Human adipose tissue-derived mesenchymal stem cells protect kidneys from cisplatin nephrotoxicity in rats

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First published December 28, 2011; doi:10.1152/ajprenal.00060.2011.—Cisplatin has multiple cellular targets and modes of action that lead to nephrotoxicity. This suggests novel therapies that act at multiple cisplatin target sites may be effective. We tested whether human adipose tissue-derived mesenchymal stem cells (Ad-MSCs) can affect multiple target sites and protect against cisplatin-induced kidney damage. Rats were divided into four groups: control, infused with Ad-MSCs, injected with cisplatin, and cisplatin followed by infusion of Ad-MSCs. Animal survival and renal function were decreased and histological damage was increased in cisplatin-treated rats at day 3. Infusion of Ad-MSCs ameliorated renal dysfunction and tissue injury caused by cisplatin, leading to increased survival. Apoptotic cell death in the kidney was significantly reduced by infusion of Ad-MSCs. Activation of p53, JNK, and ERK and the expression of inflammation-related molecules were also decreased in the kidney that received Ad-MSCs. Very few Ad-MSCs were detected in the kidney. Conditioned medium from cultured Ad-MSCs had renoprotective functions in vivo and in vitro. Renal dysfunction and tissue damage caused by cisplatin were significantly reduced in rats treated with Ad-MSCs-conditioned medium. The viability of cultured renal proximal tubular cells exposed to cisplatin was also improved by coculture with Ad-MSCs or with conditioned medium. Release of proinflammatory mediators induced by cisplatin was inhibited in coculture with Ad-MSCs. Our results show that human Ad-MSCs exert a paracrine-protective effect on cisplatin nephrotoxicity at multiple target sites and suggest that human Ad-MSCs might be a new therapeutic approach for patients with acute kidney injury.

CISPLATIN IS A WIDELY USED chemotherapeutic agent for the treatment of several human malignancies. However, the clinical use of cisplatin as a chemotherapeutic agent is severely limited by serious adverse effects, particularly nephrotoxicity. Because cisplatin has multiple cellular targets, blocking its effect on a single target may only provide partial protection against nephrotoxicity (30). This suggests that novel or multifaceted strategies may be required to improve clinical outcomes in patients treated with cisplatin. Cell-based therapeutic approaches have several potential advantages over specific drugs or growth factors in the treatment of complex disorders such as acute kidney injury (AKI). This is due to the broad functional repertoire of cells, including the secretion of various bioactive mediators, integration into host tissues, and differentiation within the injured organ. An increasing body of evidence suggests that mesenchymal stem cells (MSCs) possess potential in the treatment of AKI (1, 12, 23, 25, 40).

MSCs-based therapies are currently being investigated for the treatment of AKI, although the mechanisms involved remain controversial. Numerous recent studies have demonstrated that in vitro expanded MSCs have therapeutic effects in experimental models of AKI (12–14, 24–26, 40). For example, the infusion of MSCs can protect and accelerate the recovery from AKI induced by cis-platinum (25, 26), glycerol (12, 13), and ischemia-reperfusion injury (24, 40). Some results have described the ability of MSCs to localize within the damaged kidney and promote morphological and functional recovery (12, 25). However, the homing of MSCs to sites of tissue injury, integration, and differentiation into tubular cells was rare or absent in models of AKI (6, 24, 39, 40). It is generally considered that, when administered after organ injury, MSCs exert their beneficial effects via complex paracrine and endocrine actions, such as immunomodulation, secretions of growth factors and cytokines, and mitogenic, antiapoptotic, and anti-inflammatory responses (6, 14, 31, 40–42).

MSCs were first isolated from bone marrow (8), and most AKI studies have used MSCs of this origin. Adipose tissue is also a valid reservoir of mesenchymal progenitors (10, 47, 48). MSCs from adipose tissue are easy to obtain, raise no ethical concerns about the stem cell source, and can be rapidly expanded to produce the requisite number of cells (19, 47). Adipose tissue-derived MSCs (Ad-MSCs) have many of the characteristics of their bone marrow counterparts, including similar morphology, extensive proliferation potential, and the ability to undergo multilineage differentiation (19, 38, 44). However, the yield of MSCs from adipose tissue was reported as 40-fold higher than from bone marrow (19). Stromal cells derived from adipose tissue protected mice from cisplatin-induced AKI (1), suggesting that Ad-MSCs may also be suitable for AKI therapy.

In the present study, we sought to assess whether Ad-MSCs of human origin may be a therapeutic option in the treatment of AKI. We used Sprague-Dawley rats with cisplatin-induced AKI. We evaluated the effects of Ad-MSCs on cisplatin-induced AKI in Sprague-Dawley rats, focusing on two aspects of disease. First, we focused on the renoprotective effects of Ad-MSCs in cisplatin-induced renal injury and nephrotox-
icity and whether Ad-MSC treatment could 1) reduce mortality, 2) protect against functional and structural renal injury, 3) decrease renal tubular apoptosis, 4) inhibit activation of signaling molecules, and 5) suppress inflammation. Second, we aimed to identify mechanisms of action of Ad-MSCs in AKI by testing whether 1) infused Ad-MSCs can migrate and/or integrate into the injured renal tissue and differentiate into injured cells, 2) Ad-MSCs exert paracrine effects in vivo and in vitro, and 3) factors in host cells are affected by the action of Ad-MSCs.

MATERIALS AND METHODS

Ad-MSC culture. Ad-MSCs were isolated from human fat tissue. Adipose tissue was obtained from the abdominal fat prepared in patients who had undergone general abdominal surgery at Gyeongsang National University Hospital. All eligible patients or their parents provided their written informed consent, and permission to isolate the MSCs from fat tissues was given by the Gyeongsang National University Hospital Institute Review Board (MR7404–130). Culture methods were based on the method of Bi et al. (1) and modified. The tissue was washed with PBS followed by extracellular matrix digestion in 0.075% collagenase (Sigma, St. Louis, MO) for 1 h at 37°C. The sample was neutralized with DMEM containing 10% FBS and centrifuged at 2,300 rpm for 15 min. The pellet was resuspended in PBS, passed through a 70-μm cell strainer, and centrifuged. The cells were cultured in MSC expansion medium (catalog no. SCM015, Millipore, Billerica, MA) with antibiotics, and media were removed, nonadherent cells were taken away 24 h later, and adherent cells were maintained up to passage 7 in fresh MSC expansion medium.

Phenotypic analysis. Flow cytometry analysis for MSC phenotype was done using FCM500 (Beckmann). Cells were harvested and washed in flow cytometry buffer and incubated for 20 min in flow cytometry buffer containing fluorescein-conjugated monoclonal antibodies directed against differentiation of MSC antigens (CD29, CD49d, CD105, Chemicon) and against hematopoietic antigens (CD34 and CD45, Miltenyl Biotech). Cells at passages 3–5 were used for in vivo experiments.

Animal treatment. Male Sprague-Dawley rats (200–230 g) were maintained in a 12:12-h light–dark cycle in a temperature- and humidity-controlled facility. Standard rat chow and water were provided ad libitum. Animal studies were conducted according to the Gyeongsang National University Guide for Care and Use of Laboratory Animals. Animals were divided into four groups. The control group received a single injection of saline, intraperitoneally (n = 2). The conditioned medium (CM) group was given a single injection of CM (4 ml/animal) intraperitoneally (n = 5). The cisplatin group was given a single intraperitoneal injection of cisplatin (10 mg/kg body wt) and an equal volume of regular culture medium instead of CM (n = 5). For preparation of CM from Ad-MSC culture, Ad-MSCs in a 75-cm² culture flask were cultivated in 15 ml of fresh complete media (MSC expansion medium, Millipore). The medium was harvested 72 h later (cells were grown to 90% confluence 72 h later), centrifuged to remove floating cells at 3,000 rpm for 10 min, collected (CM), and frozen at −70°C for in vivo and in vitro experiments. CM was intraperitoneally (4 ml/each animal) injected into rats at 1 and 2 days after cisplatin injection. All animals were euthanized at day 3 after cisplatin injection. Blood and tissue samples were collected for analysis of renal function and tissue damage. Animal treatments were performed a total of five times with five individual cultured MSCs. For a survival test, a total of 22 rats/experiment were used and the test was terminated at day 7, intentionally. Three independent experiments were performed.

Assessment of renal function. Serum samples were examined for blood urea nitrogen (BUN) and serum creatinine (Bayer) in an Autoanalyzer (ADIVA 1650, Bayer) using standard diagnostic kits.

Renal histology and damage scoring. Five-micrometer sections were stained with hematoxylin and eosin. Tubular injury was defined as tubular epithelial necrosis, cast formation, intratubular debris, and loss of the brush border. Tubular injury was scored by grading the percentage of affected tubules under a high-power field (×400): 0, 0%; 0.5, <10%; 1, 10–25%; 2, 26–50%; 3, 51–75%; and 4, 75–100%. To score injured tubules, whole tubular numbers per field were considered as standard under ×400 magnification. The grading percentage was calculated in each field as follows: injury score (%) = (numbers of injured tubules/number of whole tubules) × 100. At least 10 areas in the cortex per slide were randomly selected.

Terminal uridine deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay. Detection of DNA fragmentation was performed using a kit (Roche, Indianapolis, IN). A semiquantitative analysis was performed by counting the number of terminal uridine deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)-positive cells per field, in the renal tissue, at ×400 magnification. At least 10 areas in the cortex per slide were randomly selected.

Immunoblot analysis. Kidneys were removed and homogenized in lysis buffer. Sixty micrograms of protein were loaded. Blots were probed with primary antibodies to polyclonal anti-cleaved caspase-3 and -9, p-ERK, p-p38, p-p53, and p-JNK (Cell Signaling Technology, Beverly, MA) and polyclonal anti-Bax (Santa Cruz Biotechnology, Santa Cruz, CA). The primary antibody was visualized using secondary antibodies with an ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ).

Labeling and preparation of Ad-MSCs for transplantation. Ad-MSCs were maintained in MSC expansion medium with antibiotics and passed at 90% confluence. Three days before transplantation, Ad-MSCs were supplemented with 2 μM bromodeoxyuridine (BrDU; Sigma) to label cells. Cells were detached with trypsin/EDTA, washed with RPMI-1640 twice, and resuspended at 500 μl (5 × 10⁶ cells) in RPMI-1640. Cells at passage 5 were used for in vivo experiments. The number of BrDU-positive cells was counted. Ten randomly selected kidney sections were chosen, 20–30 tubules were screened per field, and quantitative data for MSC homing are represented as BrDU signal-containing cells (brown). The number of BrDU-positive cells was counted at magnification ×400.

In vivo therapeutic effect of conditioned medium from Ad-MSC culture on cisplatin nephrotoxicity. Animals were divided into four groups. The control group received a single injection of saline, intraperitoneally (n = 2). The conditioned medium (CM) group was given a single injection of CM (4 ml/animal) intraperitoneally (n = 5). The cisplatin group was given a single intraperitoneal injection of cisplatin (10 mg/kg body wt) and an equal volume of regular culture medium instead of CM (n = 5). The cisplatin and CM group received cisplatin and was given CM at 1 and 2 days after cisplatin (n = 10). For preparation of CM from Ad-MSC culture, Ad-MSCs in a 75-cm² culture flask were cultivated in 15 ml of fresh complete media (MSC expansion medium, Millipore). The medium was harvested 72 h later (cells were grown to 90% confluence 72 h later), centrifuged to remove floating cells at 3,000 rpm for 10 min, collected (CM), and frozen at −70°C for in vivo and in vitro experiments. CM was intraperitoneally (4 ml/each animal) injected into rats at 1 and 2 days after cisplatin. All animals were euthanized at day 3 after cisplatin injection. Blood and tissue samples were collected for analysis of renal function and tissue damage. This experiment was performed a total of three separate times with three individual cultured MSCs. For a survival test, a total of 22 rats/experiment were used and the test was terminated at day 7, intentionally. Three independent experiments were performed.

In vitro therapeutic effect of conditioned medium from Ad-MSC culture on cisplatin nephrotoxicity. Human kidney proximal tubular cells (HK cells, ATCC) were exposed to 8 μM cisplatin for 6 h, cisplatin was washed out twice with PBS, and cells were incubated in conditioned medium from Ad-MSCs, which were maintained at 90% confluence in MSC expansion medium. After 24 and 72 h, viability of HK cells was assessed by water-soluble tetrazolium salt-1 (WST-1) assay. Experiments were performed in the presence or absence of conditioned medium with HK cells subjected to cisplatin.
In vitro therapeutic effect of Ad-MSCs on cisplatin-treated HK cells. HK cells were exposed to 8 μM cisplatin in α-MEM medium with 5% FBS for 6 h in 24-well plates, and cisplatin was washed out twice with PBS. Ad-MSCs (5 × 10^4 cells/insert) were loaded in Transwell inserts, applied onto HK cells, and the coculture was maintained in α-MEM medium with 5% FBS for 24 and 72 h. Experiments were performed in the presence or absence of Ad-MSCs with HK cells subjected to cisplatin. After 24 and 72 h, HK cells were stained with trypan blue dye for cell viability, and cell supernatants were harvested for cytokine analysis (TNF-α and IL-1β, SinglePlex system, Bio-Rad).

Statistical analysis. Data are expressed as means ± SD. Animal survival is presented using Kaplan-Meier survival curves. Statistical analysis was conducted using Sigma Plot 7.0 (SPSS, Chicago, IL). Data were evaluated using one-way ANOVA and t-tests. P < 0.05 was considered statistically significant.

RESULTS

Phenotypic analysis of human Ad-MSCs. Ad-MSCs were grown in culture as previously described (1, 47, 48), and flow cytometric analysis was performed to confirm the identity of the cells as Ad-MSCs. Cultured Ad-MSCs expressed high levels of the MSC-specific markers CD29, CD49d, and CD105, as reflected by M1 portion (Fig. 1C) but did not express the hematopoietic markers CD34 and CD45 (Fig. 1B). Ad-MSCs at passages 3–5 were used for all in vivo experiments.

Therapeutic effect of Ad-MSCs against cisplatin treatment. The use of cisplatin causes severe side effects, including vomiting, neurotoxicity, ototoxicity, and nephrotoxicity. We investigated whether the infusion of Ad-MSCs affected survival, the key clinical outcome in AKI. Eighty percent of cisplatin-treated rats given saline died within 4 days, reaching 100% at 7 days (Fig. 2). Survival was prolonged in rats given an infusion of Ad-MSCs, with 20% survival at 7 days (Cis vs. Cis+MSCs, P < 0.001). Compared with data from other studies, our use of a higher dose may have lead to high rate of early mortality at day 4 after cisplatin injection. However, the overall survival rate was statistically higher in the Ad-MSCs group as reflected by the P value. This effect may highlight the late survival 4 days after injection. The survival proportion was still 20% at day 10 when the experiment was intentionally terminated. This result suggests that administering Ad-MSCs protected against cisplatin-induced nephrotoxicity and promoted late overall survival but did not protect against early death.

Ad-MSCs protect against renal functional impairment induced by cisplatin. Ad-MSC infusion markedly protected cisplatin-treated rats from renal function impairment, with significantly lower BUN and serum creatinine levels at 3 days compared with rats given saline (132 vs. 64 mg/dl, P < 0.005 for BUN; 2.2 vs. 1.2 mg/dl, P < 0.005 for creatinine) (Fig. 3). These results indicate that Ad-MSCs protect kidneys against cisplatin-induced nephrotoxicity and ameliorate renal dysfunction.

Reduced cisplatin-induced renal tubular injury following Ad-MSC infusion. The kidneys of cisplatin-treated rats given saline showed AKI-associated tubular lesions at 3 days, consisting of tubular necrosis, hyaline casts, loss of brush border, intratubular debris, and flattening of the tubular epithelium (Fig. 4A). The controls and the Ad-MSCs only group did not show any renal structural changes. Ad-MSC treatment mark-
Ad-MSC treatment decreases activity of key signaling pathways in cisplatin-induced AKI. Many signaling pathways are involved in cisplatin nephrotoxicity. Blockade of p53 can protect against cisplatin-induced nephrotoxicity including tubular cell death in various experimental models (7, 17, 18, 35, 45). As shown in Fig. 6, activation of p53, ERK, and JNK, but not p38, by cisplatin was clearly decreased in the Ad-MSCs group.

Ad-MSCs decrease inflammation in cisplatin-induced AKI. TNF-α is a key mediator in the inflammatory response triggered by cisplatin and signals through the receptors (32, 46). In models of cisplatin-induced injury, TNF-α is produced predominantly by resident kidney cells and not by infiltrating inflammatory cells (36). To investigate whether Ad-MSCs are involved in TNF-α-mediated renal injury, we measured TNF-α mRNA in the kidney. Figure 7A shows that the cisplatin-induced increase in TNF-α expression in the kidney was significantly decreased by Ad-MSC treatment. To determine the involvement of the key NF-κB inflammatory signaling pathway, IkB-α phosphorylation was analyzed by immunoblot analysis (Fig. 7B). Cisplatin increased the level of phosphorylated IkB-α, which was partly reversed by the infusion of Ad-MSCs. Similarly, the expression of cyclooxygenase (COX)-2, which is regulated by NF-κB signaling, was also decreased in the Ad-MSCs group. These results suggest that Ad-MSCs ameliorate inflammation in renal tissue resulting from cisplatin treatment.

Existence of Ad-MSCs in renal tissue. Migration toward injured sites is the first step in stem cell-mediated tissue regeneration. We explored the capacity of BrdU-labeled Ad-MSCs to home to the kidney at day 3 after cisplatin treatment. BrdU-stained Ad-MSCs were rarely localized within the tubular epithelium (arrow) (Fig. 8, A and B). The number of positive cells was counted as averaging 1.5 in 20 renal tubules (Fig. 8C). BrdU-positive cells, on the other hand, were also found in the lung, with no BrdU-positive cells detected in the liver at same time point (data not shown). The rare migration of Ad-MSCs into the kidney suggests that the primary therapeutic effect of these cells is paracrine in nature.

Paracrine effects of Ad-MSCs in vivo. We hypothesized that Ad-MSCs might release factors that prevent renal injury induced by cisplatin. To test this hypothesis, we studied the effects of CM from cultured Ad-MSCs on renal function in cisplatin-treated rats. CM was injected intraperitoneally at days 1 and 2 after cisplatin injection. Blood samples were harvested 3 days later to determine renal function, and the kidneys were isolated for morphological analysis. Injection of CM (Cis+CM) led to a reduction in BUN (P < 0.05) and serum Cr (P < 0.05) compared with rats treated with cisplatin alone (Cis) (Fig. 9A). Most importantly, renal tissue injury was also reduced in the CM treatment group (Cis+CM) (Fig. 9B). Seventy percent of cisplatin-treated rats given cisplatin died within 4 days, reaching 100% at 6 days. Survival was prolonged remarkably in rats given an injection of CM, with 30% survival at 7 days when the experiment was intentionally terminated (Fig. 9C). These results suggest that soluble factors released from Ad-MSCs might be protective against cisplatin-induced nephrotoxicity.

Protective effects of Ad-MSCs in vitro. To establish an in vitro system to test mechanisms of action of Ad-MSCs, human kidney proximal tubular cells (HK cells) were cultured in
cisplatin. Renal tubules (proximal tubular cells, in particular) are the major sites of cell injury and death in cisplatin nephrotoxicity. HK cells were exposed to cisplatin for 6 h and, after the cisplatin was washed out, the HK cells were cocultured with Ad-MSCs in a Transwell system for 24 and 72 h. The viability of HK cells cocultured with Ad-MSCs was significantly higher than cisplatin-treated HK cells without Ad-MSCs at both 24 and 72 h (Cis vs. Cis/H11001 MSC, 55 vs. 88%, \( P < 0.05 \) for 24 h, 37 vs. 70%, \( P < 0.005 \) for 72 h) (Fig. 10A). CM from Ad-MSCs also increased the viability of HK cells compared with the control medium at both 24 and 72 h (Cis vs. Cis/H11001 MSC, 43 vs. 63%, \( P < 0.05 \) for 24 h, 42 vs. 64%, \( P < 0.005 \) for 72 h) (Fig. 10B).

Inhibition of inflammatory cytokine release. Cisplatin-induced renal toxicity is mediated by inflammatory cytokines, including TNF-\( \alpha \) and IL-1\( \beta \), produced by renal parenchymal cells. HK cells cocultured with Ad-MSCs showed significantly lower production of TNF-\( \alpha \) and IL-1\( \beta \) compared to cisplatin-treated HK cells without Ad-MSCs at both 24 and 72 h (Cis vs. Cis/H11001 MSC, 63 vs. 35%, \( P < 0.005 \) for 24 h, 58 vs. 32%, \( P < 0.005 \) for 72 h) (Fig. 10C). CM from Ad-MSCs also significantly decreased the production of TNF-\( \alpha \) and IL-1\( \beta \) compared to the control medium at both 24 and 72 h (Cis vs. Cis/H11001 MSC, 59 vs. 37%, \( P < 0.005 \) for 24 h, 54 vs. 33%, \( P < 0.005 \) for 72 h) (Fig. 10D).

A: Protective effects of Ad-MSCs on renal morphology in cisplatin-induced nephrotoxicity. Hematoxylin and eosin staining was performed on sections of rat kidneys 3 days after cisplatin injection (\( \times 200 \) magnification). Group labeling was same as in Fig. 3. A: the kidney from the Ad-MSCs group (MSC) showed a similar histology to the control kidney (Con). Kidney sections from Ad-MSC rats showed mild casts and distinct tubular necrosis. Kidney sections from Cis + MSC rats showed mild casts and tubular necrosis (Cis + MSC). B: tubular injury was defined as tubular epithelial necrosis, cast formation, loss of brush border, and intratubular debris. Injury scoring was based on the percentage of affected tubules in a high-power field (\( \times 400 \)), as described in MATERIALS AND METHODS. Cisplatin-treated kidneys had severe tubular injury in all scoring categories, whereas Ad-MSCs-infused cisplatin kidneys showed significantly less injury than cisplatin only. \( \ddagger P < 0.001 \), \* \( P < 0.05 \), \** \( P < 0.005 \) compared with the Cis group.

B: Protective effect of Ad-MSCs on cisplatin-induced apoptosis. Cisplatin-induced apoptosis was detected by terminal uridine deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. A: TUNEL-positive cells were stained with dense fluorescence-containing spots (arrows, \( \times 400 \) magnification) and counted as described in MATERIALS AND METHODS. Significantly different from cisplatin group, \** \( P < 0.005 \). B: expression levels of apoptosis-related proteins. Immunoblot analysis was carried out with a specific antibody against Bax and cleaved caspase-3 and -9. \( \beta \)-Actin was used as a loading control. Blots shown are representative of 3 separate experiments. Lane for Con, \( n = 3 \); lane for MSC, \( n = 3 \); lane for Cis, \( n = 3 \); lane for Cis + MSC, \( n = 4 \).
cells (32, 46). To determine whether Ad-MSCs have anti-inflammatory properties, we analyzed the release of TNF-α and IL-1β in coculture with Ad-MSC- and cisplatin-treated HK-2 cells in a Transwell for 24 and 72 h. The release of TNF-α and IL-1β was significantly inhibited in HK cells cocultured with Ad-MSCs for both 24 and 72 h (Fig. 11).

These data suggest that Ad-MSCs ameliorate cisplatin toxicity for renal proximal cells by the reduction of TNF-α and IL-1β release.

DISCUSSION

The use of bone marrow and cord blood MSCs for treating AKI has been previously reported (25–27). There is also a single report describing the ability of stromal cells from adipose tissue to improve survival, lower BUN values, and decrease renal tubular damage in cisplatin-treated mice (1). However, our study is the first report to describe the ability of human Ad-MSCs of adipose origin for the treatment of cisplatin-induced AKI at multiple levels, including regulating cytokine production and intracellular signaling cascades in rats (not immunodeficient mice). Infusion of Ad-MSCs resulted in a broad therapeutic effect, including reductions in apoptotic cell death, renal signaling, inflammation, and the release of toxic cytokines, improved renal function and tissue morphology, and increased survival. This suggests that combination or multifaceted strategies may deliver superior clinical outcomes. For this reason, the broad mechanism of action of human Ad-MSCs may be particularly advantageous in the treatment of cisplatin-induced AKI.

Apoptosis is a major cause of tubular cell loss in cisplatin-induced AKI (2), and attenuation of tubular cell apoptosis leads to amelioration of renal dysfunction in AKI (3, 28, 43). Moreover, apoptosis was evident within 3 days of cisplatin
injection, which correlated temporally with the onset of renal dysfunction (11). These findings suggest that the inhibition of apoptosis may be a powerful therapeutic strategy for the prevention and treatment of cisplatin nephrotoxicity. Our results showed that Ad-MSCs protect from apoptosis in the kidney in AKI, as shown by a significant reduction in the number of TUNEL-positive cells and the reduced expression of cleaved caspase-9 and -3. An increase in cleaved caspase-9 is associated with the mitochondria-mediated apoptosis signaling pathway. The influence of Ad-MSCs on the mitochondrial apoptosis pathway was further demonstrated by showing that these cells decreased the expression of the proapoptotic molecule Bax.

p53 is also involved in cisplatin-induced apoptosis of renal cells (45). p53 is activated by cisplatin in renal tubular cells, and its activation correlates with cisplatin-induced apoptosis. Inhibition of p53 can block apoptosis induced by cisplatin (17, 45). p53 promotes apoptosis by upregulating the expression of proapoptotic genes, leading to the permeabilization of the mitochondrial outer membrane and release of apoptogenic factors (16, 37). Treatment with Ad-MSCs attenuated the activation of p53, and the results suggest that Ad-MSCs might be effective in preventing the apoptosis of renal cells induced by cisplatin. Also, we hypothesized that treatment with Ad-MSCs may affect multiple targets of cisplatin, including the ERK, JNK/SAPK, and p38 signaling pathways, which are activated in experimental models of cisplatin nephrotoxicity (15, 20, 29, 35). ERK and JNK activation was increased in cisplatin-treated kidneys, an effect that was attenuated by the infusion of Ad-MSCs, in parallel with a reduction in apoptosis. ERK activation is known to promote inflammation and apoptosis (18).

One of the mechanisms for cisplatin-induced nephrotoxicity is mainly mediated by cytokines produced by renal parenchymal cells (4, 5, 34, 36, 46). Especially, treatment with pharmacological inhibitors and antibodies against TNF-α, or genetic targeting of TNF, is associated with marked resistance to cisplatin-induced renal injury and tubular cell death (32, 33, 43). Our results showed that a cisplatin-induced increase in TNF-α expression in the kidney was significantly decreased by treatment with Ad-MSCs. In addition to the effects on TNF-α, we also observed reduced levels of the inflammatory molecules phosphorylated IκB-α and COX-2, suggesting a broad down-regulation of inflammation by Ad-MSCs. These findings show that Ad-MSCs affect multiple targets in cisplatin-induced nephrotoxicity.

The functional recovery that occurred following Ad-MSC infusion was too rapid to be explained by renal tubular regeneration from the infused cells, and we considered that it might be achieved through tubular protection and/or enhanced renal repair. Our results showed that Ad-MSCs protect from apoptosis in the kidney in AKI, as shown by a significant reduction in the number of TUNEL-positive cells and the reduced expression of cleaved caspase-9 and -3. An increase in cleaved caspase-9 is associated with the mitochondria-mediated apoptosis signaling pathway. The influence of Ad-MSCs on the mitochondrial apoptosis pathway was further demonstrated by showing that these cells decreased the expression of the proapoptotic molecule Bax.

Fig. 9. Effects of Ad-MSC conditioned medium on cisplatin-induced renal dysfunction and tissue damage. A: rats were divided into 4 groups. The control group (Con; n = 2) was injected with saline solution. The conditioned medium (CM) group was given a single injection of CM (4 ml/each animal ip; n = 5). The cisplatin group (Cis; n = 5) was treated with a single dose of cisplatin (10 mg/kg ip) and regular culture medium. The last group received CM (ip) at days 1 and 2 after cisplatin injection (Cis+CM; n = 10). Blood was collected for the measurement of BUN and serum creatinine (Cr). Values are means ± SD. Significantly different from the cisplatin group, *P < 0.05 and **P < 0.005. B: renal damage following cisplatin treatment was improved in rats that received CM, as described in A. C: animal survival is presented using Kaplan-Meier survival curves. Test was terminated intentionally at day 7.
function. Indeed, numerous studies have shown that organ protection by administered stem cells is primarily due to paracrine mechanisms rather than the replacement of damaged cells by differentiated stem cells (9, 21, 22, 40). We hypothesized that Ad-MSCs exert beneficial paracrine actions on the injured kidney by releasing biologically active factors. CM from cultured Ad-MSCs provided protection in our animal model of cisplatin-induced nephrotoxicity, suggesting that Ad-MSCs do release protective factors. This was further supported by in vitro studies showing that coculture with Ad-MSCs, or exposure to CM, protected cultured human renal proximal tubular cells from cisplatin toxicity. Therefore, we pointed to a paracrine mechanism of action rather than to direct repopulation of cells in damaged tissue. However, we cannot say firmly that the protective effects of CM are equivalent to Ad-MSC treatment in vivo and in vitro. Further in-depth studies will be required to clarify this point.

We used an in vitro experimental system to investigate potential mechanisms of action of Ad-MSCs. Renal tubular cells exposed to cisplatin release inflammatory cytokines such as TNF-α and IL-1β. In our study, the release of these cytokines was significantly inhibited in HK cells cocultured with Ad-MSCs, similar to the results obtained using cord-blood MSCs (27). Our results suggest that human Ad-MSCs secrete paracrine-acting factors that can protect from cisplatin-induced nephrotoxicity and human Ad-MSCs can exert anti-inflammatory action by suppressing the release of TNF-α and IL-1β from renal tubular cells. There is an acute criticism, whereby the effects of intravenous infusion of Ad-MSCs and intraperitoneal injection of CM as well as cisplatin itself are systemic. We did not investigate any systemic changes like inflammatory mediators or cytokines, and we tried to limit our study to changes in the kidney. Because nephrotoxicity is a serious consequence of aggressive therapy with cisplatin, this side effect has indeed limited the clinical use of cisplatin in 25–30% of patients, even after the first dose. Multiple factors and pathways are involved in acute kidney injury resulting from cisplatin treatment, suggesting that a
cell-based therapeutic strategy is particularly attractive for this condition. The consequences of long-term pathological differentiation of engrafted cells also need to be considered in assessing cell therapy, although cell-based therapeutic approaches have several potential advantages for treating AKI. The present study is the first to show that human Ad-MSCs have a positive effect via multiple mechanisms, leading to improved kidney function after cisplatin-induced AKI in rats (not immunodeficient mice). It is natural that autologous or allogeneic rather than xenogenic, transplantation is more practical and available. However, we consider that xenogenic transplantation using human adipose tissue-originated cells is a prerequisite for the ultimate goal, which is clinical trials conducted in a safe and ethical manner. Thus our results suggest that human Ad-MSCs are a possible future option for the cellular therapy for AKI.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES
9. Gneechi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noizeux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 11: 367–368, 2005.
21. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 94: 678–685, 2004.


