Efficiency of Endovenous Versus Arterial Administration of Mesenchymal Stem Cells for Ischemia-Reperfusion–Induced Renal Dysfunction in Rats

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ABSTRACT

Background. Ischemia followed by reperfusion leads to acute renal failure in both native kidneys and a renal allograft. Our previous study found that transplantation of mesenchymal stem cells (MSCs) ameliorated ischemia-reperfusion (I/R)-induced kidney dysfunction by increasing the activities of antioxidant enzymes. The purpose of this study was to evaluate whether intra-arterial versus intravenous administration was more effective.

Methods. Renal ischemia was induced by clamping the right renal vessels for 60 minutes after removal of the left kidney. MSCs (1×10^6) were administered through either the tail vein (TV) or the renal arter (RA), followed by reperfusion. We evaluated kidney function as well as tissue activities of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px). Histopathologic and immunohistochemical examinations were performed. To tracking MSCs in vivo, they were transfected with firefly luciferase and monomeric red fluorescent protein reporter genes (fluc-mrfp). MSC retention and survival were assessed using bioluminescence imaging. We observed the effects of MSCs (1×10^6, 2×10^6, and 5×10^6) on IR injury.

Results. MSC infusion via either the tail vein or the renal artery significantly improved kidney function at days 1, 3, and 5 as indicated by lower urea and creatinine levels compared with vehicle controls ($P < .05$). I/R induced a reduction in renal tissue SOD activity but GSH-PX was significantly improved by MSCs ($P < .05$) on day 1. Treatment with MSCs also significantly reduced renal tissue MDA levels that had been otherwise increased by renal I/R injury ($P < .05$). The above parameters were similar between the TV and the RA groups. Histological examination revealed kidneys from MSC-treated rats to show fairly normal morphology. The percentages of proliferating cell nuclear antigen (PCNA)-positive cells were higher in the MSC groups: $16.83 \pm 4.62\%$, $19.17 \pm 6.21\%$, and $2.17 \pm 1.16\%$ for the TV, RA, and control groups, respectively. There was no significant dose-related difference among MSC groups. Bioluminescence imaging demonstrated most MSCs to be lost within 7 days after either intravenous or intra-arterial infusion.

Conclusions. MSCs ameliorated I/R-induced acute renal failure in rats with similar efficiency whether infused either through the TV or the RA. There was no dose-dependent responses.

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ISCHEMIA/reperfusion (I/R) injury is a major cause of acute renal failure (ARF),[1] which is unavoidable in many clinical situations such as kidney transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery, and elective urologic operations. In these situations, I/R injury initiates a complex, interrelated sequence of events, resulting in injury to and eventual death of kidney cells.[2,3] Several factors have been demonstrated to contribute to the pathophysiological changes during renal I/R injury, including vascular or microvascular injury, endothelial dysfunction, accelerated cell necrosis, granulocyte activation, and modulation of the nitric oxide/angiotensin II axis.[4,5] Reactive oxygen species (ROS) play important roles in the development of I/R-related delayed graft function.[6,7] Under normal conditions, naturally occurring antioxidant enzymes in the kidney counteract the cellular effects of oxygen-free radicals.[8] But toxic oxygen radicals cannot be totally eradicated during I/R.[9] The remaining ROS inevitably cause cell death by apoptosis.[10] Although many agents have been shown to counteract the actions of ROS, none of them is approved to reduce renal oxidative stress and to prevent or treat renal ischemic injury.[11,12]

Mesenchymal stem cells (MSCs) are multipotent elements that are able to differentiate into a variety of cells, including osteoblasts, chondrocytes, and adipocytes, MSCs are nonimmunogenic and immunosuppressive, possessing the ability to migrate to sites of tissue injury and inflammation to participate in tissue repair.[13] In the past several years, many clinical trials have shown beneficial effects of MSCs in settings of acute myocardial ischemia and acute liver damage.[14–16] Although no similar clinical trial has been reported for acute kidney injury (AKI), the potential role of MSCs in this disorder is under intensive investigation in animal models of ischemia- or chemical-induced ARF.[17–22] In these studies paracrine effects of MSCs have been demonstrated to be responsible for protective and regenerative effects, Recently, in a rodent model of AKI,[23] we demonstrated that MSCs alleviated I/R injury by increasing the activities of antioxidant enzymes.

The administration route of MSCs must allow them to travel to target organs and tissues, The most commonly used routes for MSC administration include systemic delivery (intravenous injection), site-directed delivery (interventional therapy), and directive local injection. Attracted by signals from injured and inflammatory tissues, MSCs may migrate specifically to the site of damage. However, after systemic administration they are prone to being trapped in tissues laden with capillaries, such as lung, spleen, and liver. Thus, site-directed delivery is believed to be superior to systemic intravenous injection.[24] In the present study, we compared the effects of 2 administration routes of MSCs to ameliorate I/R-induced kidney dysfunction and to increase the activities of superoxide dismutase (SOD) and other antioxidant enzymes of renal tissue. We hypothesized that site-directed delivery of MSCs to the injured kidney via the renal artery might be more effective than systemic delivery to alleviate I/R-induced injury.

MATERIALS AND METHODS

Animals

We purchased male Sprague-Dawley rats of 40–60 g or 250–300 g and ages 4–6 weeks and 8–10 weeks from Shanghai Laboratory Animal Center (Shanghai, China). They were housed under light and dark cycles with free access to food and tap water. All protocols were approved by our institutional animal ethics committe.

Isolation, Characterization, and Lentiviral Transfection of MSCs

Isolated MSCs were cultured as previously described.[19,23] To obtain bone marrow from Sprague-Dawley rats (weight range, 40–60 g; age range, 4–6 weeks), the animals were humanely killed and femurs and tibias aseptically removed. After flushing from the shaft of the bone with Dulbecco's Modified Eagle Medium medium (Sigma, USA), containing 0.1% flow cytometry standard (Invitrogen, USA) plus penicillin/streptomycin, the bone marrow was passed through a 200-μm sterile filter to yield a single-cell suspensioin. Mononuclear cells isolated there from Ficoll-Paque (Sigma) density-gradient centrifugation were plated in DMEM plus 10% FCS and penicillin-streptomycin. After elements adhered to the plate bottom for 48 hours, nonadherent cells were removed. The medium was changed every 3 days. At 80% confluence, cells harvested with 0.25% trypsin and 0.02% EDTA (ethylene diaminetetrac acid) were replated, yielding passages 4–6 for in vitro and in vivo experiments. Cell surface marker expression was analyzed using flow cytometry (FACS Canto; Becton Dickinson, San Jose, Calif, United States). Anti-CD29-FITC, anti-CD44-FITC, anti-CD105-FITC, anti-CD45-PE, and anti-CD34-PE were purchased from Beckman Coulter (USA). Differentiation potential was evaluated by culturing cells under conditions for adipogenic and osteogenic differentiation, as previously described.[25]

The MSCs were lentivirally transduced to express both firefly luciferase (Fluc) and monomeric red fluorescent protein (mRFP). Briefly, MSCs were seeded in a 6-well plate at 1×10^5 cells/well. Then, the serum-free OptiMEM (lnvitrogen) medium and polybrene were added to a final concentration of 8 μg/mL (Sigma Chemical Co.) After removing the medium and adding OptiMEM (1.5 mL per well), the virus was thawed and added at a multiplicity of infection of 10. After 48 hours of incubation, the medium was replaced with fresh α-MEM/10% fetal bovine serum, as previously reported.[26]

Surgery and Experimental Protocol

Rats (weight range, 250–300 g; age range, 8–100 weeks) were anesthetized by intramuscular injection of xylazine (10 mg/kg) and ketamine (70 mg/kg), Via midline incision the left kidney was removed and uninephrectomized hosts randomly divided into a sham-operated, a control, a vehicle-treated, a tail vein (TV) and a renal artery (RA) group (n = 8 each). To induce ischemic ARF, the right RA and vein were occluded with a nontraumatic clamp for 60 minutes. At the end of the ischemic period, the clamp was released for reperfusion. Rats in the TV group received 1×10^6 MSCs via the TV 1 minute after the start of reperfusion. Those in the RA group received 1×10^6 MSCs via the RA using a microcatheter. To optimize the kidney infusion, a vascular clamp was used to temporarily occlude the aorta between the origins of