Effect of preemptive treatment with human umbilical cord blood-derived mesenchymal stem cells on the development of renal ischemia-reperfusion injury in mice

Hye Ryoun Jang,1 Ji Hyeon Park,1 Ghee Young Kwon,2 Jung Eun Lee,1 Woosong Huh,1 Hye Jin Jin,3 Soo Jin Choi,3 Wonil Oh,3 Ha Young Oh,1 and Yoon-Goo Kim1

1 Nephrology Division, Department of Medicine, Samsung Medical Center, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Korea; 2 Department of Pathology, Samsung Medical Center, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Korea; and 3 Biomedical Research Institute, MEDIPOST Company Limited, Seoul, Korea

Submitted 21 October 2013; accepted in final form 18 August 2014

MATERIALS AND METHODS

C57BL/6 mice were purchased from Orient Bio (Seongnam, Kyonggi-do, Korea). All mice were 8- to 10-wk-old male mice and were housed in a specific pathogen-free barrier facility. The Samsung Medical Center Animal Care and Use Committee approved all experiments.

Mice were anesthetized with an intraperitoneal injection of ketamine (15 mg/kg, Yuhan, Seoul, Korea) and xylazine (25 mg/kg, Bayer, Leverkusen, Germany). After an abdominal midline incision, both renal pedicles were bluntly dissected and clamped with a microvascular clamp (Roboz Surgical Instrument, Gaithersburg, MD) for 27 min. During these procedures, mice were kept well hydrated with warm sterile saline at a constant temperature (37°C). After the clamps were removed, the wounds were sutured, and mice were allowed to recover.
recover with free access to chow and water. Cohorts of mice were euthanized on days 1 and 2 after surgery.

In the treatment group, 1 × 10^6 HUCB-MSCs were injected intraperitoneally 24 h before surgery and during reperfusion. The dosage of cells was determined based on previous studies that used similar MSCs in a murine IRI model (6, 10, 31). The same volume of medium was injected intraperitoneally at the same time point in the control group. In comparison experiments for injection route or dose escalation, 1 × 10^6 HUCB-MSCs were injected intraperitoneally or intravenously via the tail vein, and 3 × 10^6 HUCB-MSCs were injected intraperitoneally 24 h before surgery and during reperfusion.

Preparation of HUCB-MSCs. HUCB-MSCs were isolated and cultivated from human umbilical cord blood, as previously described (22, 27).

MSCs are positive for human leukocyte antigen-ABC, CD73, CD90, and CD105 but negative for CD14, CD34, CD45, and human leukocyte antigen-DR. HUCB-MSCs differentiate into various cell types, such as osteoblasts, chondrocytes, and adipocytes, with the in vitro induction by specific osteogenic, chondrogenic, and adipogenic differentiation stimuli.

HUCB-MSCs were labeled with PKH-26 (Sigma, St. Louis, MO) to analyze the trafficking of these cells into the postischemic kidney.

Measurement of blood urea nitrogen and serum creatinine levels. Blood urea nitrogen (BUN) and creatinine levels were measured in serum samples obtained on days 1 and 2 after bilateral renal IRI using colorimetric kits (BUN: Fujifilm, Bedford, UK; creatinine: ARBOR Assays, Ann Arbor, MI) according to the manufacturers’ recommended methods. Baseline BUN and creatinine levels were measured at 7 days before IRI surgery.

Tissue histological analysis. Kidneys were harvested after exsanguination. Tissue sections were fixed with 10% buffered formalin followed by paraffin embedding and stained with hematoxylin and eosin. Renal tubular damage was scored by a renal pathologist who was blinded to the experimental groups.

Immunofluorescence analysis for the detection of PKH-26-labeled HUCB-MSCs. A piece of kidney harvested after exsanguination was immediately soaked in 25% sucrose solution and frozen in a mold. Frozen tissue sections were fixed with chilled 95% ethyl alcohol. After being dried at room temperature for 30 min, slides were washed five times with phosphate buffer solution. Mounting medium (20 μl) containing 4',6-diamidino-2-phenylindole (DAPI; Vectashield, Vector Laboratories, Peterborough, UK) was applied to the slides. The next day, slides were incubated for 1 h at room temperature, KMNCs were collected from the interface of 36% and 2.5% FBS, and 2.5 mg/kg of each was administered into the peritoneal cavity following four groups: the control group received IRI surgery and sterile PBS only, the VEGF inhibitor group received IRI surgery and VEGF inhibitor, the HUCB-MSC + isotype control group received IRI surgery and HUCB-MSC treatment with the isotype control, and the HUCB-MSC + VEGF inhibitor group received IRI surgery and HUCB-MSC treatment with VEGF inhibitor. All mice were euthanized 2 days after IRI.

Flow cytometry analysis of kidney-infiltrating mononuclear cells. Kidney mononuclear cell (KMNC) isolation was performed according to previously described methods (1). Briefly, decapsulated kidneys were immersed in RPMI buffer (Mediatech, Manassas, VA) containing 5% FBS and mechanically disrupted using Stomacher 80 Biomaster (Seward, Worthing, West Sussex, UK). Samples were strained, washed, and resuspended in 36% Percoll (Amersham Pharmacia Biotech, Piscataway, NJ) followed by gentle overlaying onto 72% Percoll. After being centrifuged at 1,000 g for 30 min at room temperature, KMNCs were collected from the interface of 36% and 72% Percoll. Afterward, KMNCs were washed twice and counted on a hemocytometer using trypan blue exclusion.

To minimize nonspecific antibody binding, isolated KMNCs were preincubated with anti-CD16/CD32 Fc receptor blocking antibody for 10 min. Thereafter, cells were incubated with anti-mouse anti-CD3, CD4, CD8, CD19, CD25, CD45, CD69, and NK1.1 (all from BD Biosciences) for 25 min at 4°C, washed with FACS buffer, and fixed with 1% paraformaldehyde solution. Acquisition and analyses of four-color immunofluorescence staining were performed using a FACSCalibur instrument (BD Biosciences, San Jose, CA) and FACS Flow 4 Flow Research (De Novo Software, Los Angeles, CA), respectively. Each assay included at least 10,000 gated events.

To calculate the percentage of CD45-positive cells in total cells in the postischemic kidney, the whole field of the slide was scanned and analyzed with TissueFAXS (TissueGnostics) (30, 36).

Multiplex protein array. A panel of cytokines and chemokines was measured in whole kidney protein extracts using the Milliplex MAP Mouse Cytokine/Chemokine kit (Luminex, Austin, TX) according to the manufacturer’s instructions. This multiplexed, particle-based, flow cytometric assay uses anti-cytokine monoclonal antibodies linked to microspheres incorporating distinct properties of two fluorescent dyes. Our assay was designed to detect and quantify IL-2, IL-6, IL-10, IL-17, interferon (IFN)-γ, monocyte chemoattractant protein (MCP)-1, regulated on activation, normal T cell expressed and secreted (RANTES), TNF-α, and VEGF. The value for each cytokine was normalized by dividing the raw cytokine concentration (in pg/ml) by the kidney protein concentration (in mg/ml) measured by the Bradford method.

Baseline data of the multiplex protein array of kidney tissue were obtained from normal mouse kidneys.

Cytokine and chemokine assays of HUCB-MSCs. The rat alveolar macrophage cell line NR8383 [American Type Culture Collection (ATCC)] was cultured in RPMI buffer supplemented with 50 μg/ml penicillin and 10% FBS. Cells were maintained at 37°C in a humidified atmosphere of 5% CO2. To induce activation of NR8383, as a positive control, lipopolysaccharide (LPS; 1 μg/ml, Sigma) was added. HUCB-MSCs were cocultured with LPS-exposed NR8383 for 72 h. After 72 h, coculture supernatants were collected for the measurement of cytokines. Rat IL-1α, rat IL-6, rat cytokine-induced neutrophil chemoattractant (CINC)-1, and human VEGF were quantified in cell culture supernatants by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer’s protocols. Results were acquired by measuring absorbance at 450 nm using a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA).

Administration of VEGF inhibitor. Neutralizing antibody of mouse VEGF with cross reactivity of human VEGF and control IgG (normal mouse IgG1) were purchased from Anglo-Proteomie (Boston, MA). Both VEGF inhibitor and control IgG were diluted with sterile PBS, and 2.5 mg/kg of each was administered into the peritoneal cavity during reperfusion and 24 h after surgery. Mice were divided into the following four groups: the control group received IRI surgery and sterile PBS, and the VEGF inhibitor group received IRI surgery and VEGF inhibitor, the HUCB-MSC + isotype control group received IRI surgery and HUCB-MSC treatment with the isotype control, and the HUCB-MSC + VEGF inhibitor group received IRI surgery and HUCB-MSC treatment with VEGF inhibitor. All mice were euthanized 2 days after IRI.
Statistical analyses. All data are expressed as means ± SE. Group means were compared using the Mann-Whitney test or ANOVA followed by Newman-Keuls post hoc analysis (using GraphPad Prism version 5). Statistical significance was determined when the P value was less than 0.05.

RESULTS

HUCB-MSCs attenuate early functional and structural renal injury after IRI. Serum BUN and creatinine were increased markedly after bilateral IRI surgery but were significantly lower in the HUCB-MSC-treated group than in controls on days 1 and 2 (Fig. 1A).

Histological evaluation of the postischemic kidneys was performed by assessing the proportion of necrotic, damaged, and intact tubules. The proportion of necrotic and damaged tubules tended to be lower in the cortex of the HUCB-MSC-treated group than in controls and was significantly lower in the medulla of the HUCB-MSC-treated group than in controls (Fig. 1B). The proportion of intact tubules tended to be higher than controls in the cortex of the HUCB-MSC-treated group and was significantly higher than controls in the medulla of the HUCB-MSC-treated group (Fig. 1C).

HUCB-MSCs traffic into postischemic kidneys. To examine the trafficking of HUCB-MSCs into postischemic kidneys, HUCB-MSCs were labeled with PKH-26 dye. By fluorescent microscopy, the proportion of PKH-26-labeled HUCB-MSCs was significantly increased in postischemic kidneys of the HUCB-MSC-treated group, whereas there were no PKH-26-positive cells in postischemic kidneys of the control group (control group vs. HUCB-MSC-treated group: day 1, 0.0 ± 0.00 vs. 1.5% ± 0.39% of total DAPI-positive cells, P < 0.05, and day 2, 0.0 ± 0.00 vs. 2.2% ± 0.57% of total DAPI-positive cells, P < 0.01; Fig. 2).

HUCB-MSCs do not reduce leukocyte trafficking into postischemic kidneys. Leukocyte trafficking into postischemic kidneys was examined by immunohistochemical staining for CD45 antigen and compared with baseline values in normal kidneys. The number of CD45-positive leukocytes was in-

Fig. 1. Renal functional and structural injury after ischemia-reperfusion injury (IRI) is reduced by human umbilical cord blood-derived mesenchymal stem cell (HUCB-MSC) treatment. A: the blood urea nitrogen (BUN) level was lower in the HUCB-MSC treatment group than in the control group on both days 1 and 2. The increase in serum creatinine (Cr) after IRI was significantly attenuated in the HUCB-MSC treatment group. B: tubular damage in the cortex of the postischemic kidney tended to be less in the HUCB-MSC treatment group compared with the control group on day 2 after IRI. In the medulla of the postischemic kidney, tubular damage was significantly decreased and the percentage of intact tubules was higher in the HUCB-MSC treatment group compared with the control group. C: hematoxylin and eosin (H&E) staining of postischemic kidneys. Arrows indicate dilated tubules with casts, and arrowheads indicate necrotic tubules. Magnification: ×200. *P < 0.05 compared with the control group. n = 12–15/group.
creased significantly in postischemic kidneys after IRI surgery, but there were no differences between control and HUCB-MSC-treated groups in the numbers of CD45-positive leukocytes as a proportion of total cells (Fig. 3).

HUCB-MSCs suppress IFN-γ expression and preserve VEGF expression in postischemic kidneys. The expression of proinflammatory and anti-inflammatory cytokines or chemokines in postischemic kidneys was analyzed and compared with baseline values in normal kidneys. Postischemic kidneys of the HUCB-MSC-treated group expressed higher levels of IL-2, IL-6, and IL-17 on day 1 than those of the control group. However, intrarenal IFN-γ levels on day 2 were significantly lower in the HUCB-MSC-treated group compared with the control group (Fig. 4A). The expression of MCP-1 was increased after IRI, but there were no differences between groups. There were no differences in the expression of IL-10 between groups. The expression of VEGF was significantly decreased in postischemic kidneys of the control group, but this reduction in VEGF after IRI was significantly attenuated on day 1 in postischemic kidneys of the HUCB-MSC-treated group (Fig. 4B).

Anti-inflammatory activity of HUCB-MSCs. The humoral effect of HUCB-MSCs was evaluated using the rat alveolar macrophage cell line NR8383 (ATCC). Severe inflammatory conditions were simulated in culture because a robust inflammatory response after IRI induces renal injury, and HUCB-MSCs are hypothesized to exert anti-inflammatory effects on the postischemic kidney. After macrophages were activated with LPS, they were cocultured with HUCB-MSCs. ELISA was performed to detect rat IL-1, rat IL-6, rat CINC-1, and human VEGF in culture supernatants. ELISA revealed increased levels of the rat proinflammatory cytokines IL-1α, IL-6, and CINC-1 in supernatants of NR8383 cells after LPS exposure. Compared with this positive control, the secretion of IL-1α, IL-6, and CINC-1 was decreased in supernatants of HUCB-MSC-treated cells (Fig. 5A). When macrophages exposed to LPS were cocultured with HUCB-MSCs for 72 h, the level of human VEGF in supernatants reached an average level of 105.5 pg/ml, which was >50-fold higher than the VEGF level produced from naïve HUCB-MSCs at 72 h (Fig. 5B).

The injection route and dose escalation of HUCB-MSCs do not potentiate the renoprotective effect of HUCB-MSCs. Functional and structural changes after IRI were simultaneously compared in the control group and three different treatment groups as follows: intraperitoneal injection of 1 × 10^6 or 3 ×
10^6 HUCB-MSCs and intravenous injection of 1 × 10^6 HUCB-MSCs.

Serum BUN and creatinine were comparable in the three different treatment groups, although both values were significantly lower in all treatment groups compared with the control group on day 2 (Fig. 6A).

The proportion of necrotic and damaged tubules was lower in both the cortex and medulla of the three treatment groups compared with the control group (Fig. 6B). However, there were no differences in the proportions of damaged and intact tubules among the three treatment groups (Fig. 6C), although increased trafficking of PKH-26-labeled HUCB-MSCs was found in the 3 × 10^6 HUCB-MSC intraperitoneal injection group compared with the 1 × 10^6 HUCB-MSC intravenous injection group (Fig. 6D).

**VEGF inhibitor abolish the renoprotective effect of HUCB-MSCs.** Simultaneous treatment of VEGF inhibitor or control IgG with HUCB-MSCs was performed to investigate the significance of VEGF in mediating the renoprotective effect of HUCB-MSCs. The VEGF inhibitor group, which received IRI surgery with VEGF inhibitor administration, showed more severe renal injury compared with the control group, which received IRI surgery with PBS administration only. Both serum BUN and creatinine were higher in the HUCB-MSC + VEGF inhibitor group compared with the HUCB-MSC + isotype control group, which received simultaneous treatment with both control IgG and HUCB-MSCs (Fig. 7A).

The proportion of necrotic and damaged tubules tended to be lower in the cortex and medulla of the HUCB-MSC +
Fig. 4. HUCB-MSCs suppress the increase in interferon (IFN)-γ and attenuate the decrease in VEGF in the postischemic kidney. A: compared with the control group, the expression of IL-2, IL-6, and IL-17 in the postischemic kidney was increased in the HUCB-MSC group on day 1, whereas the expression of IL-2 and IFN-γ was decreased in the HUCB-MSC group on day 2. There were no differences between groups in the expression of monocyte chemoattractant protein (MCP)-1, regulated on activation, normal T cell expressed and secreted (RANTES), and TNF-α. B: the expression of VEGF rapidly decreased after IRI, but HUCB-MSCs attenuated this decrease in the postischemic kidney. Baseline data of the multiplex protein array of kidney tissue were obtained from normal mouse kidneys. *P < 0.05 compared with the control group. n = 6–10/group.
isotype control group than in both the control and HUCB-MSC + VEGF inhibitor groups (Fig. 7B). The proportion of intact tubules tended to be higher in the cortex of the HUCB-MSC + isotype control group and was significantly higher in the medulla of the HUCB-MSC + isotype control group than both the control and HUCB-MSC + VEGF inhibitor groups (Fig. 7C).

HUCB-MSCs reduce the infiltration of NK T cells and increase regulatory T cells in postischemic kidneys. The percentages of total lymphocytes, total T cells, total B cells, CD4 and CD8 T cells, activated CD4 and CD8 T cells, and activated B cells were comparable in the control, HUCB-MSC, and HUCB-MSC + VEGF inhibitor groups on day 2 after IRI (Table 1). However, the percentage of NK T cells among total lymphocytes was lower in postischemic kidneys of the HUCB-MSC group compared with the control and HUCB-MSC + VEGF inhibitor groups (Fig. 8A). The percentage of regulatory T cells was higher in the HUCB-MSC group compared with the control and HUCB-MSC + VEGF inhibitor groups (Fig. 8B).

**DISCUSSION**

Our study demonstrates that in mice, early treatment with HUCB-MSCs reduces initial functional and structural renal injury after IRI. HUCB-MSCs traffic into the postischemic kidney after IRI and attenuate the rapid decrease in VEGF there, although HUCB-MSCs do not alter leukocyte infiltration into the postischemic kidney. These data reveal the potential of early HUCB-MSC therapy to improve clinical outcomes in high-risk patients with predictable serious ischemic kidney injury.

Although IRI results in ischemic AKI in both native kidneys and renal allografts, IRI is more problematic in renal allografts because it causes DGF in various clinical settings, and DGF increases the risk of acute rejection and contributes substantially to the development of chronic allograft nephropathy (39, 44). Avoiding or minimizing DGF in recipients of renal allografts has been a critical issue, especially for patients who receive allografts from cadaveric donors or extended criteria donors. The clinical importance of IRI as a substantial contrib-
utor to DGF has prompted many rigorous studies investigating the pathogenesis and early diagnosis of IRI and investigating novel therapies for IRI. Numerous studies using murine IRI models have demonstrated that both the innate and adaptive immune systems mediate renal injury after IRI and reported that the modulation of major cellular and humoral components, including T cells, B cells, macrophages, NK T cells, NK cells, and complement, can alter the renal outcome after IRI (3, 5, 11, 19, 23, 35, 40, 47, 48, 54–56). However, altering these cellular or humoral components is frequently not feasible and may result in unexpected adverse effects or inconsistent outcomes in experimental or clinical settings (4, 17, 42, 50). Modifying the proinflammatory micromilieu of postischemic kidneys to more favorable conditions that attenuate ongoing renal tissue...

Fig. 6. Administration route and dose escalation of HUCB-MSCs do not potentiate the renoprotective effect. A: serum BUN and Cr levels of the three treatment groups were significantly lower than the control group but comparable between the three different treatment groups on day 2 after IRI. B: the proportion of necrotic and damaged tubules was significantly lower in the three treatment groups compared with the control group. However, there were no differences in the proportion of damaged and intact tubules among the three treatment groups. C: H&E staining of postischemic kidneys. Arrows indicate dilated tubules with casts, and arrowheads indicate necrotic tubules. Magnification: ×200. D: trafficking of HUCB-MSCs evaluated with the percentage of PKH-26-labeled HUCB-MSCs among total DAPI using TissueFAXS was comparable between 1 × 10^6 intravenous and intraperitoneal groups. The trafficking of HUCB-MSCs was significantly higher in the 3 × 10^6 intraperitoneal group than the 1 × 10^6 intravenous group. *P < 0.05 compared with the control group. n = 5–6/group.
damage and hasten tubular regeneration seems more feasible and more critical.

HUCB-MSCs show low immunogenicity and have immunomodulatory activity, suppressing production of proinflammatory cytokines and augmenting levels of anti-inflammatory cytokines and chemokines (7, 12, 15, 45). HUCB-MSC treatment has been studied in various disease models including cerebral ischemic stroke, osteoarthritis, glioma, carbon tetrachloride-induced liver cirrhosis, and acute respiratory distress syndrome (9, 24–26, 28, 37, 43). In the present study, HUCB-MSCs administered intraperitoneally during reperfusion trafficked into the postischemic kidney, prevented the VEGF decrease in the postischemic kidney on day 1, and reduced the expression of IFN-γ in the postischemic kidney on day 2. Exogenous MSCs have been previously reported to traffic to the injured kidney by means of CD44 (16). In an in vitro experiment using cell culture, the VEGF-secreting ability of HUCB-MSCs under proinflammatory conditions was con-

Fig. 7. VEGF inhibitor cotreatment with HUCB-MSCs abolishes the renoprotective effect of HUCB-MSCs after IRI. A: serum BUN and Cr levels of the HUCB-MSC + isotype control group were significantly lower than the control and HUCB-MSC + VEGF inhibitor groups on both days 1 and 2 after IRI. B: tubular damage in the cortex of the postischemic kidney tended to be less in the HUCB-MSC + isotype control group compared with the control and HUCB-MSC + VEGF inhibitor groups on day 2 after IRI. In the medulla of the postischemic kidney, the proportion of intact tubules was higher in the HUCB-MSC + isotype control group compared with the control and HUCB-MSC + VEGF inhibitor groups.

C: H&E staining of postischemic kidneys. Arrows indicate dilated tubules with casts, and arrowheads indicate necrotic tubules. Magnification: ×200. *P < 0.05 compared with the control group. n = 5–9/group.

HUCB-MSCs IN THE INJURY PHASE OF RENAL IRI

F1157
firmed. HUCB-MSC treatment also increased IL-10 expression in the postischemic kidney on day 1, although this was not significant. These data suggest that early treatment with HUCB-MSC modulates the micromilieu of the postischemic kidney in mice to an anti-inflammatory condition and consequently attenuates tubular injury, despite the species barrier. VEGF has been reported to have beneficial effects on kidney allografts that underwent IRI (33) and to play a key role in the recovery from IRI (49). Early treatment with VEGF has also been reported to preserve the vascular structure after IRI (34). Several studies reported that bone marrow- or adipose tissue-derived MSCs reduce renal injury after IRI in murine models, and a small number of studies have investigated the effects of HUCB-MSCs in a rat model of renal IRI. Although most of these previous studies have reported paracrine effects of MSCs as the mechanism of their renoprotective effects, the key mediator of these paracrine effects was not identified, and the effects of HUCB-MSC on the postischemic kidney were not investigated thoroughly. The present study showed that HUCB-MSCs exert beneficial effects on the postischemic kidney in mice without evidence of rejection and that the main mechanism of the renoprotective effects of HUCB-MSCs seems their ability to secrete VEGF in proinflammatory conditions. The present study showed that VEGF inhibitor cotreatment with HUCB-MSCs abolished the renoprotective effect of HUCB-MSCs after IRI. This result may support the critical role of VEGF as a mediator of renoprotective effects of HUCB-MSCs in the postischemic kidney, although it was possible that HUCB-MSCs were injected into the mice with more severe injury induced by VEGF inhibitor than the control group. The suppressive effects of HUCB-MSCs on production of proinflammatory cytokines such as IL-6 and TNF-α have been demonstrated in cell culture, but they were not reproduced at the tissue level in the in vivo experiment. The production of sufficient VEGF by HUCB-MSCs to halt the rapid decline in VEGF in the postischemic kidney was consistent both in vitro in cell culture and in vivo in tissue. Furthermore, NK T cells were decreased and regulatory T cells were increased in postischemic kidneys of the HUCB-MSC treatment group. NK T cells have already been identified as important effector cells that contribute to the pathogenesis of renal IRI (18, 35, 53). Regulatory T cells have also been reported to play beneficial roles in both injury and repair phases of renal IRI (14, 29). The renoprotective effects of HUCB-MSC treatment in renal IRI may be mediated by immunomodulatory functions of HUCB-MSCs, especially the expression of VEGF and the infiltration of NK T cells and regulatory T cells into postischemic kidneys.

Regarding the administration route and the dosage of HUCB-MSCs, there were no differences in the renoprotective effects of HUCB-MSCs among the three different treatment groups: the intraperitoneal injection group, the intravenous injection group, and the high-dose intraperitoneal injection group. The high-dose HUCB-MSC treatment group showed increased trafficking but failed to confer greater protection in postischemic kidneys. These results might be caused by the proinflammatory phenotypes of locally activated HUCB-MSCs inducing IL-1 and TNF-α in certain conditions (8).

Diverse treatment protocols with different cell dosages have been reported in previous studies investigating the effects of MSCs from different sources in animal AKI models (51, 52). Many previous studies used MSCs originating from bone marrows of the rat, mouse, and human in a murine ischemic AKI model induced by IRI surgery and murine toxic AKI models induced by glycercol or cisplatin injection. MSC dosages ranged from 0.1 × 10⁶ to 9 × 10⁶, and relatively higher dosage of MSCs were used for intraperitoneal injection. Previous studies using the same HUCB-MSCs as our study in a murine acute lung injury model and severe brain injury model (24). A total of 1 × 10⁶ (40 × 10⁶/kg) HUCB-MSCs were intraperitoneally injected in the present study to increase the trafficking efficiency of systemically injected HUCB-MSCs. A total of 1–2 × 10⁶ bone marrow-derived MSCs were intravenously injected twice in a phase I clinical trial for the patients with allograft rejection after kidney transplantation (41). Further studies are required to find the most adequate dosage of HUCB-MSCs in preventing or treating renal IRI.

HUCB-MSCs have similar potential for multilineage differentiation, immune modulation, tumor tropism, and nursing function as bone marrow-derived MSCs (2, 32). The important
Advantages of HUCB-MSCs compared with other MSCs are that HUCB-MSCs can be collected less invasively and possess consistent and excellent immune modulating function with low immunogenicity. Therefore, HUCB-MSCs are worth evaluating as a novel cell therapy for renal IRI mediated by robust inflammatory responses, and our study strongly supports the therapeutic potential of HUCB-MSCs in renal IRI.

A few limitations of this study deserve consideration. First, only early treatment with HUCB-MSCs was tested. Various ways of administering HUCB-MSCs should be evaluated to find the most effective strategy. HUCB-MSCs were injected intraperitoneally during reperfusion in our study to investigate the effects of early treatment in mice. One recent study (8) reported better graft outcomes of early treatment with mesen-

---

**Fig. 8.** HUCB-MSCs decrease the infiltration of natural killer (NK) T cells and increase the trafficking of regulatory T cells into postischemic kidneys. **A:** on day 2 after IRI, the infiltration of NK T cells decreased and the proportion of regulatory T cells among total T cells increased in the HUCB-MSC treatment group. **B:** The infiltration of NK T cells and regulatory T cells of the HUCB-MSC + VEGF inhibitor group was comparable to the control group. *P < 0.05 compared with the control group. n = 4–6/group.
chymal stromal cells by changing the injection timing. Second, the paracrine effects of HUCB-MSCs on the expression of proinflammatory cytokines and leukocytes in the postischemic kidney need to be investigated further. In the present study, early treatment with HUCB-MSCs did not reduce the infiltration of leukocytes such as CD4 T cells, well-known pathogenic effector cells in renal IRI, and the expression of some major proinflammatory cytokines during the early renal injury phase after IRI. However, the long-term paracrine effects of HUCB-MSCs on the postischemic kidney also need to be evaluated. In conclusion, the present study demonstrates that HUCB-MSCs traffic into the postischemic kidney of mice, prevent the rapid decrease of VEGF in the postischemic kidney by secreting VEGF, and consequently attenuate early renal injury during the initial injury phase of IRI. Our data strongly support the therapeutic potential of HUCB-MSCs as a novel cell therapy with low immunogenicity that alters the micromilieu of the postischemic kidney.

ACKNOWLEDGMENTS

The authors thank Kyung Hee Choi and Ji Woo Kim (Samsung Biomedical Research Institute) and Seung-yeul Lee and Ik-soon Jang (Korea Basic Science Institute) for technical assistance.

GRANTS

This work was supported by Samsung Biomedical Research Institute Grant GL1B22612, Korean Society of Nephrology Grant PH1130221, and Korean Health Technology Research & Development Project, Ministry of Health & Welfare, Republic of Korea Grant HI13C-1263-020013.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

28. Kim SM, Lim JY, Park SJ, Jeong CH, Oh JH, Jeong M, Oh W, Park SH, Sung YC, Jeun SS. Gene therapy using TRAIL-secreting human...


