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Intra-renal arterial injection of autologous bone marrow mesenchymal stromal cells ameliorates cisplatin-induced acute kidney injury in a rhesus *Macaque mulatta* monkey model

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Abstract

Background. Clinically, acute kidney injury (AKI) is a potentially devastating condition for which no specific therapy improves efficacy of the repair process. Bone marrow mesenchymal stromal cells (BM-MSCs) are proven to be beneficial for the renal repair process after AKI in different experimental rodent models, but their efficacy in large animals and humans remains unknown. This study aims to assess the effect of autologous rhesus *Macaque mulatta* monkey BM-MSC transplantation in cisplatin-induced AKI. *Methods.* We chose a model of AKI induced by intravenous administration of 5 mg/kg cisplatin. BM-MSCs were transplanted through intra-arterial injection. The animals were followed for survival, biochemistry analysis and pathology. *Results.* Transplantation of 5×10^6 cells/kg ameliorated renal function during the first week, as shown by significantly lower serum creatinine and urea values and higher urine creatinine and urea clearance without hyponatremia, hyperkalemia, proteinuria and polyuria up to 84 d compared with the vehicle and control groups. The superparamagnetic iron oxide nanoparticle-labeled cells were found in both the glomeruli and tubules. BM-MSCs markedly accelerated Foxp3+ T-regulatory cells in response to cisplatin-induced damage, as revealed by higher numbers of Foxp3+ cells within the tubuli of these monkeys compared with cisplatin-treated monkeys in the control and vehicle groups. *Conclusions.* These data demonstrate that BM-MSCs in this unique large-animal model of cisplatin-induced AKI exhibited recovery and protective properties.

Key Words: *acute kidney injury, bone marrow mesenchymal stromal cells, cisplatin, intra-renal arterial injection*

Introduction

Acute kidney injury (AKI) in humans is a protean syndrome of varying severity that primarily results from ischemic and/or toxic injury. AKI occurs in up to 5% of hospitalized patients (1) and is a major cause of morbidity and mortality (2). Administration of cisplatin, an anticancer agent, is accompanied by a high incidence of nephrotoxicity and renal dysfunction. Currently, most renal-protective approaches for AKI are only partially beneficial, and patients

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either remain on dialysis or progress to chronic kidney disease (3).

Recent studies have tested stem cell–based therapy, which provides the exciting prospect of a powerful treatment to repair acute organ damage by virtue of the unique stem cell tropism and pro-regenerative capacity (4). Transplantation of human bone marrow mesenchymal stromal cells (BM-MSCs) in cisplatin-induced AKI has been shown to improve disease symptoms (5,6), decrease cisplatin nephrotoxicity *in vitro* and *in vivo* and enhance survival of mice with AKI (7).

Most studies have been conducted on AKI models that used BM-MSCs from rodents. Therefore, we believe that the development of alternative large-animal models would benefit the evaluation of stem cell therapeutic potential in the kidneys.

We developed a model of cisplatin-induced nephrotoxic injury in the adult rhesus *Macaca mulatta* monkey that presented with a typical pattern of AKI injury that closely mimicked AKI in humans. Furthermore, we have shown that transplanted BM-MSCs can engraft at the site of injury and ameliorate the outcome of AKI.

Methods

Cell culture and characterization of BM-MSCs

Bone marrow aspirates (3–5 mL) were collected from the monkeys' iliac crests after animals were anesthetized with intramuscular injections of ketamine (10 mg/kg) and xylazine (0.4 mg/kg). We isolated mononuclear cells (MNCs) with the use of lymphodex (gravity: 1.077–1.080; Inno-Train Diagnostik, Kronberg, Germany). MNCs were washed and seeded at 1×10^6 cells/cm^2 in Minimum Essential Medium Eagle Alpha Modification (Life Technologies, Grand island, NY, USA) medium that contained 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 1% L-glutamine (Life Technologies) and 1% penicillin-streptomycin (pen/strep) liquid (Life Technologies). Mesenchymal potential of MSCs was confirmed by adipogenic, osteogenic and chondrogenic differentiation.

We used the fibroblast-like colony-forming unit assay to evaluate primary cells grown on a tissue culture dish. Total BM-MNCs were plated at a density of 10^5–10^6 cells/cm^2. After 14 d, the capability of BM-MSCs to form fibroblast-like colonies was assessed. Contrast-phase microscopy after crystal violet staining was used for colony formation evaluation.

The expression of membranous antigen on BM-MSCs was analyzed by a FACSCalibur argon laser cytometer (BD Pharmingen, San Jose, CA, USA) and WinMDI 2.9 software. BM-MSCs were incubated with antibody (1:20 dilution) for 30 min at 4°C. Binding of antibodies to CD73, CD44, CD29, CD34, CD11b, CD14 (BD Pharmingen), CD105 (Inc, Minneapolis, MN, USA), CD3 (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), CD19 (eBioscience, San Diego, CA, USA) conjugated to phycoerythrin, CD45 and CD31 (BD Pharmingen) and CD90 (Dako, Glostrup, Denmark) conjugated to fluoresceine isothiocyanate were analyzed by means of flow cytometry.

For karyotyping, BM-MSCs from the primary passages were exposed to phytohemagglutinin (Life Technologies) for 15–17 h and then were treated with colcemid (Life Technologies), detached with 0.125 μL trypsin (Life Technologies) and treated with 0.075 mol/L KCl in a 37°C water bath. Cells were fixed and spread onto slides. Air-dried slides were stained with freshly prepared 10% Giemsa stain in a Gurr buffer (Invitrogen, Carlsbad, CA, USA) for 30 min.

Characterization and in vitro *recognition of labeled cells with superparamagnetic iron oxide–protamine sulfate complex*

Nanomag-D–SPIO nanoparticles (79–00–201, Micromod Partikeltechnologie GmbH, Rostoc, Germany) were used for contrast-enhanced magnetic resonance (MR) nephrography. These nanoparticles were dextran-coated iron oxide particles that had a mean diameter of 20 nm. The solid content of the nanoparticles was 10 mg/mL and their Fe concentration was 2.4 mg/mL. We conducted MR phantom studies with the use of an aqueous solution of superparamagnetic iron oxide–protamine sulfate (SPIO) compounds in 500-μL plastic tubes with an iron concentration that ranged from 0–200 μg Fe/mL.

SPIO at a concentration of 200 μg Fe/mL and protamine sulfate (ProS; 90 μg/mL) were put into a mixing tube that contained serum-free medium. ProS was prepared as a fresh stock solution of 10 mg/mL at the time of use. For attached cells, SPIO-ProS complexes were applied directly to the cells followed by the addition of an equal volume of the serum-free medium to the cells, which resulted in a final concentration of 100 μg SPIO and 45 μg ProS per milliliter of medium. The cells were incubated for 2 h at 37°C. After incubation, we added 10% FBS, 1% L-glutamine and 1% pen/strep to the cells, which were then incubated for 48 h. The iron-labeled cells were stained with Prussian blue dye (Accustain Iron Stain Kit, Sigma-Aldrich, Steinheim, Germany). For the transmission electron microscopic (TEM) studies, the SPIO-labeled cell pellet ($1.5–2 \times 10^6$) was fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate-buffered saline (PBS) (pH 7.4) for 2 h. After washing with PBS, cells were post-fixed with 1% osmium tetroxide for 1.5 h, again