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Brief Communication

Mesenchymal Stem Cells Ameliorate Renal Inflammation in Adriamycin-induced Nephropathy

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ABSTRACT

Mesenchymal stem cells (MSCs) ameliorate the renal injury in Adriamycin (ADR)-induced nephropathy, but the mechanisms underlying their efficacy remain incompletely understood. In this study, we demonstrated that MSCs increased the survival, recovered body weight loss, and decreased proteinuria and serum creatinine levels in ADR-treated mice. MSCs also prevented podocyte damage and renal fibrosis by decreasing the expression of fibronectin, collagen 1α1, and α-smooth muscle actin. From a mechanistic perspective, MSCs inhibited renal inflammation by lowering the expression of CCL4, CCL7, CCL19, IFN-α/β, TGF-β, TNF-α, and chitinase 3-like 1. In summary, our data demonstrate that MSCs improve renal functions by inhibiting renal inflammation in ADR-induced nephropathy.

Keywords: Mesenchymal stem cells; Inflammation; Podocytes; Fibrosis

INTRODUCTION

Adriamycin (ADR)-induced nephropathy is a murine model of human focal glomerulosclerosis, which is characterized by podocyte damage, glomerular sclerosis, fibrosis, and tubulointerstitial inflammation (1). ADR is an anti-neoplastic anthracycline isolated from *Streptomyces peucetius* and has been widely used for the treatment of patients with different cancers; however, it causes severe nephrotoxicity because of the high accumulation in the kidney (2). ADR is toxic to endothelial cells and podocytes, resulting in a subsequent change in glomerular filtration with an increase in serum creatinine and proteinuria levels (1,3,4). ADR also exacerbates fibrosis as indicated by an increase in the accumulation of extracellular matrix in the tubulointerstitium (5). Histological assessment of the kidneys of ADR-injected mice shows severe tubulointerstitial inflammation with marked infiltration of $CD4^+$ T cells, $CD8^+$ T cells, B cells, and inflammatory M1 macrophages and low infiltration of Tregs (6).

Mesenchymal stem cells (MSCs) are multi-potent cells capable of differentiating into osteoblasts, chondrocytes, and adipocytes (7,8). MSCs secrete wide range of soluble factors that are beneficial for tissue repair, anti-fibrosis, anti-apoptosis, and immunomodulation (9). Several studies have shown that MSCs ameliorate renal injury in ADR-, cisplatin-,

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Conflict of Interest
Kim KS and Lee TY are employees of Corestem Inc. and others declare no conflict of interest.

Abbreviations
ADR, Adriamycin; CHI3L1, chitinase 3-like 1; Col1α1, collagen 1α1; Ct, threshold cycle; KO, knockout; MSC, mesenchymal stem cell; PAS, periodic acid-Schiff; RT-qPCR, RT-quantitative PCR; RQ, relative quantification; TBST, Tris-buffered saline; WT, wild-type; α-SMA, α-smooth muscle actin

Author Contributions
Conceptualization: Kim HS, Han SB, Kim KS, Bae SC; Data curation: Lee HK, Park EJ, Jeon HW, Kang YJ, Lee TY, Park JH; Formal analysis: Kim HS, Lee TY, Park JH; Investigation: Lee JS, Kim HS, Lee JS, Lee HK, Park EJ, Jeon HW, Kang YJ; Methodology: Lee JS, Kim HS, Lee JS, Lee HK, Park EJ, Jeon HW, Kang YJ; Project administration: Han SB; Resources: Lee TY, Kim KS, Bae SC; Writing - original draft: Kim HS, Han SB; Writing - review & editing: Park JH, Han SB.

and adenine-treated animal models and an ischemia-reperfusion injury model (8,10-15). However, the mechanisms underlying their efficacy have not been fully elucidated. In this study, we examined how MSCs ameliorate renal injury in ADR-treated mice.

MATERIALS AND METHODS

MSCs

Human bone marrow-derived MSCs were obtained from Corestem Inc. (Seoul, Korea) (16). In brief, bone marrow was aspirated from the posterior iliac crest of healthy donors and mononuclear cells were isolated using Ficoll (Ficoll-Paque Premium; GE Healthcare Bio-Sciences AB, Uppsala, Sweden) density gradient centrifugation. These cells were cultured in CSMB-A06 medium (Corestem Inc.) containing 10% FBS (BD Biosciences, Franklin Lakes, NJ, USA), 2.5 mM L-glutamine, and penicillin/streptomycin (WELGENE Inc., Gyeongsan, Korea) in a 7% CO_2 incubator at 37°C. When the cultures reached 80% confluence, MSCs were harvested using 0.125% trypsin-EDTA (Thermo Fisher Scientific, Waltham, MA, USA). MSCs had high expression levels of CD29, CD44, CD73, and CD105 and low expression levels of CD34 and CD45. All human MSC studies were approved by the Institutional Review Board of Hanyang University Hospital and were carried out in accordance with their approved guidelines; all participants provided written informed consents.

ADR-induced nephropathy

Male BALB/c and C57BL/6 mice were purchased from Samtako (Osan, Korea). Chitinase 3-like 1 (CHI3L1) knockout (KO) mice on C57BL/6 background were provided by Dr. Lee CG (Brown University, Providence, RI, USA) (17). All mice were used at the age of 6 to 8 weeks. Mice were housed in specific pathogen–free conditions at 21°C–24°C with 40%–60% relative humidity under a 12 h light/dark cycle (n=6–7). Mice were intravenously injected with PBS or 10 mg/kg of ADR (Santa Cruz Biotechnology Inc., Dallas, TX, USA) on day 0 (18). ADR-treated mice were intravenously injected with PBS or MSCs (1×10^6 cells/mouse) on day 7 and 14. Survival was examined every day and body weights were checked every week. Urine and serum were collected on day 28 and stored at −70°C until use. Proteinuria level was measured with a kit from Thermo Fisher Scientific and serum creatinine level was measured with a kit from Alpha Diagnostic International (San Antonio, TX, USA). All animal studies were approved by the Chungbuk National University Animal Experimentation Ethics Committee and were carried out in accordance with the approved guidelines.

RT-quantitative PCR (RT-qPCR) and ELISA

Total RNA was isolated using TRIzol Reagent (Thermo Fisher Scientific). cDNA was synthesized from 1 μg total RNA using an RT kit (Bioneer, Daejeon, Korea). The levels of mRNAs of diverse molecules were examined by qPCR with SYBR Green Master Mix (Qiagen, Hilden, Germany) and a StepOnePlus real-time PCR System (Applied Biosystems, Foster City, CA, USA) (19). The relative quantification (RQ) of mRNA levels in each sample was calculated based on its threshold cycle (Ct) in comparison to the Ct of the housekeeping gene β-actin. CHI3L1 levels in serum were measured by ELISA (Bio-Techne, Minneapolis, MN, USA).

Renal histology

Kidneys were fixed in 10% neutral buffered formalin solution (Sigma Aldrich, St. Louis, MO, USA) and then embedded in paraffin. Tissue sections (4-μm thick) were stained with periodic acid-Schiff (PAS) or Masson's trichrome and viewed though a microscope. Glomerular size

was measured using image analysis program of AxioVision microscope (Zeiss, Oberkohen, Germany). The glomerular sclerotic index was scored using a previously described method: L0, no lesion; L1, 1 to 24% sclerotic; L2, 25% to 49% sclerotic; L3, 50% to 74% sclerotic; and L4, 75% to 100% sclerotic. The glomerulosclerosis score was obtained using the following equation (20):

(1×Number of L1 glomeruli+2×Number of L2 glomeruli+3×Number of L3 glomeruli+4×Number of L4 glomeruli)×100%/Total number of glomeruli

Fifty glomeruli were analyzed per section. The tubular damage was scored as follow: 0, no fibrosis; 1, very mild focal dilation of tubules; 2, larger number of dilated tubules with widening of the interstitium; 3, fairly extensive dilation of tubules with cystic formation and widening of interstitium; and 4, entire atrophy of tubules (21). Kidney sections were also immunostained with antibodies against mouse desmin (Dako, Santa Clara, CA, USA). Positively stained cells appeared brown (1).

Podocytes

Kidneys from male BALB/c mice at 9 wk of age were minced into small pieces and digested with 2 mg/ml of collagenase (Sigma Aldrich) in RPMI-1640 medium containing 10% FBS at 37°C for 40 min (22). The specimens were then passed through a 100-μm cell strainer (BD Biosciences), and treated with ACK lysis buffer to remove red blood cells (22). Upon washing with medium, Specimens were treated with 0.5 mg/ml of collagenase and dispase II (Sigma Aldrich) and 0.075% trypsin (Sigma Aldrich) in medium at 37°C for 20 min. Single cells were finally obtained by passing the samples through a 25-μm filter to remove incompletely dissociated renal tissues. For the purification of podocytes, single cells were incubated with CD31 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany), and CD31-positive endothelial cells were then excluded using the MACS system with MS columns (Miltenyi Biotec). The CD31-negative fractions, which passed through the magnetic columns, were subsequently subjected to the second MACS separation using anti-nephrin antibody (R&D Systems, Minneapolis, MN, USA). The cells were labelled with 10 μg of biotin-conjugated antibody recognizing nephrin, followed by incubation with Streptavidin MicroBeads and separation using the MACS system with MS columns. The positive fractions were collected and cultured in collagen I-coated dishes.

Western blotting

Whole cell lysates were prepared as previously described (23). Detergent-insoluble material was removed, and equal amounts of protein were fractionated by 10% SDS-PAGE and transferred to pure nitrocellulose membranes. Membranes were blocked with 5% (w/v) skim milk in 0.05% (v/v) Tween-20 in Tris-buffered saline (TBST) for 1 h and then incubated with an appropriate dilution of primary antibody in 5% (w/v) BSA in TBST for 2 h. Blots were incubated with biotinylated secondary antibody for 1 h and then with HRP-conjugated streptavidin for 1 h. Signals were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Anti-mouse antibodies against BAX and Bcl2 were purchased from Cell Signaling Technology (Danvers, MA, USA).

Statistics

Data are presented as mean±SEM of at least three independent *in vitro* experiments performed in triplicate or >6 mice. To determine statistical significance, p values were calculated using one-way ANOVA (GraphPad Software, San Diego, CA, USA) (24).

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RESULTS

MSCs ameliorate renal injury in ADR-treated mice

We examined the therapeutic effects of MSCs on renal injury in ADR-treated mice. ADR was injected once on day 0 and MSCs were transplanted on days 7 and 14, and the outcome was analyzed on day 28. MSC treatment showed the prolonged survival rate of ADR-treated mice: chemically-untreated control mice survived 100%, ADR-treated mice survived 57%, and ADR+MSC-treated mice survived 83% (**Fig. 1A**). Body weight (**Fig. 1B**) and kidney weight (**Fig. 1C**) of ADR-treated mice decreased, and MSCs prevented these decreases. ADR increased the levels of proteinuria (**Fig. 1D**) and serum creatinine (**Fig. 1E**), and these effects were prevented by MSCs. PAS and Masson's trichrome staining data showed that ADR-increased glycoprotein accumulation (arrow ①), decreased capsular space (arrow ②), dilated tubules (arrow ③), and increased collagen accumulation (arrow ④) in the glomeruli; altogether, these data demonstrated that ADR caused pathological lesions in the glomeruli, characterized by the increase of glomerular size, glomerular sclerosis, and tubular damage; in contrast, the pathologic changes in MSC-treated mice were milder (**Fig. 1F**). These data suggest that ADR impairs renal functions, which can be ameliorated by MSCs.

MSCs protect podocytes from ADR toxicity

We also examined expression levels of desmin, which is expressed in damaged podocytes. ADR strongly increased the kidney expression of desmin; MSCs suppressed this increase (**Fig. 1G**). To examine the direct effect of MSCs on podocytes, we isolated podocytes and co-cultured them with MSCs in the presence of ADR. ADR strongly increased apoptotic BAX expression and decreased anti-apoptotic Bcl-2 expression in podocytes, and these effects were reversed at the mRNA and protein levels (**Fig. 1H**). It was also confirmed by Western blot. Unfortunately, we could not use flow cytometric analysis because of low cell numbers. These data suggest that MSCs protect podocytes from the damage of ADR *in vivo* and *in vitro*.

MSCs inhibit renal inflammation in ADR-treated mice

Next, we examined whether MSCs could prevent renal inflammation in ADR-treated mice. ADR increased gene expression of inflammatory cytokines, including TNF-α, TGF-β, and IFN-α/β, and MSCs significantly inhibited this increase (**Fig. 2A**). We also examined the effects of MSCs on the expression of CHI3L1, since CHI3L1 is highly expressed by intra-renal macrophages and plays a key role in inflammation, fibrosis, and repair after unilateral ischemia-reperfusion injury of the kidney (25). ADR increased gene expression of CHI3L1 in the kidney (**Fig. 2B**) and protein levels in the serum (**Fig. 2C**), and these effects were abolished by MSCs. Since the pathogenic role of CHI3L1 in ADR-induced nephropathy has not been studied yet, we further studied whether CHI3L1 is associated with renal inflammation by using KO mice. We injected ADR into CHI3L1 KO mice and measured proteinuria levels 28 days later. Unlike in wild-type (WT) mice, ADR only weakly increased proteinuria levels in CHI3L1 KO mice, confirming the pathogenic role of CHI3L1 as a pro-inflammatory cytokine in ADR-induced nephropathy (**Fig. 2D**). ADR also increased the gene expressions of most chemokines, and this increase was inhibited by MSCs (**Fig. 2E**). However, some variations were observed: we found strong inhibition of CCL4, CCL7, CCL19, and CXCL11 expression, but weak inhibition of others (**Fig. 2E**). We also examined whether MSCs would prevent renal fibrosis in ADR-treated mice. ADR strongly increased mRNA expression levels of the representative extracellular matrix components, fibronectin and collagen 1α1 (Col1α1), and myofibroblast surrogate marker α-smooth muscle actin (α-SMA) in the kidney; all these effects of ADR were inhibited by MSCs (**Fig. 2F**). Overall, our data suggest that MSCs have anti-inflammatory and anti-fibrotic activities.

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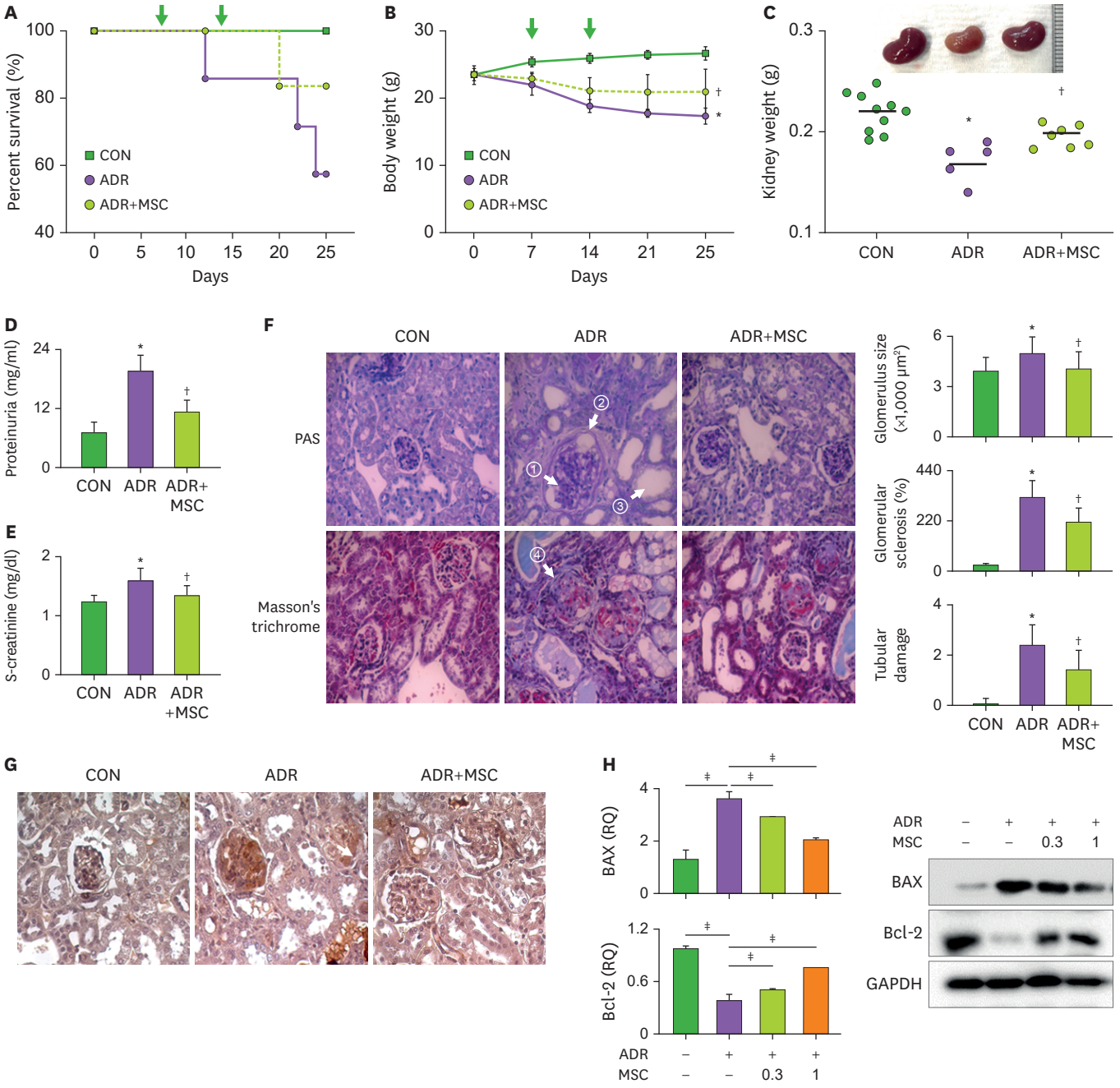

Figure 1. MSCs ameliorate renal injury in ADR-treated mice. (A-G) Male BALB/c mice were intravenously injected with 10 mg/kg of ADR on day 0, and MSCs (1×10^6 cells/mouse) on days 7 and 14 (arrows). Mice were sacrificed on day 28 to determine (A) survival rate, (B) body weight, (C) kidney morphology and weight, (D) proteinuria level, and (E) serum creatinine level. (F) Kidney sections were stained with PAS or Masson's trichrome stains. The amounts of glycoprotein (arrow ①), the size of capsular spaces (arrow ②), and the size of tubules (arrow ③), and the amounts of collagens (arrow ④) in the glomeruli were examined. Glomerular size, glomerular sclerotic index, and tubular damage score are presented. (G) Expression of the podocyte injury marker desmin was measured by immunohistochemistry. Each group are presented as CON (chemically-untreated control mice; n=6), ADR (ADR-treated mice; n=6), and ADR+MSC (ADR and MSC-treated mice; n=7), respectively. (H) Podocytes were isolated from normal BALB/c mice and were cultured with MSCs and/or 0.5 μg/ml of ADR for 24 h. Gene and protein expression of apoptotic (BAX) and anti-apoptotic (Bcl-2) molecules were measured by RT-qPCR and Western blot, respectively.
*p<0.05 CON versus ADR; †p<0.05 ADR versus ADR+MSC; ‡p<0.05.

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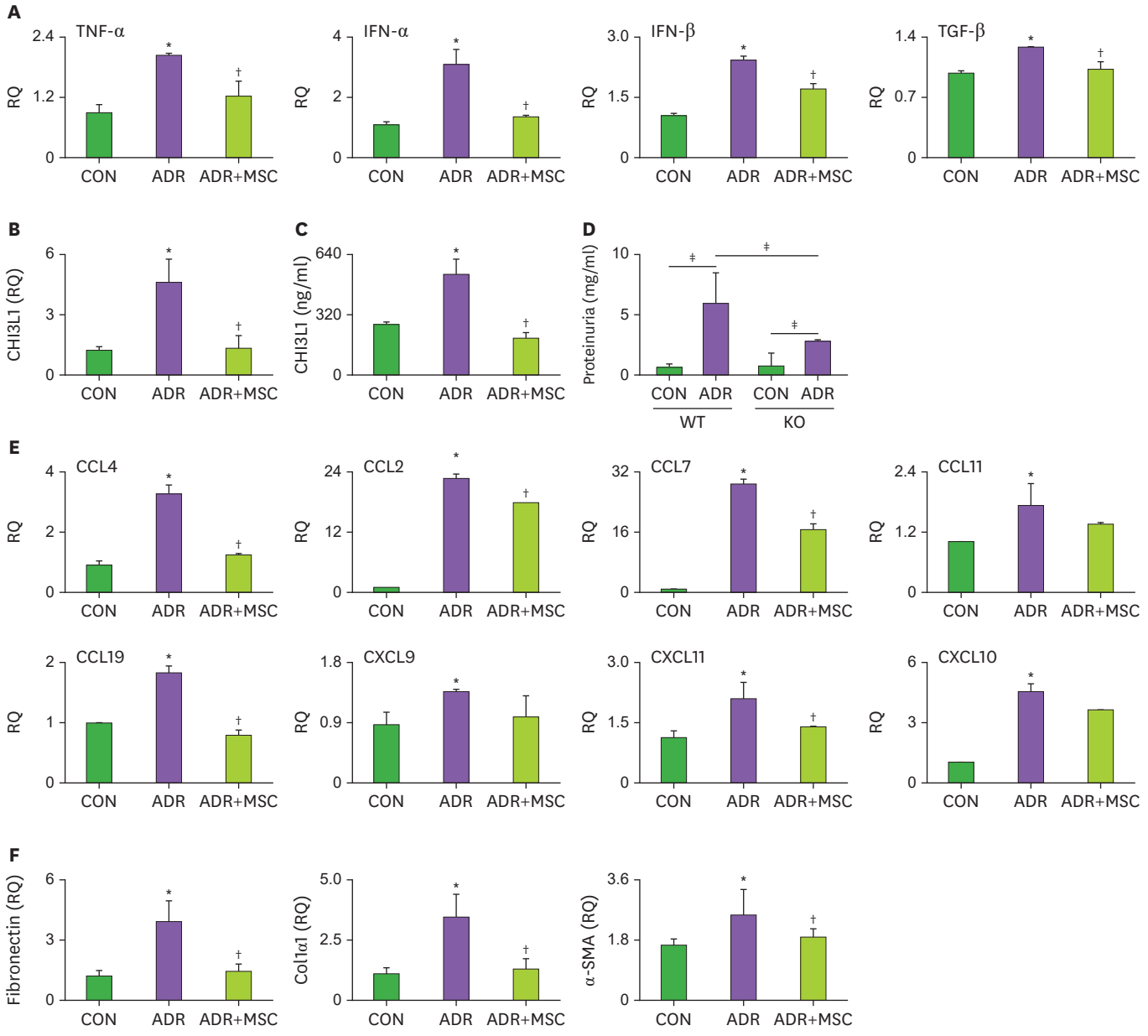

Figure 2. MSCs inhibit renal inflammation in ADR-treated mice. (A-C, E, and F) Male BALB/c mice were intravenously injected with 10 mg/kg of ADR on day 0, and MSCs (1×10^6 cells/mouse) on days 7 and 14. Mice were sacrificed on day 28. The amounts of mRNA of cytokines (A), CHI3L1 (B), chemokines (E), and extracellular proteins, such as fibronectin, Col1α1, and α-SMA (F) were measured by RT-qPCR. (C) The level of CHI3L1 in serum was measured by ELISA. Each group are presented as CON (chemically-untreated control mice; n=6), ADR (ADR-treated mice; n=6), and ADR+MSC (ADR and MSC-treated mice; n=7), respectively. (D) ADR was injected to WT male C57BL/6 or CHI3L1 KO mice (C57BL/6 background) on day 0 and proteinuria level was measured on day 28. Each group are presented as CON (chemically-untreated control mice; n=4) and ADR (ADR-treated mice; n=4), respectively.
*p<0.05 CON versus ADR; †p<0.05 ADR versus ADR+MSC; ‡p<0.05.

DISCUSSION

Our data suggest that MSCs ameliorate ADR-induced renal injury by suppressing inflammation. In several renal injury models, MSCs inhibit the expression of inflammatory cytokines, including IL-1α, IL-6, IFNs, and TNF-α, and augment the expression of anti-inflammatory cytokines, such as IL-4 and IL-10 (26-28). MSCs also inhibit the expression of

chemokines, such as CCL2, CCL3, CCL5, CXCL10, CXCL12, CXCL13, and CX3CL1, which are highly expressed in the kidney of patients or mice with systemic lupus erythematosus (24,28). Consistently, our data demonstrate that MSCs inhibit the expression of IFNs, TGF-β, TNF-α, CCL4, CCL7, CCL19, and CXCL11, although they did not affect the expression of IL-4 and IL-10 in our experiments (data not shown). Our data suggest that MSCs ameliorate renal injury by inhibiting inflammation via reduction in chemokine and cytokine expression in ADR-induced nephropathy. An interesting finding might be the increase of CHI3L1 in the kidney and serum of ADR-treated mice. CHI3L1 is a highly conserved glycoprotein expressed in a variety of cell types, including macrophages, neutrophils, chondrocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, and epithelial cells (29,30). The expression of CHI3L1 is regulated by various cytokines, including IL-1β, IL-6, IL-13, IFN-γ, and TNF-α (29). Circulating levels of CHI3L1 are augmented in patients with asthma, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, sarcoidosis, chronic obstructive lung disease, infectious diseases, atherosclerosis, diabetes, and cancer (29-32). However, the biological roles of CHI3L1 in renal injury are largely unknown. In the present study, we provide 2 data to predict the role of CHI3L1 in renal inflammation: i) CHI3L1 expression is increased in the kidney and serum of ADR-treated mice and ii) renal injury caused by ADR is very weak in CHI3L1-KO mice. These data suggest that CHI3L1 might be a pro-inflammatory cytokine involved in the pathogenesis of renal injury in ADR-induced nephropathy.

ADR shows the toxicity to podocytes, which might be resulted in the renal inflammation (1,3,4). Podocytes are highly specialized cells maintaining the glomerular filtration barrier, and podocyte injury is observed in various renal diseases (33,34). Several studies showed that MSCs inhibited podocyte apoptosis via epithelial growth factor, VEGF, CCAAT/enhancer binding protein-α, and β-catenin in ADR-induced and a diabetic nephropathy (3,4,34). Consistently, our data demonstrate that MSCs significantly decrease the expression of the podocyte injury marker desmin in the kidney of ADR-treated mice and significantly inhibit pro-apoptotic BAX expression in ADR-treated podocytes in an *in vitro* co-culture setting.

Renal fibrosis is the final common pathway of renal diseases, such as diabetic, hypertensive, toxic, ischemic, or autoimmune diseases of kidney (35). Renal fibrosis in ADR-induced nephropathy is complexly associated with toxic damage to podocytes and glomerular structure, oxygen free radical accumulation, and inflammation (35). MSCs ameliorate renal fibrosis by inhibiting macrophage infiltration and TGF-β expression and by increasing matrix metalloproteinase expression (5,26). In agreement with these data, our data suggest that MSCs prevent the expression of fibronectin, Col1α1, and α-SMA by inhibiting podocyte damage, inflammation, and TGF-β expression.

In conclusion, we demonstrate the beneficial effect of MSCs on renal injury in ADR-induced nephropathy and suggest podocyte protection, anti-fibrosis, and anti-inflammation capabilities of MSCs as potential mechanisms. However, further studies are still required to address the role of chemokines, cytokines, and CHI3L1 in the kidney of ADR-treated mice. It will be interesting to study which cells produce chemokines, cytokines, and CHI3L1 and which cells are affected by these inflammatory factors. Furthermore, it will be also worthy studying how MSCs regulate these effector and target cell types. MSCs are well known to Tregs, B cells, NK cells, macrophages, and dendritic cells by producing the soluble factors, such as TGF-β, prostaglandin E_2, and indoleamine 2,3-dioxygenase, and by expressing FasL and PD-L1 for contact-dependent inhibition (16). These data will help to reveal how MSCs inhibit renal inflammation in ADR-induced nephropathy in greater detail.

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REFERENCES

1. Lee VW, Harris DC. Adriamycin nephropathy: a model of focal segmental glomerulosclerosis. *Nephrology (Carlton)* 2011;16:30-38.
PUBMED | CROSSREF
2. Nogueira A, Pires MJ, Oliveira PA. Pathophysiological mechanisms of renal fibrosis: a review of animal models and therapeutic strategies. *In Vivo* 2017;31:1-22.
PUBMED | CROSSREF
3. Zhang H, Ren R, Du J, Sun T, Wang P, Kang P. AF1q contributes to Adriamycin-induced podocyte injury by activating Wnt/β-catenin signaling. *Kidney Blood Press Res* 2017;42:794-803.
PUBMED | CROSSREF
4. Zoja C, Garcia PB, Rota C, Conti S, Gagliardini E, Corna D, Zanchi C, Bigini P, Benigni A, Remuzzi G, et al. Mesenchymal stem cell therapy promotes renal repair by limiting glomerular podocyte and progenitor cell dysfunction in Adriamycin-induced nephropathy. *Am J Physiol Renal Physiol* 2012;303:F1370-F1381.
PUBMED | CROSSREF
5. Huuskes BM, Wise AF, Cox AJ, Lim EX, Payne NL, Kelly DJ, Samuel CS, Ricardo SD. Combination therapy of mesenchymal stem cells and serelaxin effectively attenuates renal fibrosis in obstructive nephropathy. *FASEB J* 2015;29:540-553.
PUBMED | CROSSREF
6. Mahajan D, Wang Y, Qin X, Wang Y, Zheng G, Wang YM, Alexander SI, Harris DC. $CD4^+CD25^+$ regulatory T cells protect against injury in an innate murine model of chronic kidney disease. *J Am Soc Nephrol* 2006;17:2731-2741.
PUBMED | CROSSREF
7. Najar M, Krayem M, Meuleman N, Bron D, Lagneaux L. Mesenchymal stromal cells and Toll-like receptor priming: a critical review. *Immune Netw* 2017;17:89-102.
PUBMED | CROSSREF
8. Ma H, Wu Y, Zhang W, Dai Y, Li F, Xu Y, Wang Y, Tu H, Li W, Zhang X. The effect of mesenchymal stromal cells on doxorubicin-induced nephropathy in rats. *Cytotherapy* 2013;15:703-711.
PUBMED | CROSSREF
9. Fitzsimmons RE, Mazurek MS, Soos A, Simmons CA. Mesenchymal stromal/stem cells in regenerative medicine and tissue engineering. *Stem Cells Int* 2018;2018:8031718.
PUBMED | CROSSREF
10. Ma H, Wu Y, Xu Y, Sun L, Zhang X. Human umbilical mesenchymal stem cells attenuate the progression of focal segmental glomerulosclerosis. *Am J Med Sci* 2013;346:486-493.
PUBMED | CROSSREF
11. Yokote S, Katsuoka Y, Yamada A, Ohkido I, Yokoo T. Effect of adipose-derived mesenchymal stem cell transplantation on vascular calcification in rats with adenine-induced kidney disease. *Sci Rep* 2017;7:14036.
PUBMED | CROSSREF
12. Lange C, Tögel F, Ittrich H, Clayton F, Nolte-Ernsting C, Zander AR, Westenfelder C. Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. *Kidney Int* 2005;68:1613-1617.
PUBMED | CROSSREF
13. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, Rottoli D, Angioletti S, Benigni A, Perico N, et al. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. *J Am Soc Nephrol* 2004;15:1794-1804.
PUBMED | CROSSREF
14. Bi B, Schmitt R, Israilova M, Nishio H, Cantley LG. Stromal cells protect against acute tubular injury via an endocrine effect. *J Am Soc Nephrol* 2007;18:2486-2496.
PUBMED | CROSSREF
15. Tögel F, Weiss K, Yang Y, Hu Z, Zhang P, Westenfelder C. Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. *Am J Physiol Renal Physiol* 2007;292:F1626-F1635.
PUBMED | CROSSREF

16. Lee HK, Kim HS, Kim JS, Kim YG, Park KH, Lee JH, Kim KH, Chang IY, Bae SC, Kim Y, et al. CCL2 deficient mesenchymal stem cells fail to establish long-lasting contact with T cells and no longer ameliorate lupus symptoms. *Sci Rep* 2017;7:41258.
PUBMED | CROSSREF

17. Kim DH, Park HJ, Lim S, Koo JH, Lee HG, Choi JO, Oh JH, Ha SJ, Kang MJ, Lee CM, et al. Regulation of chitinase-3-like-1 in T cell elicits Th1 and cytotoxic responses to inhibit lung metastasis. *Nat Commun* 2018;9:503.
PUBMED | CROSSREF

18. Wang Y, Wang YP, Tay YC, Harris DC. Progressive Adriamycin nephropathy in mice: sequence of histologic and immunohistochemical events. *Kidney Int* 2000;58:1797-1804.
PUBMED | CROSSREF

19. Lee YH, Seo JW, Kim YG, Moon JY, Kim JS, Jeong KH, Kim BM, Kim KW, Yang CW, Kim CD, et al. Validation study of an operational tolerance signature in Korean kidney transplant recipients. *Immune Netw* 2018;18:e36.
PUBMED | CROSSREF

20. Peng X, Xu H, Zhou Y, Wang B, Yan Y, Zhang X, Wang M, Gao S, Zhu W, Xu W, et al. Human umbilical cord mesenchymal stem cells attenuate cisplatin-induced acute and chronic renal injury. *Exp Biol Med (Maywood)* 2013;238:960-970.
PUBMED | CROSSREF

21. Uehara Y, Numabe A, Hirawa N, Kawabata Y, Iwai J, Ono H, Matsuoka H, Takabatake Y, Yagi S, Sugimoto T. Antihypertensive effects of cicletanine and renal protection in Dahl salt-sensitive rats. *J Hypertens* 1991;9:719-728.
PUBMED | CROSSREF

22. Murakami A, Oshiro H, Kanzaki S, Yamaguchi A, Yamanaka S, Furuya M, Miura S, Kanno H, Nagashima Y, Aoki I, et al. A novel method for isolating podocytes using magnetic activated cell sorting. *Nephrol Dial Transplant* 2010;25:3884-3890.
PUBMED | CROSSREF

23. Lee HK, Kim KH, Kim HS, Kim JS, Lee JH, Ji A, Kim KS, Lee TY, Chang IY, Bae SC, et al. Effect of a combination of prednisone or mycophenolate mofetil and mesenchymal stem cells on lupus symptoms in MRL.*Fas*^{lpr} mice. *Stem Cells Int* 2018;2018:4273107.
PUBMED | CROSSREF

24. Lee JH, Lee HK, Kim HS, Kim JS, Ji AY, Lee JS, Kim KS, Lee TY, Bae SC, Kim Y, et al. CXCR3-deficient mesenchymal stem cells fail to infiltrate into the nephritic kidney and do not ameliorate lupus symptoms in MRL.*Fas*^{lpr} mice. *Lupus* 2018;27:1854-1859.
PUBMED | CROSSREF

25. Montgomery TA, Xu L, Mason S, Chinnadurai A, Lee CG, Elias JA, Cantley LG. Breast regression protein-39/chitinase 3-like 1 promotes renal fibrosis after kidney injury *via* activation of myofibroblasts. *J Am Soc Nephrol* 2017;28:3218-3226.
PUBMED | CROSSREF

26. Semedo P, Correa-Costa M, Antonio Cenedeze M, Maria Avancini Costa Malheiros D, Antonia dos Reis M, Shimizu MH, Seguro AC, Pacheco-Silva A, Saraiva Camara NO. Mesenchymal stem cells attenuate renal fibrosis through immune modulation and remodeling properties in a rat remnant kidney model. *Stem Cells* 2009;27:3063-3073.
PUBMED | CROSSREF

27. Wise AF, Ricardo SD. Mesenchymal stem cells in kidney inflammation and repair. *Nephrology (Carlton)* 2012;17:1-10.
PUBMED | CROSSREF

28. Wu HJ, Yiu WH, Li RX, Wong DW, Leung JC, Chan LY, Zhang Y, Lian Q, Lin M, Tse HF, et al. Mesenchymal stem cells modulate albumin-induced renal tubular inflammation and fibrosis. *PLoS One* 2014;9:e90883.
PUBMED | CROSSREF

29. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, He CH, Takyar S, Elias JA. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. *Annu Rev Physiol* 2011;73:479-501.
PUBMED | CROSSREF

30. Nielsen TL, Plesner LL, Warming PE, Pallisgaard JL, Dalsgaard M, Schou M, Høst U, Rydahl C, Brandi L, Køber L, et al. YKL-40 in patients with end-stage renal disease receiving haemodialysis. *Biomarkers* 2018;23:357-363.
PUBMED | CROSSREF

31. Nadkarni GN, Rao V, Ismail-Beigi F, Fonseca VA, Shah SV, Simonson MS, Cantley L, Devarajan P, Parikh CR, Coca SG. Association of urinary biomarkers of inflammation, injury, and fibrosis with renal function decline: the ACCORD trial. *Clin J Am Soc Nephrol* 2016;11:1343-1352.
PUBMED | CROSSREF

32. Ziatabar S, Zepf J, Rich S, Danielson BT, Bollyky PI, Stern R. Chitin, chitinases, and chitin lectins: emerging roles in human pathophysiology. *Pathophysiology* 2018;25:253-262.
PUBMED | CROSSREF

33. Wang S, Li Y, Zhao J, Zhang J, Huang Y. Mesenchymal stem cells ameliorate podocyte injury and proteinuria in a type 1 diabetic nephropathy rat model. *Biol Blood Marrow Transplant* 2013;19:538-546.
PUBMED | CROSSREF

34. Zhong F, Wang W, Lee K, He JC, Chen N. Role of C/EBP-α in Adriamycin-induced podocyte injury. *Sci Rep* 2016;6:33520.
PUBMED | CROSSREF

35. Szalay CI, Erdélyi K, Kökény G, Lajtár E, Godó M, Révész C, Kaucsár T, Kiss N, Sárközy M, Csont T, et al. Oxidative/nitrative stress and inflammation drive progression of doxorubicin-induced renal fibrosis in rats as revealed by comparing a normal and a fibrosis-resistant rat strain. *PLoS One* 2015;10:e0127090.
PUBMED | CROSSREF