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Bone Marrow Mesenchymal Stem Cells Reduce Intestinal Ischemia/Reperfusion Injuries in Rats

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Background. **Adult stem cells are promising novel therapies in regenerative medicine. We investigated effects of bone marrow-derived mesenchymal stem cells (MSCs) on intestinal mucosal permeability impaired by ischemia/reperfusion (I/R).**

Methods. **We used a common I/R model in rats to induce intestinal injury by clamping and unclamping the superior mesenteric artery (SMA) in female Sprague-Dawley rats. MSCs were directly injected into the small intestinal submucosa of the syngenic female rats. Control group were injected with the same volume of 0.9% sodium chloride. Small intestine samples were examined for the engraftment of donor-derived mesenchymal stem cells (MSCs) by Y chromosome *in situ* hybridization analysis. The small intestinal permeability and histomorphologic alternations were measured to evaluate the therapeutic effect of MSCs transplantation.**

Results. **Small intestinal permeability and villi injuries were significantly reduced in the MSCs administrated group compared with control group. MSCs administration accelerated the recovery of the intestinal barrier dysfunction.**

Conclusion. **We concluded that submucosal infusion of MSCs might exert protective effects on the integrity of intestinal barrier.** © 2011 Elsevier Inc. All rights reserved.

Key Words: **mesenchymal stem cells (MSCs); transplantation; ischemia/reperfusion (I/R); intestine.**

INTRODUCTION

Ischemia/reperfusion (I/R) injuries in the gut have been an important clinical issue. The small intestine is composed of labile cells that are easily injured by ischemia and reperfusion. Prognosis for patients with occlusion of the superior mesenteric artery remains very poor even after revascularization, with mortality rates between 60% and 100% [1]. Ischemia/reperfusion (I/R) injury is still the biggest obstacle to improving outcomes for intestinal transplantation [2]. The mechanisms and treatment for I/R injuries in the intestine have been under extensive research. Oxygen-free radical formation, release of iron storage, inflammatory cytokines, complement activation, neutrophils infiltration, and enteric bacteria translocation are all thought to be involved [3]. Treatments, such as ischemic preconditioning (IPC), antioxidants, free-radical scavengers, nitric oxide (NO) supplementation, anti-complement therapy, anti-leukocyte therapy, glutamine supplementation, and glycine supplementation are still inadequate [4].

Bone marrow-derived mesenchymal stem cells (MSCs) are fibroblast-like, pluripotent, adult stem cells within the bone marrow. MSCs are adherent to plastic and can readily grow in the laboratory. MSCs not only give rise to mesoderm cells [5, 6], but also have been reported to differentiate down all three germ cell lines [7]. This capacity of MSCs is enhanced in the setting of tissue injuries, suggesting a possible role for MSCs in regenerative medicine. Devine *et al.* [8] transplanted allogeneic MSCs to primates *via* the intravenous route and demonstrated that MSCs distributed to the gastrointestinal tract and proliferated within these tissues. Semont *et al.* [9] discovered that infused MSCs engrafted into irradiated intestine and might link to injury recovery. Mesenchymal stem cells have also been shown to have immunomodulatory capabilities [10, 11] by secreting several growth factors, such as

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vascular endothelial growth factor (VEGF), insulin-like growth-1 (IGF-1), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and transform growth factor alpha (TGF-α). Those factors play an important role in the inhibition of apoptosis, enhancement of angiogenesis, stimulation of mitosis, proliferation, and differentiation of organ intrinsic precursor or stem cells [12]. Studies in the ischemia/reperfusion rodent model found that MSCs could be beneficial *via* paracrine production of the above growth factors and anti-inflammatory cytokines [13]. Increasing evidences suggested that the therapeutic potential of MSCs could be applied to I/R injured tissue such as heart, brain, and kidneys of experimental animals.

The potential of MSCs of rodent origin in intestinal ischemia/reperfusion has been under investigation. In the present study, we aimed to evaluate the influence of exogenous MSCs treatment on intestinal mucosal villous injury and intestinal barrier function after superior mesenteric ischemia/reperfusion. We demonstrated that MSCs transplantation could improve intestinal barrier function and attenuate intestinal ischemia and reperfusion injuries.

MATERIALS AND METHODS

Animals

MSCs were isolated from 4-wk old male Sprague-Dawley rats. The recipients were female Sprague-Dawley rats weighing 180–220 g. They were housed in plastic-bottomed wire-lidded cages and maintained on a 12:12-h light/dark cycle in a temperature-controlled room (25°C) with free access to water and rat chow. All animals were acclimatized at least 7 d before use. The experimental procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals, the Animal Research Committee of Nanjing University, Jiangsu, China.

MSCs Preparation and Differentiation Assay *In Vitro*

MSCs isolation and expansion was performed according to a previously described method [14]. In brief, we euthanized 4-wk old male Sprague-Dawley rats and harvested the femurs and tibias in a sterile fashion. Cavities of the femurs and tibias were flushed with sterile PBS and centrifugation (400 g, 5 min). Cell pellets were plated in culture flasks (200,000 cells per cm^2) with DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Nonadherent cells were removed after 72 h. MSCs were recovered by their capacity to adhere highly to plastic culture dishes. MSCs phenotypes were confirmed by the typical spindle-shaped appearance, and by differentiation into osteocytes and adipocytes with specific differentiation media [15]. For flow cytometry assay, MSCs were incubated with anti-rat CD90/fluorescein isothiocyanate (FITC) (Invitrogen, Carlsbad, CA) and CD45/PE antibody (Invitrogen) for 1 h; fluorescence was analyzed by flow cytometry (FACSCalibur; Becton, Dickinson, Franklin Lake, NJ). MSCs at passage 3 were used for all experiments.

Experimental Design and Surgical Procedure

Sixty female rats were randomly assigned to three experimental groups, 20 rats per group, and all animals were fasted for 12 h with free access to water before operation. Operative procedures were performed using standard sterile technique under general anesthesia with ketamine (100 mg/kg, i.p.). All intestinal I/R rats were subjected to laparotomy using a midline incision about 3 cm and identified the principal branches of superior mesenteric artery (SMA). In sham group, rats only underwent laparotomy and freed blunt dissection SMA, without clamping the SMA. In MSCs group, animals' SMA was occluded with an atraumatic microvascular clamp for 45 min. Just after the clamp was released, 1×10^7 male rat MSCs suspended in 0.5 mL serum free DMEM were submucosa-injected into the intestine at 10 different points. In vehicle group, animals received the same surgical procedure as MSCs group and 0.5 mL normal saline was submucosa-injected into the intestine at 10 different points. The abdomen was then closed and the animals recovered with free access to tap water and standard pellet rat chow. At the end of the observation period (4 and 7 d after the operation), 10 rats of each group were euthanized, and blood samples and about 5-cm ileum segment of each rat was collected. Plasma was separated by centrifugation and stored at –80°C until analysis. Intestinal samples were fixed in 4% paraformaldehyde for histopathologic analysis and *in situ* hybridization.

Detection of Donor MSCs in the Recipient Intestines

We used a Y chromosome *in situ* hybridization method (SRY gene detection reagent, Haoyang Biological Manufacture, Tianjin, China) to confirm the presence and distribution of MSCs in recipients. Intestinal biopsies were fixed in 4% paraformaldehyde, routinely processed, and embedded in paraffin wax. Sections were treated with xylene, graded alcohols (95%–80%–60%–30%) and PBS. After incubation with deionized water containing 3% H_2O_2 for 10 min at room temperature, SRY reagent B (concoction fluid) was added for another 10 min. Sections were then washed with 0.1 mol TBS (PH 7.8) for 5 min, 0.1 mol TBS for 20 min at 95$\sim$100°C, 0.1 mol cold TBS and 0.2×SSC for 5 min. The SRY reagent A (hybridization fluid) was used to incubate the sections for 4–8 h in humid box at room temperature. After washing with 2×SSC, 0.2×SSC, and 0.1 mol TBS(PH 7.8) at 37°C, the reagent C (POD transforming agent) was added to slides for 45 min at 37°C. Slides were developed in SRY reagent F (3, 3′-diaminobenzidine), counterstained with hematoxylin, and mounted with either an aqueous mounting medium or 60% glycerol. The nucleus stained in brown was defined as Y chromosome positive cells. Sections of each animal were examined under light microscope by two independent pathologists. Evaluation was performed by counting Y chromosome positive cells in at least 10 HPF (×400) per section. Date was expressed as percentage of Y positive cells to total 200 of cells for each HPF.

Histological Measurement of Intestinal Mucosal Injury

The serial 2-cm samples were taken from terminal ileum and fixed with 10% neutral formalin. Tissues were processed, embedded, and stained with hematoxylin and eosin. Three paraffin sections were prepared from each tissue sample. Two pathologists who were blinded to the source of the slides analyzed each slide. The degree of histopathologic changes was graded semiquantitatively using the histologic injury scale previously described by Chiu *et al.* [16], 0, normal mucosal villi; 1, development of subepithelial space, usually at the apex of the villi with capillary congestion; 2, extension of the subepithelial space with moderate lifting of the epithelial layer from the lamina propria; 3, massive epithelial lifting down the sides of the villi and ulceration at the villous tips; 4, denuded villi with dilated capillaries and increased cellularity of the lamina propria; and 5, degradation and disintegration of the lamina propria, hemorrhage, and ulceration. A minimum of six randomly chosen fields from each rat were evaluated and averaged to determine mucosal damage.

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