phenobarbital sodium (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd, Shanghai, China) was added to drinking water (300 mg/L) 1 week before CCl_4 administration. For 6 weeks, 2 mL/kg CCl_4 solution (40% in olive oil) was injected subcutaneously twice a week (12 times). Masson trichrome staining (IHC WORLD, Woodstock, MD, USA) was performed to evaluate hepatic fibrosis induced by CCl_4.

Cell culture and identification of MSCs

Two rats were sacrificed by cervical dislocation and soaked in 75% alcohol solution (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd) for 10 min. The femur was dislocated from the tibia and placed in sterile pre-warmed Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich, Dorset, UK) supplemented with 2% fetal bovine serum (FBS; Hangzhou Biology Technology Company) and 40 IU heparin (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd). Epiphyses of femurs and tibias were removed, and the marrow was flushed out by using a syringe filled with medium. The suspension was centrifuged at 1000 rpm for 10 min, re-suspended in 10 mL of low-glucose DMEM and passed through a 70-μm nylon mesh and plated in 75-cm^2 flasks. After culturing for 24 h, supernatant containing non-adherent cells was removed, and fresh medium was added. After the cells had grown to about 80% confluency for 2–3 days, they were passaged 2–5 times by being detached with 0.25% parenzyme (Hangzhou Biology Technology Company, Zhejiang, China)/1 mmol/L ethylenediaminetetraacetic acid (Hangzhou Biology Technology Company) at 37°C for 5 min and replaced (13,14).

After the third-generation MSCs covered approximately 70% confluency in culture flask, flow cytometry was used to confirm the identity of MSCs (15,16). Briefly, MSCs were prepared in 5×10^6–1×10^7/mL with low-glucose DMEM containing 10% FBS. Centrifuge tubes coated previously with anti-CD29-PE, anti-CD166-PE, anti-CD105-PE, anti-CD44-PE, anti-CD34-FITC and anti-CD45-FITC (5–50 μL, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were pipetted with 40 μL MSC suspension followed by 50 μL inactivated normal rabbit serum (Hangzhou Biology Technology Company) (1:20) and incubated at 4°C for 30 min. MSCs were re-suspended, washed two times with phosphate-buffered saline (PBS; Hangzhou Biology Technology Company), combined with 50 μL goat anti-rat fluorescein isothiocyanate-labeled substrate (Hangzhou Biology Technology Company), mixed well and incubated at 4°C for 30 min. Into every tube 1 mL fixing solution was added, and 10 samples were subjected to flow cytometry. Three-color flow cytometry was employed using an enzymatic amplification staining kit (Flow-Amp Systems; Tebu-bio, Le Perray en Yvelines, France). Fluorescent labeling was analyzed using a FACSCalibur flow cytometer (Becton, Dickinson and Company). Final results were analyzed by software (Coulter Epics XL-MCL, EXPOTM32; Beckman Coulter, Brea, CA, USA).

Adipogenic and osteogenic differentiation of MSCs

Adipogenic and osteogenic differentiation was performed using MSCs of passage 3. To induce adipogenic differentiation, 10% FBS, 1 mg/L dexamethasone (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd), 0.5 mmol/L 3-isobutyl-1-methylxanthine (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd), 10 mg/L insulin (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd) and 100 mmol/L indomethacin (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd) in high-glucose DMEM was used. Oil red O staining was used as an indicator of intracellular lipid accumulation at day 20. To induce osteogenic differentiation, 10% FBS, 10^{-7} mol/L dexamethasone, 10 mmol/L β-glycerophosphate (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd) and 50 mg/L ascorbic acid (Shanghai No. 1 Biochemical & Pharmaceutical Co, Ltd) in low-glucose DMEM was used. Alkaline phosphatase staining was performed on day 14, and alizarin red staining was performed on day 21 to assess the level of extracellular matrix calcification (12,14).