Published in final edited form as:

Exp Mol Pathol. 2013 June ; 94(3): 466–473. doi:10.1016/j.yexmp.2013.03.002.

Transplantation of Bone Marrow-derived MSCs Improves Cisplatin-induced Renal Injury through Paracrine Mechanisms

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Abstract

Mesenchymal stem cells (MSCs) have been reported to preserve renal function in various models of acute kidney injury (AKI). Different routes were used to transplant MSCs but the role of cell transplantation routes in directing outcomes has been unknown. In the present study, we evaluated organ bio-distributions of transplanted MSCs, and correlated survival of transplanted cells with outcomes in mice with cisplatin-induced AKI. We found that after intravenous administration MSCs were largely localized in pulmonary capillaries and only a minute fraction of MSCs entered kidneys and the cells survived only transiently. Therefore, we also transplanted MSCs via intraperitoneal and renal subcapsular routes. Transplanted MSCs survived longer in peritoneal cavity and renal subcapsular space. Interestingly, when MSCs transplantation was followed by cisplatin-induced AKI, renal morphology and renal functions were better preserved, irrespective of the cell transplantation route. As transplanted MSCs did not migrate to kidneys from either peritoneal cavity or renal subcapsular space, this finding suggested that migration of cells was not required for the beneficial response. The possibility of indirect mechanisms was confirmed when administration of the conditioned medium from MSCs also protected renal tubular cells from cisplatin-induced cytotoxicity. We identified presence of over forty regulatory cytokines in the conditioned medium obtained from MSCs. Since paracrine factors released by transplanted cells accounted for improvements, it appears that the route of cell

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transplantation is not critical for realizing benefits in AKI of cell therapy with MSCs. Studies of specific cytokines secreted by MSCs will help to obtain new therapeutic mechanisms for renal protection.

INTRODUCTION

Many chemotherapeutic drugs, including platinum derivatives, e.g., cisplatin, produce dose-dependent nephrotoxicity (Pabla and Dong, 2008), which often restricts cancer therapies (Yao et al., 2007). Renal proximal tubular epithelial cells are particular targets of cisplatin-induced acute kidney injury (AKI) (Jordan and Carmo-Fonseca, 2000; Pabla and Dong, 2008). Inflammation, oxidative stress, and apoptosis are all manifestations of cisplatin toxicity in renal tubular epithelial cells (Faubel et al., 2007; Jordan and Carmo-Fonseca, 2000). However, molecular mechanisms underlying cisplatin-induced AKI are not well understood. Recently, the possibility of overcoming cisplatin-induced AKI by cell-based therapies was suggested (Ozawa et al., 2008). In this area, use of donor bone marrow (BM)-derived mesenchymal stem cells (MSCs) gained considerable interest, in large part because these cells may be readily isolated and expanded in culture conditions (Bussolati et al., 2009; Herrera et al., 2007; Morigi et al., 2008).

MSCs are non-hematopoietic cells that represent 0.01–0.001% of total BM cells (30) with the ability to differentiate into adipocytes, osteoblasts or chondrocytes (Bussolati et al., 2009; Herrera et al., 2007; Tropel et al., 2004). Moreover, MSCs may be capable of generating additional cell types, e.g., endothelial cells, renal tubular cells, hepatocytes, etc (Roobrouck et al., 2011; Singaravelu and Padanilam, 2009). Recently, therapeutic potential of MSCs was investigated in animal models of kidney disease, including cisplatin-induced AKI (Eliopoulos et al., 2010; Faubel et al., 2007; Herrera et al., 2007; Morigi et al., 2008). The possibilities were that transplanted MSCs could either directly replace damaged cells or could indirectly induce cell regeneration through paracrine signals (Kunter et al., 2007; Morigi et al., 2008; Zarjou et al., 2011). The ability of transplanted MSCs to home into sites of injury and to secrete beneficial factors with antiapoptotic, anti-inflammatory, mitogenic, or angiogenic properties was in agreement with both of these possibilities (Bussolati et al., 2009; Morigi et al., 2008). However, the relative contributions of these processes and the identity of putative factors released by MSCs that may protect from cisplatin-induced AKI remain to be defined and explained.

In studies of AKI, MSCs have typically been administered intravenously (IV) but due to their much larger size versus that of pulmonary capillaries, transplanted MSCs should be entrapped in pulmonary capillary bed (Allers et al., 2004; Gao et al., 2001; Gholamrezanezhad et al., 2011), which would prevent their distributions to kidneys. We considered that this complication could be avoided by administering MSCs in sites, such as peritoneal cavity or subcapsular space of kidneys, where paracrine benefits of transplanted MSC could be demonstrated (Eliopoulos et al., 2010; Li et al., 2011; Zarjou et al., 2011).

In the present study, we evaluated the benefits of transplanted MSCs on cisplatin-induced AKI in mice. We determined whether MSCs would confer renal tubular protection either by directly entering tissues or by paracrine signals emanating from extrarenal sites. We examined biodistributions of transplanted MSCs in kidneys and other organs by labeling MSCs with Indium-111 (¹¹¹In) oxine. This permitted localization of transplanted MSCs in various organs. Furthermore, paracrine effects of MSCs were evaluated by subcapsular and intraperitoneal transplantation of cells. Our results indicated that MSCs delivered by three distinct routes, i.e., IV, intraperitoneal (IP) and renal subcapsular space (SCS) provided renal protection from cisplatin-induced AKI. The beneficial responses were not due to entry of

transplanted MSCs into kidneys but were instead because of paracrine signaling from extrarenally located transplanted MSCs.

MATERIAL AND METHODS

Animals

The Animal Care and Use Committees of the Albert Einstein College of Medicine and the Feinstein Institute of Medical Research approved study protocols. C57BL/6J and C57BL/6-TgCAG-EGFP/10sb/J mice (GFP mice) with ubiquitous expression of green fluorescent protein (GFP) were originally purchased from Jackson Labs (Bar Harbor, ME). Mice aged 6–8 weeks were used and were housed under standard lighting conditions with unlimited access to pelleted food and water.

Isolation and characterization of mouse MSCs

Primary MSCs were isolated and expanded from long bones of C57BL/6J and GFP mice according to the method described previously (Short et al., 2009; Zhu et al., 2012) with minor modifications. Briefly, compact bones were excised into fragments and suspended in Mesencult basal medium (StemCell Technologies Inc; Vancouver, CA) containing 10% FBS and 1 mg/ml of collagenase II (Sigma-Aldrich, USA), followed by digestion for 1–2 hours at 37°C. The released cells were discharged and bone fragments were incubated in Mesencult basal medium with stimulatory supplements (StemCell Technologies Inc, CA) in a humidified 37°C incubator with 4% O₂ and 5% CO₂ for 3 days. The nonadherent cells and tissue debris were removed at 3 days by changing medium and replacing with fresh medium. The cells were used for studies after three to five passages in culture. To analyze cellular phenotype, MSCs were stained with PE-conjugated monoclonal antibodies specific for CD11b, CD14, CD34, CD86, CD90, and CD105 (BD Pharmingen) and analyzed by flow cytometry (Becton Dickinson).

Cisplatin-induced AKI and cell therapy with MSCs

Mice were given either 12 mg/kg cisplatin (Sigma Chemical Co., St. Louis, MO) subcutaneously or vehicle (saline) alone. Cisplatin was freshly dissolved in sterile normal saline to 1.2 mg/ml. MSCs were transplanted 24 hours before cisplatin by these different procedures, i.e., IV (5×10^5) via tail vein, SCS (1×10^6) via laparotomy and IP (4×10^6) with microcarriers (Cytodex 3, Sigma). To demonstrate changes in renal morphology and blood, kidneys and blood samples were collected on days 3, 5 and 8 after cisplatin. Cytodex 3 microcarriers with transplanted cells in peritoneal cavity were also collected when mice were sacrificed.

Biodistribution studies

MSCs were radiolabeled in normal saline with 150 megabecquerels (MBq) of ¹¹¹In-oxine (Mallinckrodt Inc., Maryland Heights, MO) for 20 minutes at room temperature. Radiolabeling efficiency was determined by measuring activity in cell pellets and pooled supernatants. Cell viability after radiolabeling was determined by trypan blue dye exclusion. C57BL/6J mice were given 5×10^5 radiolabeled MSCs via tail vein. Mice were grouped (n = 3–5 each) for studying cell biodistributions and engraftment 1 hour, 1d, 3d and 7d after transplantation of MSCs. One hour after cell transplantation, total body images were acquired for 10 minutes in 64 × 64 × 16 matrix at zoom factor of 2 with gamma camera (Argus; ADAC Laboratories, Milpitas, CA). Subsequently, mice were sacrificed and lungs, liver, spleen, kidneys, heart, bones and intestines were excised for organ activity. Total radioactivity was used as denominator for determining fractional distribution of activity in each organ. To verify engraftment of cells, other animals were sacrificed 1, 3 and 7 days

after MSCs transplantation. For cryosections, tissue samples were frozen in methylbutane cooled to -80°C .

Identification of transplanted MSCs

Genomic DNA was extracted with DNeasy Tissue Kit (Qiagen, Milpitas, CA). Transplanted MSC-GFP cells were verified by PCR for GFP (forward primer: 5'-CGTAAACGGCCACAAGTTCA-3', reverse primer: 5'-CGCTTTACTTGTACAGCTCGT-3'). The integrity of complementary DNA was verified by amplifying β -actin (forward primer: 5'-ATGGGTCAGAAGGACTCCTACG-3', reverse primer: 5'-AGTGGTACGACCAGAGGCATAC-3'). PCR was for 40 cycles with denaturation at $95^{\circ}\text{C} \times 30$ seconds, annealing at $55^{\circ}\text{C} \times 30$ seconds, and extension $72^{\circ}\text{C} \times 1$ minute. PCR products were resolved in 1.5% agarose gels containing ethidium bromide.

Transplanted MSC-GFP cells were identified by immunostaining for GFP. Cryosections of 5 μm thickness were fixed in 4% paraformaldehyde in PBS for 10 minutes at 4°C and blocked in 3% goat serum for 1 hour at room temperature. Primary rabbit anti-GFP antibodies (Molecular Probes, 1:300) were applied for 1 hour at room temperature followed by FITC-conjugated goat anti-rabbit IgG for 30 minutes. Sections were counterstained with DAPI-Antifade.

Histological evaluation of nephrotoxicity

Kidneys were removed and fixed in 10% buffered formalin for 24 hours and then transferred to 100% ethanol. Tissues were embedded in paraffin to cut 5 μm thick sections and stained with hematoxylin and eosin and Periodic Acid Schiff (PAS). The extent of injury in cortex and outer medulla was graded by identifying fractions of tubules with loss of brush borders, necrosis, cast formation, or dilatation, as follows: 0 = none, 1 = $<10\%$, 2 = $10\text{--}25\%$, 3 = $26\text{--}45\%$, 4 = $46\text{--}75\%$, and 5 = $>75\%$. At least 20 microscopic fields were reviewed per tissue section under high power with blinded observers.

BUN assay

Serum BUN was measured as previously described (Cheng et al., 2009). Briefly, 0.15 mL Urease Buffer Reagent (U-3383, Sigma) was added to 50 μL serum for 20 minutes at room temperature followed by 0.3 mL Phenol Nitroprusside Solution (P-6994, Sigma), 0.3 mL alkaline hypochlorite solution (A-1727, Sigma), and 1.5 mL deionized water, in that order. The mixture was incubated for 30 minutes at room temperature and absorbance at 540 nm was measured. Standards stock solution was prepared at the concentration of 10 mg/mL urea (U-5128, Sigma).

Conditioned medium and cytoprotection assay with renal tubular cells

For harvesting conditioned medium (CM), MSCs were cultured to confluence and then shifted to serum-free medium (Mesencult basal medium, StemCell Technologies Inc) for 24 hours. Mouse renal proximal tubular cell line (MTC) were incubated with 8, 10, 15, 25 and 50 μM of cisplatin with or without MSC conditioned medium for 24 hours at 37°C in 5% CO_2 . Cells were cultured at density of 5×10^5 cells/ cm^2 in 24-well culture dishes. Growth inhibition of cells was evaluated by thiazolyl blue (MTT) utilization assays. All experiments were carried out in triplicate and were repeated twice for reproducibility ($n=3$).

Cytokine content of CM

The CM harvested from MSCs was analyzed by mouse cytokine antibody array to probe for 97 cytokines (RayBiotech, Norcross, GA). Briefly, samples from CM and basal medium were incubated with membranes for 1 hour at room temperature. After washing, membranes

were incubated with biotin-conjugated anti-cytokine primary antibodies followed by washes and development with HRP-conjugated streptavidin. Chemiluminescence was used for signal detection. Pixel densities of cytokine spots were analyzed using Image J software (NIH, Bethesda). The density of cytokine spots with conditioned medium versus basal medium was compared. Signal intensity was averaged and graded from 0 (absent) to 4+ (maximal intensity).

Statistical Analysis

Data were expressed as means $\pm$ standard deviation. Significance was determined by Student's *t* test or analysis of variance with SigmaStat 3.1 (Systat Software Inc., Point Richmond, CA); *P* < 0.05 was considered significant.

RESULTS

Properties of MSCs

After incubation of compact bones over 3 days, spindle-shaped cells migrated out of bone fragments. These MSCs were homogenously positive for the mesenchymal markers CD90, CD105, but negative for hematopoietic markers CD11b, CD14, CD34, and costimulating molecules CD86 (not shown).

Organ biodistributions of transplanted MSCs

MSCs efficiently incorporated ^{111}In -oxine with labeling efficiencies of 85% to 95%. The viability of MSCs did not change after radiolabeling as shown by trypan blue dye. At one hour after IV injection of ^{111}In -labeled MSCs in controls, radioactivity was largely confined to the lungs, indicating that the transplanted cells were trapped in pulmonary capillaries (Fig. 1A). Analysis of excised organs confirmed that most of the activity was concentrated in the lungs (67.2%), followed by liver (12.5%), spleen (11.4%) and kidneys (5.4%) (Fig. 1B). In mice with cisplatin-induced AKI, the organ distribution of ^{111}In -labeled MSCs injected IV was similar to that in control mice (Fig. 1C and 1D).

GFP sequences in lungs, liver, spleen and kidneys were detected by PCR after intravenous administration of MSC-GFP (1 hour and 1 day; Fig. 2A). These findings correlated with the organ biodistribution results determined with radiolabeled MSCs. However, GFP sequences were not detected (in these organs) after 7 days of administration of MSC-GFP. The presence of transplanted GFP+ MSCs in various organs was also verified by GFP immunostaining. Transplanted MSCs were identified in lungs, liver and kidneys 1 day after IV transplantation (Fig. 2B). However, after 7 days, MSCs were undetectable in lungs or kidneys (data not shown). These findings indicated that MSCs transplanted through IV route were rapidly cleared without any engraftment in tissues.

Effects of MSCs transplanted IV on cisplatin-induced AKI

In control mice, MSCs transplantation did not alter renal function as indicated by unchanged BUN values (Fig. 3A) as well as by normal renal histology profile (Fig. 3B). In mice treated with cisplatin alone, BUN reached highest levels after 3 days (Fig. 3C). By contrast, BUN levels were significantly lower in mice treated with IV MSCs followed by administration of cisplatin compared with mice receiving vehicle and cisplatin, i.e., $29.6 \pm \text{mg/dl}$ vs $17.6 \pm 1.7 \text{ mg/dl}$, *p* < 0.05 (Fig. 3C). By day 8 after cisplatin, BUN levels in mice receiving MSCs and cisplatin had returned to baseline, whereas mice receiving vehicle and cisplatin continued to show high BUN levels ($22.5 \pm 1.2 \text{ mg/dl}$ vs $16.7 \pm 0.9 \text{ mg/dl}$, *p* < 0.05; Fig. 3C). Renal histology was characterized by less tubular injury and cast formation at days 3 and 5 post-cisplatin in mice receiving MSCs (Fig. 3D). Mice receiving MSCs + cisplatin displayed lower (*p* < 0.05) renal histology scores at all time

points (3, 5, and 8 days) when compared to mice receiving vehicle + cisplatin (vehicle + cisplatin: at 3 day, 2.7 ± 0.6 ; at 5day, 2.0 ± 0.4 ; at 8 day, 1.9 ± 0.4 vs. MSCs + cisplatin: at 3day, 1.3 ± 0.5 ; at 5 day, 0.9 ± 0.3 ; and 8 day, 0.2 ± 0.1).

Effects of MSCs transplanted in renal subcapsular space (SCS) or peritoneal cavity on cisplatin-induced AKI

In recipients of MSCs transplanted SCS, after 3 days of administration of cisplatin, BUN levels were lower when compared with vehicle + cisplatin-treated mice (20.2 ± 1.8 mg/dl vs. 32.5 ± 1.2 mg/dl, $p < 0.05$; Fig. 4A). Mice treated with MSCs displayed lower ($p < 0.05$) kidney damage (Fig. 4B; renal score, MSCs + cisplatin, 1.6 ± 0.3 vs. cisplatin + vehicle, 2.9 ± 0.4). To evaluate survival of transplanted MSCs, we studied additional animals after transplanting GFP+ MSCs in SCS. We found GFP+ MSCs survived under renal capsule but without migrating to parenchyma of kidneys (Fig. 4C).

We further examined whether MSCs contributed through paracrine signaling after IP administration of GFP+ MSCs with Cytodex 3 microcarriers in mice which were followed by cisplatin administration. In mice receiving MSCs + cisplatin, BUN levels were also lower compared with vehicle-treated mice (MSCs+Cis, 19.2 ± 1.8 mg/dl vs. Cis, 31.5 ± 1.8 mg/dl; $p < 0.05$; Fig. 5A). At day 3 (post cisplatin), mice with IP-MSCs displayed attenuated kidney damage in the form of diminished tubular cell injury and cast formation (Fig. 5B; renal score, IP-MSCs + Cis, 1.3 ± 0.2 vs. Cis, 3.1 ± 0.8 , $p < 0.05$). We verified that GFP+ transplanted cells were present in peritoneal cavity of mice (Fig. 5C). However, transplanted GFP+ cells were not found in parenchyma of kidneys. Taken together, these results indicated that subcapsular or IP transplantation of MSCs provided protection against cisplatin-induced AKI through paracrine mechanisms.

Analysis of CM from MSCs

To demonstrate whether MSCs secreted protective factors, we analyzed mouse tubular cells (MTC) exposed to cisplatin for 24 hours with or without CM. The viability of MTCs cultured with CM was greater ($p < 0.05$) after cisplatin treatment when compared to cells cultured with basal medium (Fig. 6). These results indicated that MSCs exerted protective effect on renal tubular cells through paracrine mechanisms.

Analysis of CM from MSCs identified over 40 cytokines (Table 1). Multiple cytokines were expressed at high levels, e.g., IL-6, KC, CXCL16, decorin, Galectin-1, Gas 6, MCP1, MIP1 α , MIP2, osteopontin, osteoprotegerin, RANTES, sTNFR1, VCAM1, etc.; Furthermore, MSCs moderate or low levels expressed GCSF, VEGF, HGF, IL-10, EGF, IGF, etc., which have been shown rescued mice from cisplatin-induced renal failure (Bengatta et al., 2009; Deng et al., 2010; Deuse et al., 2009; Imberti et al., 2007; Iwasaki et al., 2005; Tögel et al., 2005; Yuan et al., 2011) in previous reports.

DISCUSSION

The present study provides insights into the potential of MSCs for cell-based therapy in general, and prevention of cytotoxicity from chemotherapeutic drugs in particular. We demonstrated that transplantation of MSCs conferred protection against cisplatin-induced AKI. This beneficial effect was independent of the route of cell transplantation. Intravenously transplanted MSCs were entrapped in pulmonary capillaries initially; however, they were rapidly eliminated. Although subcapsular or peritoneally transplanted MSCs survived over longer periods but they did not enter renal parenchyma. Thus, it appeared that transplanted MSCs provided protection against cisplatin-induced AKI via paracrine signaling rather than by replacement of the damaged cells. This notion was further

supported by the ability of MSCs to stimulate the release of multiple cytokines that would have contributed to its renoprotective role.

Understanding of bio-distribution of transplanted MSCs is required for the treatment of systemic diseases (Allers et al., 2004; Gao et al., 2001; Gholamrezanezhad et al., 2011). Since ^{111}In -oxine radiolabeling human liver stem/progenitors (Cheng et al., 2009) as well as rat MSCs (Gao et al., 2001; Gholamrezanezhad et al., 2011) did not alter cell viability, we also chose mouse MSCs radiolabeling with ^{111}In -oxine. As expected it was efficient and did not alter cell viability after labeling. Moreover, it demonstrated biodistribution successfully, which was further confirmed by imaging, organ activity, DNA PCR, and tissue immunostaining methods. The radiolabeled MSCs were predominantly distributed in lungs and liver (1 hour after intravenous infusion); it may be related to the relative large size of MSCs. Since the vast majority of transplanted MSCs were cleared from all organs within 7 days, it indicated that MSCs failed to engraft in tissues. In previous studies, transplanted MSCs were recruited to the specific sites (Morigi et al., 2004) including kidneys (Morigi et al., 2004; Tögel et al., 2005) which led to the interactions between MSCs receptors (CXCR4, CD44, PDGF-R) and relevant renal cell ligands (SDF-1, hyaluronic acid, PDGF) (Burton et al., 1999; Herrera et al., 2007; Hung et al., 2007). However, these types of specific interaction are not possible if cells cannot be delivered to the site of injury because of their vascular entrapment in pulmonary capillaries.

To overcome the disadvantage of systemic IV administration of MSCs, alternative approaches for delivering MSCs in AKI have been investigated, including local delivery of cells through renal artery (Behr et al., 2007), and directly into renal parenchyma (Kim et al., 2008). However, direct injection of cells into kidneys led to bleeding and tissue destruction (Thirabanasak et al., 2010). Although intra-renal arterial injection of MSC led to engraftment of MSC in experimental models of chronic kidney disease (Kunter et al., 2007; Quimby et al., 2011), the invasive nature of the procedure limits clinical application of such an approach.

We evaluated subcapsular and IP injection of MSCs in cisplatin-induced AKI. Advantages of extra-renal cell delivery such as IP transplantation not only avoided vascular trapping as in lungs but also provided beneficial systemic effects on organs besides kidneys (Bi et al., 2007; Eliopoulos et al., 2010; Zarjou et al., 2011); additionally, one could administer large numbers of cells compared to other delivery routes (Eliopoulos et al., 2010). In the past, IP injection of MSCs or MSC-derived conditioned medium mitigated cisplatin-induced renotoxicity (Bi et al., 2007; Eliopoulos et al., 2010; Li et al., 2011; Zarjou et al., 2011). However, in those studies, MSCs were injected without extracellular matrix support. In our experience, use of microcarrier scaffolds markedly improved survival of transplanted cells in peritoneal cavity (Cheng et al., 2009). On that account, we transplanted MSCs intraperitoneally with microcarriers for cisplatin-induced AKI. Despite inability of transplanted MSCs to enter kidneys after IV, SCS or IP transplantation, transplanted MSCs exerted beneficial effects on cisplatin-induced AKI; these findings suggested that this effect of transplanted MSCs happened their paracrine signaling. These findings are consistent with other studies performed in the past (Eliopoulos et al., 2010; Kunter et al., 2007). Similar observations have been reported in other cells types e.g. intraperitoneally transplanted hepatocytes did not migrate into the liver, but supported organ regeneration by paracrine mechanisms (Bandi et al., 2011).

Our evaluation of conditioned medium from MSCs by a cytokine array was consistent with the ability of these cells to secrete multiple cytokines and chemokines (Chen et al., 2008; Eliopoulos et al., 2010; Yoon et al., 2010). We identified over 40 cytokines or growth factors, which were highly expressed in mouse MSCs. Although we did not explore the role

of individual factors, further studies will be appropriate for obtaining mechanistic insights into the therapeutic benefits of MSCs in AKI. This line of investigation will be critical since conditioned medium from MSCs administered IP has been reported to protect against cisplatin-induced AKI, making it reasonable to conclude that the protective effects of transplanted MSCs emanated from secretion of cytokines and growth factors (Bi et al., 2007). However, the specific factors that responsible for the protection against cisplatin-induced AKI are not clearly delineated. Nonetheless, studies where expression of insulin-like growth factor-I (IGF-I) or vascular endothelial growth factor (VEGF) was knocked down in MSCs by small interfering RNAs (siRNAs) indicated that individual paracrine factors expressed in MSCs can contribute in renal protection (Imberti et al., 2007; Tögel et al., 2005). In another study, increasing VEGF secretion enhanced protective effect of MSC in cisplatin-induced AKI (Yuan et al., 2011). Other substances, e.g., hepatocyte growth factor (HGF) and epidermal growth factor (EGF), contribute to tubular cell repair and regeneration (Deuse et al., 2009). Therefore, identification of biological substances released by MSCs that could be renoprotective will provide new directions for therapeutic applications that could possibly be accomplished pharmacologically without even requiring transplantation of cells.

Acknowledgments

Supported in part by NIH grants RO1DK084910 and RO1 DK083931 (PCS) and R01 DK071111, R01 DK088561 and P30-DK41296 (SG).

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Highlights

- Organ bio-distributions of transplanted mesenchymal stem cells (MSC).
- Transplanted MSC enhanced survival of mice with acute kidney injury.
- Transplantation of MSC via intraperitoneal and intracapsular displayed better outcome.
- This survival effect of MSCs was through their paracrine effects.
- More than 40 regulatory cytokines were identified in MSC conditioned medium.

Figure 1. Biodistributions of ^{111}In -labeled MSCs in control and cisplatin-treated mice
 ^{111}In -labeled MSCs were distributed in the indicated organs 1 hour after IV injection as shown by gamma imaging (A, C) and measurement of radioactivity in organs (B, D). Transplanted MSCs were restricted largely to lungs in control mice (A, B) as well as mice with AKI (C, D).

Fig. 2. Identification of IV transplanted MSCs 1 hour after cell transplantation

(A) Shows presence of GFP sequences in various organs by DNA PCR. (B) GFP immunostaining readily demonstrated transplanted GFP+ MSCs in lungs (green color), whereas GFP+ MSCs were rarely found in other organs, such as kidneys or liver.

Fig. 3. Effect of IV transplantation of MSCs on cisplatin-induced AKI

Blood and kidney samples were collected on days 3, 5, and 8 after administration of MSCs. (A and B) Mice receiving MSCs alone showed normal renal function as displayed by BUN levels (A) and renal histology (B). (C and D) In mice treated with cisplatin, BUN values were higher along with significant kidney damage, which was significantly attenuated in mice treated with MSCs (C and D, PAS staining, $\times 200$, * $p < 0.05$).

Fig. 4. Effect of SCS transplanted MSCs on cisplatin-induced AKI
 MSCs (1×10^6) were transplanted SCS 24 hours before cisplatin. Blood and kidney samples were collected 3 days after cisplatin. (A) BUN values decreased in mice receiving MSCs. (B) Renal histology displayed less tubular injury and cast formation in MSCs-treated mice, PAS stain, $\times 200$. (C) GFP immunostaining identified transplanted GFP + MSCs under renal capsule, $\times 200$, * $p < 0.05$.

Fig. 5. Effect of IP transplanted MSCs on cisplatin-induced AKI

MSCs (4×10^6) were transplanted IP 24 hours before cisplatin administration. Blood, kidney and transplanted cell-Cytodex 3 microcarrier conglomerates were collected 3 days after cisplatin administration. (A) BUN values were lower in mice receiving MSCs. (B) Renal histology showed less tubular injury and cast formation in mice receiving MSCs. PAS stain, $\times 200$. (C) GFP immunostaining demonstrated GFP+ MSCs in peritoneal cavity, $\times 200$, * $p < 0.05$.

Fig. 6. Effect of MSCs conditioned media (CM) on cisplatinum-induced toxicity in mouse tubular cells (MTC)

Cells were incubated with 8, 10, 15, 25 and 50 μM cisplatinum with or without MSCs CM for 24 hours. MSCs CM protected MTC from cisplatinum toxicity.

Table 1
Identification of cytokines in CM from cultured MSCs

The expression of cytokines identified in conditioned medium is listed.

High expression (3~4 +)	Moderate expression (2 +)	Low expression (1 +)
IL-6	6Ckine	ALK1
KC	Eotaxin-2	Amphiregulin
CXCL16	GCSF	CD30
Decorin	Flt-3 Ligand	Dkk-1
Galectin-1	Fractalkine	EGF
Gas6	HGF	Eotaxin
MCP-1	IL-1 α	Granzyme B
MIP-2	IL-1 γ	IGF-II
MIP-1 α	IL-4	IGFBP-5
MMP-2	IL-5	IGFBP6
Osteoponin	IL-9	IL-2 R α
Osteoprotegerin	IL-11	IL-12 p40
RANTES	IL-12p70	IL-17
sTNF RI	IL-13	IL-17B R
VCAM-1	IL-15	IL-17E
	IL-17F	IL-20
	IL-21	I-TAC
	Leptin	Leptin R
	Lungkine	MadVAM-1
	MIP-3 α	MDC
	MIG	MIP-1 γ
	Prolactin	MIP-3 β
	Pro-MMP-9	GITR
	SCF	GITR Ligand
	sTNF RII	TACI
	TWEAK R	TARC
	JAM-A	TRANCE
	VEGF	TPO
	VEGF R1	TROY
		VEGF R3