IJKD KIDNEY DISEASES

Stem Cell Therapy Ameliorates Ischemia-reperfusion Induced Kidney Injury After 24 Hours Reperfusion

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Original Paper

Introduction. The mortality rate in patients with acute kidney injury (AKI) is high. The aim of this study was to evaluate the efficacy of treatment with adipose-derived mesenchymal stem cells (AD-MSC) in renal ischemia-reperfusion (I/R) model in rats.
Methods. In this study 28 male Wistar rats were divided into four groups of control, sham, I/R24h+PBS, and I/R24h+AD-MSC. Blocking the renal arteries for 45 minutes induced renal I/R and then reperfusion was conducted for 24 hours. Parameters including urine volume, osmolarity, plasma creatinine (Cr_p), and blood urea nitrogen (BUN) were evaluated and values of creatinine clearance (C_{Cr}), absolute sodium excretion ($U_{Na}V°$), fractional excretion of sodium (FE_{Na}), absolute potassium excretion ($U_KV°$) and fractional excretion of potassium (FE_K) were calculated. The right kidney was removed to measure the malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP), as well as the left kidney for histological evaluation.
Results. I/R caused a significant increase in Cr_p, BUN, $U_{Na}V°$, FE_{Na}, FE_K, MDA, and tissue damages. In addition, the values of C_{Cr}, urine osmolarity, and FRAP level decreased significantly ($P < .05$). Following AD-MSC treatment, values of FE_{Na}, Cr_p, FEK, MDA, and tissue damages decreased significantly, while urine osmolarity increased significantly in the I/R24h + AD-MSC group compared to the I/R24h + PBS group. Furthermore, FRAP values increased significantly ($P < .001$).
Conclusion. Treatment with AD-MSC reduced tissue damage and oxidative stress while increasing antioxidant activity. In addition, it improved kidney function after 45 min ischemia and 24 h reperfusion.

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INTRODUCTION

The prevalence of acute kidney injury (AKI) is about 23.9% in adults and 13.8% in children. The AKI is defined by the decreased performance of kidneys,[1] which is determined by the reduced glomerular filtration rate (GFR), and increased concentration of plasma creatinine (Cr_p) and urea nitrogen (BUN).[2] Several factors can cause the occurrence or development of AKI, and ischemia/reperfusion (I/R) is among the most common of these factors. A number of major structures are damaged during the process of I/R including tubules, glomerulus, interstitium, and blood vessels of the kidney.[3] Following the induction of I/R, the inflammatory processes are activated which lead to the accumulation of inflammatory cells,

increased inflammation, and eventually necrosis and apoptosis by releasing inflammatory factors including cytokines, chemokines, and reactive oxygen species (ROS).[4] Due to the limitations in transplantation treatments including the lack of donor organs and low efficacy of chemical drugs, finding new methods to minimize kidney damage and mortality rate due to AKI seems necessary.[5,6] Recent developments in stem cell researches and clinical treatments based on them is a promising reason to believe that such treatments based on stem cells will be widely available.[5]

Mesenchymal stem cells (MSCs) are undifferentiated cells with the capacity of self-renewal and differentiation into different cell types.[7] The efficacy of these cells has been reported in acute and chronic diseases including diabetes,[8] osteoarthritis,[9] and renal failure.[10] MSCs especillay adipose tissue-derived mesenchymal stem cells (AD-MSCs) have reported having the ability to reduce inflammation and suppress intrinsic and acquired immune diseases, so they may be good candidates for being used in the treatment of AKI.[11] In addition, MSCs produce various factors which help the healing process in parenchymal and endothelial levels.[6] Tögel *et al*. reported that arterial injection of MSCs have anti-inflammatory effects after renal ischemia by reducing inflammatory parameters such as Interleukin-1β (IL-1β), and tumor necrosis factor alpha (TNF-α), over 24 h of reperfusion in rats.[12] In addition, Havakhah *et al*. conducted a renal artery clamping and 24 h of reperfusion in rats. Then, they injected 2×10^6 bone marrow mesenchymal stem cells (BM-MSCs) using intravenous (IV) and intraperitoneal (IP) injections. They concluded that the function of possible healing mechanism is independent of the distinction process. Their results showed that the functions of paracrine MSC, including secretion of cytokines and increased expression of growth factors, are involved in the healing process.[4] Although several studies are conducted on the effects of MSCs in renal damage caused by I/R, no study has addressed the therapeutic effects of AD-MSC after 45 minutes ischemia and 24 h reperfusion. The aim of present study was to investigate the therapeutic effects of AD-MSC on functional disturbances, tissue damages and oxidative stress caused by renal I/R model.

MATERIALS AND METHODS

Animals

The present experimental study was performed on 28 male Wistar rats weighing 180 to 200 gram. The study was conducted based on full compliance with the Helsinki Declaration and the ethical codes of laboratory animals' welfare was approved by the ministry of health and medical education (Iran) under license number IR.ARAKMU.REC.1396.113. Mature rats were obtained from the animal laboratory of Arak University of medical sciences (Iran). All tested animals were kept at a temperature of 22-24°C, and the environment condition light-dark (12 h) cycle. In addition, the rats had ad libitum access to standard water and food.[13]

Isolation and Determination of Adipose Tissue-derived Mesenchymal Cells (AD-MSCs)

Rats were anesthetized using an i.p. injection of 60 mg/kg ketamine hydrochloride (Trittau, Germany) and 6 mg/kg xylazine hydrochloride (Woerden, Netherlands). Isolation and determination of AD-MSCs were performed using the fluorescence-activated cell sorting (FACS) analysis.[14]

Test Protocol and Groups

The animals were randomly divided into 4 groups (n = 7). No intervention was performed in the control group. On the other hand, the surgery was performed on the 24h sham group, but renal vessels were not blocked, and 1 ml of PBS was injected immediately after the end of surgery. In the third group (I/R-24h + PBS), complete renal artery blockage was performed for 45 minutes on both kidneys, and then 24h of reperfusion was done along with 1 mL injection of PBS through the tail vein. In the fourth group (I/R-24h + AD-MSC) protocol was similar to the third group, but immediately after the removal of the blockage, 2×10^6 cells of AD-MSC were injected in 1 mL of PBS.

In order to induce ischemia, initially 50 mg/kg.bw of pentobarbital (Lund beck, Denmark) was injected i.p. for induction of anesthesia. Then, an aseptic longitudinal incision was performed in the abdominal area. The vessels of both kidneys were completely blocked at the same time for 45 min and then, the rats were returned to their cages after the removal of the blockage for recovery and 24h of reperfusion.[11]

Sample Collection Method

After the reperfusion stage, animals were placed in a metabolic cage and their 24h urine was collected and measured using the gravimetric method.[15] In the next stage, blood samples were collected under anesthesia from cardiac and the kidneys were removed. The left kidney was deposited in a container including 10% formalin solution for hematoxylin-eosin (H&E) tissue staining. The right kidney was transferred immediately after removing from the body to a liquid nitrogen tank to be used to measure oxidative stress by measuring malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP).

Biochemical Analysis

The values of BUN and Cr_p were measured using an auto analyzer (biotechnical, Italy). Furthermore, plasma and urine sodium and potassium were analyzed using the photometric method (BWBTech, UK). Urine osmolarity was measured using an osmometer (Osmomat 030, Germany). Parameters of creatinine clearance (C_{Cr}), absolute urinary sodium excretion ($U_{Na}V°$), fractional excretion of sodium (FE_{Na}), absolute urinary potassium excretion ($U_KV°$), and fractional excretion of potassium (FE_K) were calculated using the corresponding formulas.[16]

Measuring Oxidative Stress

In order to measure the level of MDA, the kidney tissue was homogenized in PBS and evaluated using thiobarbituric acid (TBA) test. The protocol for TBA test included adding 200 µL of homogenized solution to a tube containing acetic acid 20%, TBA 0.8%, and sodium dodecyl sulfate (SDS) 8.1%. Then, the solution was transferred to a water bath (DUBNOFF, USA) at 95°C for 60 min. In the next stage, the solution was cooled down, 4ml of n-butanol was added and the mixture was centrifuged at 4000 rpm. The light absorption of the upper layer was measured at 532 nm wave length using a spectrophotometer (Spectrolab 7500 UV, UK). Tetraethoxypropane (TEP) was used as an external standard.[17] MDA values are reported in nmol/gkw.

In order to measure FRAP, a solution was prepared using a mixture of 300 mmol acetate buffer (pH = 3.6), 10 mM of TPTZ solution (Merck, Germany) in chloride acid (40 mmol/L), and ferric chloride (2mmol/L). Then, 1.5 mL of the solution was transferred into a test tube at 37°C and 50 µL of the homogenized tissue solution were added to the tube to initiate the reaction. Absorption changes were measured at 593 nm wavelengths. The standard curve was drawn using $FeSO_4.7\ H_2O$. FRAP values are reported in µmol/gkw.[18]

Histological Study

In order to investigate the tissue damage in the kidney, cortex, external and internal medullas were studied using optical microscope. Grading of tissue damages were done considering enlargement of bowman's space, cell necrosis, vascular congestion, and proteinaceous casts formation in tubules' lumens. Grading was done in such a way that the increase in the bowman's space in rats showing the highest increase compared to the control group was considered as one hundred percent of the injury, and the percentage of dilation in remaining rats was calculated based on it. Grading of cell necrosis, vascular congestion, and casts formation were carried out as a percentage of the total damaged area of the study under the microscope.[19]

Statistical Analysis

Data were analyzed using SPSS software version 18. One-way ANOVA and Duncan post hoc tests were used to evaluate the significance of the data. The precise value of *P* was calculated using LSD test. Analysis of non-parametric data was performed using Kruskal-Wallis multiplicity and Mann-Witney tests. Data were reported as Mean ± SE and values of $P < .05$ were considered as significant.

RESULTS

The results of this study showed that 45 min ischemia and 24h reperfusion significantly increased BUN (68%), Cr_p (73%), $U_{Na}V°$ ($P < .05$), FE_{Na} ($P < .001$), and FE_K ($P < .01$) in the I/R group compared to the control one. In addition, it significantly decreased urine osmolarity ($P < .001$), and creatinine clearance ($P < .05$) compared to the control group. After treatment with AD-MSC, urine osmolarity increased by 35% ($P < .01$). Furthermore, FE_{Na}, Cr_p($P < .001$), and FE_K ($P < .01$) decreased compared to I/R group (Table 1). Also, treatment with AD-MSCs decreased MDA level (66%) significantly ($P < .001$) after its 54% increase by ischemia-reperfusion (Figure 1A). In addition, I/R resulted in a significant reduce ($P < .001$) in value of FRAP in the ischemia-reperfusion group compared

Table 1. Changes in Renal Function Parameters Due to Ischemia-reperfusion in Rats and Effect of Stem Cell Therapy

Parameter	Control	Sham 24h	I/R 24h + PBS	I/R 24h + Stem
Plasma Creatinine, mg/dL	0.52 ± 0.014	0.55 ± 0.01	2.08 ± 0.1***	1.1 ± 0.06 ***†††
BUN, mg/dL	23.6 ± 1.65	21.7 ± 0.92	69 ± 5***	60.3 ± 6.02***
Absolute Urinary Sodium Excretion, mmol/min/kg	2.56 ± 0.49	1.8 ± 0.32	3.5 ± 0.57*	2.57 ± 0.58
Absolute Urinary Potassium Excretion, mmol/min/kg	4.01 ± 0.96	3.1 ± 0..63	3.3 ± 0.59	1.59 ± 0.4
Fractional Urinary Sodium Excretion, mmol/min/kg	1.33 ± 0.3	0.82 ± 0.04	5.36 ± 0.3***	1.56 ± 0.3†††
Fractional Urinary Potassium Excretion, mmol/min/kg	72.19 ± 25.74	38.4 ± 2.4	124.2 ± 12.31**	39.38 ± 9.8††
Urine Osmolarity, mOsm/kg H_2O	1751.7 ± 119.64	1748.4 ± 158.7	866 ± 98.23***	1338.4 ± 130.7*††
Creatinine Clearance, mL/min/kg	1.67 ± 0.38	1.36 ± 0.25	0.4 ± 0.09*	1.12 ± 0.4

*P < .05, **P < .01, ***P < .001, in comparison with control group
†P < .05, ††P < .01, †††P < .001, in comparison with the I/R group

to the control (33%). After 24h of reperfusion, the results showed that the level of FRAP in the AD-MSC treated group was significantly higher than the ischemia-reperfusion group (P < .001) (Figure 1B). The induction of 45 min ischemia and 24h reperfusion resulted in a significant (P < .01) tissue damages in the ischemia-reperfusion group compared to the control. So that a grade of 2.15 was seen in the size of Bowman's space, a damage grade of 1.83 was seen in the proximal tubule, and a damage grade of 1.38 was seen in the thick ascending limb of loop of Henle compared to the control group (Table 2, Figure 2). In addition to the cortex, significant damage of the Pars Recta, thick ascending limb of loop of Henle, vascular congestion and the intratubular proteinaceous casts were seen in the internal and the external medulla of the ischemia-reperfusion group compared to the control one. Vascular congestion is shown in the ischemia-reperfusion group in Figure 2C. The tissue damage in the treated group was significantly lower than the ischemia-reperfusion group (P < .05), so that the total grade of histopathologic damage in the ischemia-reperfusion group was 17.94, which was decreased to 14.23 in the treated group (P < .05).

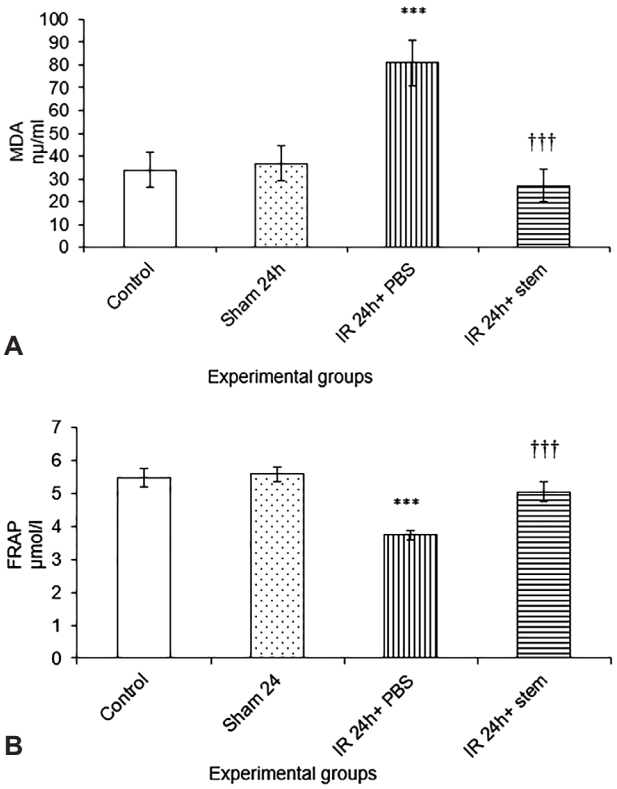

Figure 1. Tissue level of Malondialdehyde (MDA) **(A)** and ferric reducing antioxidant power (FRAP) **(B)** in different groups after 45 min ischemia and 24 hours reperfusion.
***P < .001, in comparison with the control group
†††P < .001, in comparison with the I/R group

DISCUSSION

I/R leads to tubular necrosis, increased oxidative stress and inflammation, and impaired renal function. In the present study, intravenous injection of AD-MSC after 45 min ischemia led to a relative improvement in hemodynamic and functional parameters, as well as oxidative stress and tissue damage during 24h reperfusion period.

Kidney ischemia results in rapid depletion of ATP in kidney tubular cells. Anaerobic glycolysis leads to the accumulation of lactate and acidification of cell cytosol, which eventually leads to further mitochondrial damage and cellular dysfunction.[20] In addition, lack of energy leads to dysfunction of cytoskeleton, tight junctions, reduction in cellular

Table 2. Summary of Kidney Histopathologic Damages Induced by 45 min Ischemia and 24 Hours Reperfusion and the Effect of Adipose-derived Mesenchymal Stem Cells

Histopathologic Damages	Experimental Groups			
	Control	Sham 24h	I/R 24h + PBS	I/R 24h + Stem
Cortex				
Bowman Space Enlargement	0	0.05	2.15	2.82
Proximal Tubal Damage	0	0.03	1.83	2.13
Henle Thick Ascending Limb Damage	0	0	1.38	1.66
External Medulla				
Pars Recta Damage	0	0	2.65	1.9
Henle Thick Ascending Limb Damage	0	0.03	1.56	2.61
Vascular Congestion	0	0.02	2.05	0.89
Tubular Proteinaceous Cast	0	0	1.66	0.65
Internal Medulla				
Vascular Congestion	0	0.01	2.87	0.84
Tubular Proteinaceous Cast	0	0	1.79	0.73
Total Histopathologic Score	0	0.14	17.94***	14.23†***

Grades of histopathologic injuries in rats with no intervention (Control), rats undergone surgery without renal vessels clamping and receiving PBS solution and equivalent 24h reperfusion period (Sham), rats with 45 min of ischemia and 24h of reperfusion receiving PBS (I/R 24h + PBS), and rats with 45 min of ischemia and 24h of reperfusion receiving 2 × 10^6 AD-MSC (I/R 24h + AD-MSC)
*P < .05, **P < .01, ***P < .001, in comparison with the control group
†P < .05, ††P < .01, †††P < .001, in comparison with the I/R group

polarity and brush border and impairment of membrane carriers such as Na^+/K^+-ATPase.[21] Ultimately, epithelial and endothelial cells separate from the basement membrane and obstruct tubular lumens and vessels. Increased permeability of tubules and vessels lead to accumulation of fluids in interstitium, which results in more delayed kidney reperfusion and further damage due to ischemia.[20] After 45 min ischemia and 24h reperfusion, a decrease was seen in C_{Cr}, followed by an increase in the concentrations of BUN and Cr_p in the reperfusion group compared to the control. C_{Cr} is an index of glomerular filtration rate (GFR) which is decreased following I/R and results in an increase in the concentrations of BUN and Cr_p.[3] Renal tissue ischemia promptly induces structural and functional changes in proximal tubule cells. The precise mechanism for reducing GFR after ischemia is not known, however, contractions of afferent arterioles in response to tubuloglomerular feedback, glomerular filtrated fluid leakage, and tubular obstruction are reported as mechanisms for reducing GFR.[22,23] In the present study, AD-MSC injection was able to improve Cr_p, BUN, and C_{Cr} in the treatment group in comparison to the reperfusion group. Sheashaa *et al.* reported that after the injection of 1 × 10^6 of AD-MSC, BUN and Cr_p concentrations are reduced, and GFR is increased 24 h after I/R. Part of the therapeutic mechanism of AD-MSC is probably due to the anti-inflammatory feature of it.[24] It also increases FE_K in AKI.[25] In the present study, increase in FE_K and FE_{Na} was seen in the I/R group compared to the sham group probably due to the damage to proximal tubular cells and impaired Na^+/K^+-ATPase, [26] which was decreased following the injection of AD-MSC in the treatment group compared to the reperfusion one. Steenhard *et al* injected embryonic stem cells to the isolated kidneys of mice and reported the formation of renal structures including epithelial cells, and expression of the Na^+/K^+-ATPase gene in the membrane.[27] Possibly, AD-MSC is also able to stimulate the Na^+/K^+-ATPase gene expression, which leads to improved kidney function. In addition, 45 min of ischemia resulted in decreased $U_KV°$ and urine osmolarity due to decreased GFR[3] and degradation of osmotic agents.[28] Following treatment with AD-MSC, increased urine osmolarity was seen in the treatment group compared to the reperfusion group. Franchi *et al* reported the improving effect of MSC in urine osmolarity following an injection of 2.5 × 10^6 bone marrow-derived mesenchymal stem cells (BM-MSC) to rats with polycystic kidneys (PKD) due to increased vascular density of the renal cortex.[29]

Reperfusion following ischemia due to a sudden increase in blood flow and oxygen pressure leads to vascular changes, which onsets oxidative reactions.[30,31] The process of oxidative stress begins at the early stages of the AKI induced inflammation.

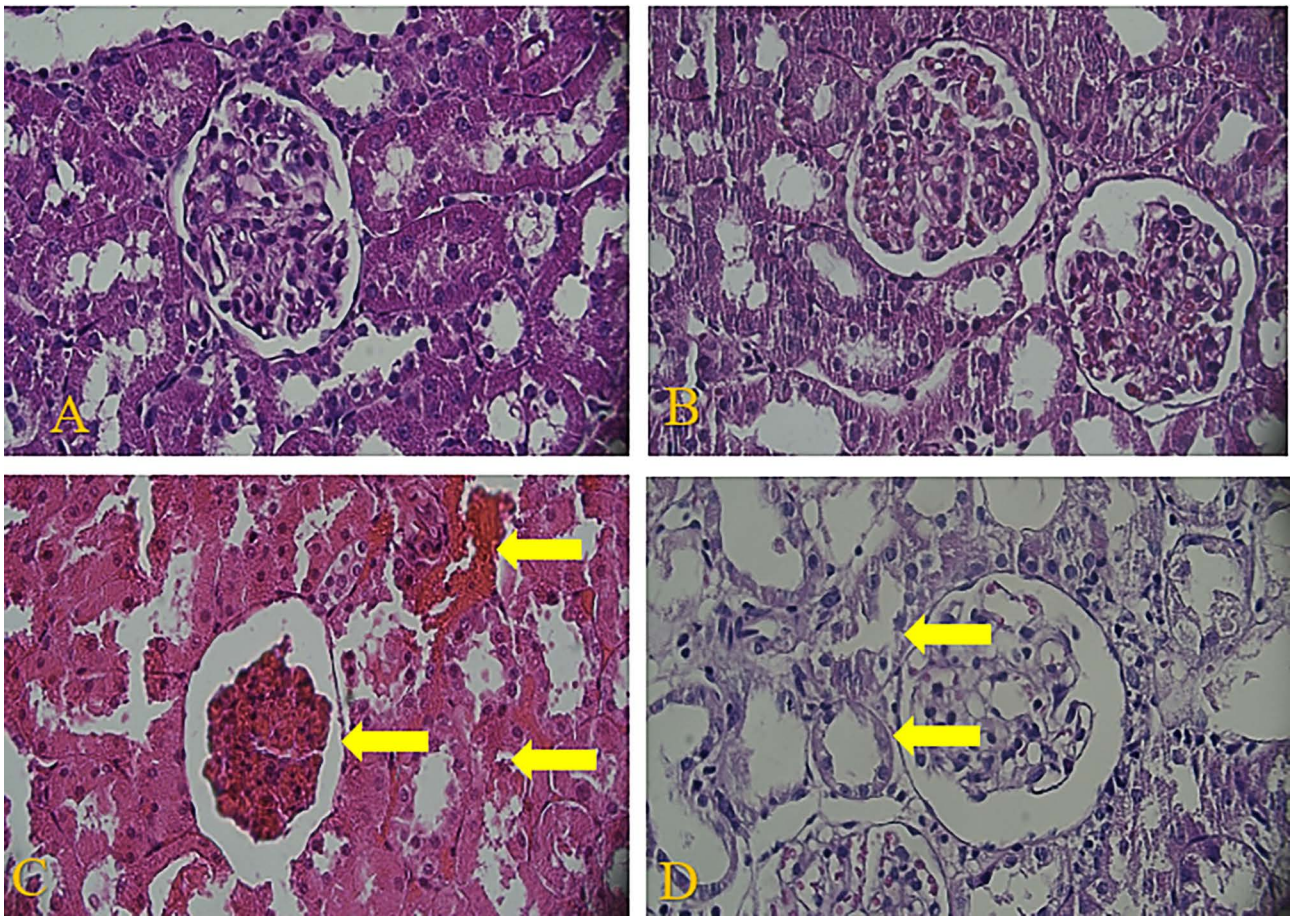

Figure 2. Cross-sectional view of the kidneys showing the size of Bowman's space and necrosis in groups: A, No intervention control; B, Sham operation without vascular obstruction and receiving the PBS; C, 45 min ischemia and 24h reperfusion and receiving PBS; and D, 45 min ischemia and 24h reperfusion and receiving 2× 10^6 AD-MSC. Magnification x 400, hematoxylin-eosin staining.

Superoxide anion, nitric oxide, and hydrogen peroxide are produced during kidney damage that can lead to the production of peroxynitrite, an important oxidant agent in protein oxidation and renal failure.[23,32] Studies have shown that MSC is able to increase the level of Hemoxygenase-1 (HO-1), which is a potent antioxidant molecule. Expression of HO-1 can also regulate the antioxidant response, as well as inflammatory and immune responses.[33] The results of this study showed that after I/R, the MDA level in the reperfusion group increased compared to the control. After administration of AD-MSC, the amount of MDA in the treatment group was reduced compared to the reperfusion group. In animal studies conducted on AKI, the antioxidant effect of MSC was measured by reducing inducible nitric oxide synthase (iNOS), endothelial NO synthase (eNOS) and 8-Hydroxy-2-Deoxy Guanosine (8- OHdG).[34] It has also been reported that MSC increases the activity of superoxide dismutase (SOD) and expression of Glutathione peroxidase (GSH-PX), a potent antioxidant enzyme.[35] Zhang *et al*. showed a decrease in MDA and 8-OHDG using an injection of stem cell-derived micro vesicles (EVs) in a 45-minute ischemic and 24 h reperfusion model in the kidney. They reported the increased expression of HO-1 and activity of Nrf2/antioxidant response element following the injection of MSC-EV.[36] As mentioned above, the anti-oxidant effect of AD-MSC has been reported in a number of studies.[34,35] In this study, the FRAP test was used to evaluate the total antioxidant capacity of the tissue. I/R damage resulted in a decrease in FRAP level. Intravenous administration of AD-MSC in rats increased FRAP in the treatment group compared to the reperfusion one. Therefore, it can be concluded that AD-MSC, with its antioxidant properties, is able to protect the kidneys against the active species of oxygen.

In the present study, I/R resulted in bowman's

space enlargement, proximal and thick ascending limb (TAL) cell injury, as well as damage to the internal and external medullas in the I/R group compared to the control. Treatment with mesenchymal stem cells was able to relatively healing the cellular damages caused by 45 minutes of ischemia and 24h of reperfusion.

MnSOD can protect cells against oxidative stress and help to their survival. Meanwhile, MSCs release IL-6, which prevents the differentiation of dendritic cells.[37] The immune regulation mechanism of the MSC is not well known. It seems that the release of soluble factors such as indoleamine 2,3-dioxygenase (IDO), transforming growth factor beta 1 (TGF-$\beta 1$), interleukin-10 (IL-10) and nitric oxide are involved in this process.[37] Gregorian *et al.* showed that 24h after a mesenchymal stem cell injection, minimal to none amount of MSCs were found in the kidney. Based on these observations, it can be concluded that the MSC treatment mechanism is often paracrine, in which a number of growth factors are expressed, including hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1), which lead to renal repair. MSC injection significantly reduced the expression of Interferon- γ (IFN-γ) and increased the expression of interleukin-10, thereby mediating the protective and suppressive effects of the immune response. In addition, MSC blocks the expression of IL-6, which is a major product of monocyte/macrophage inflammation.[38] HGF has shown to have reno protective and reno trophic effects in animal models of AKI which functions through proliferative, as well as anti-apoptotic and anti-inflammatory effects.[39] In our study, injection of AD-MSC in tail vein reduced the tissue damage in the treatment group compared to the reperfusion group. There is the possibility that AD-MSC may have improved the tissue injuries through the mentioned mechanisms.

CONCLUSIONS

In recent years, therapeutic strategies for the treatment of AKI have been directed toward cell therapy, especially the use of stem cells. The present study examined the efficacy of AD-MSC therapy in the treatment of AKI. The results of this study showed that AD-MSC was able to improve hemodynamic and functional parameters of the kidney, including Cr_p, glomerular filtration rate and urine osmolarity after 45 min ischemia and 24h reperfusion. It also reduced oxidative stress and tissue damage in the cortex and medulla of the kidneys. Therefore, AD-MSC can have a therapeutic effect on I/R-induced renal damage.

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DECLARATIONS OF INTEREST

None.

REFERENCES

1. Rewa O, Bagshaw SM. Acute kidney injury—epidemiology, outcomes and economics. Nature reviews nephrology. 2014; 10: 193.
2. Farrara A. Acute Kidney Injury. The Nursing clinics of North America. 2018; 53: 499-510.
3. Changizi-Ashtiyani S, Najafi H, Jalalvandi S, et al. Protective effects of Rosa canina L fruit extracts on renal disturbances induced by reperfusion injury in rats. Iranian journal of kidney diseases. 2013;7:290.
4. Havakhah S, Sankian M, Kazemzadeh GH, et al. In vivo effects of allogeneic mesenchymal stem cells in a rat model of acute ischemic kidney injury. Iranian journal of basic medical sciences. 2018;21:824-31.
5. De Vries DK, Schaapherder AF, Reinders ME. Mesenchymal stromal cells in renal ischemia/reperfusion injury. Frontiers in immunology. 2012;3:162.
6. Miller BL, Garg P, Bronstein B, et al. Extracorporeal Stromal Cell Therapy for Subjects With Dialysis-Dependent. Acute Kidney Injury. 2018;3:1119-1127.
7. Mollazadeh S, Neshati V, Fazly Bazzaz BS, et al. Standardized Sophora pachycarpa Root Extract Enhances Osteogenic Differentiation in Adipose-derived Human Mesenchymal Stem Cells. Phytotherapy Research. 2017;31:792-800.
8. Zhao Y, Jiang Z, Zhao T, et al. Reversal of type 1 diabetes via islet β cell regeneration following immune modulation by cord blood-derived multipotent stem cells. BMC medicine. 2012;10:3.
9. Murphy JM, Fink DJ, Hunziker EB, et al. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum. 2003; 48:3464-74
10. Mathiasen AB, Jørgensen E, Qayyum AA, et al. Rationale and design of the first randomized, double-blind, placebo-controlled trial of intramyocardial injection of autologous bone-marrow derived Mesenchymal Stromal Cells in chronic ischemic Heart Failure (MSC-HF Trial). American heart journal. 2012;164:285-91.
11. Cura-Esquivel I, Delgado-Chávez E, García-Narro J, et al. Attenuation of pro-inflammatory cytokines and oxidative stress by misoprostol in renal ischemia/reperfusion in rats. Pharmazie. 2018;73:537-540.

12. Togel F, Hu Z, Weiss K, et al. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. American Journal of Physiology-Renal Physiology. 2005;289:F31-F42.
13. Moosavi SMS, Ashtiyani SC, Hosseinkhani S. L-carnitine improves oxidative stress and suppressed energy metabolism but not renal dysfunction following release of acute unilateral ureteral obstruction in rat. Neurourol Urodyn. 2011;30:480-7.
14. Hoseini SJ, Ghazavi H, Forouzanfar F, et al. Fibroblast growth factor 1-transfected adipose-derived mesenchymal stem cells promote angiogenic proliferation. DNA Cell Biol. 2017;36:401-412.
15. Naccarato W, Treuting J, Cannon D. Gravimetric determination of urine volumes. The American journal of medical technology. 1981;47:111-2.
16. Ashour RH, Saad M-A, Sobh M-A, et al. Comparative study of allogenic and xenogeneic mesenchymal stem cells on cisplatin-induced acute kidney injury in Sprague-Dawley rats. Stem cell research & therapy. 2016;7:126.
17. Changizi-Ashtiyani S, Najafi H, Kabirinia K, etal. Oral omega-3 administration cause to reduction of renal dysfunction induced by ischemia/reperfusion in rats. Iran J Kidney Dis. 2012;6:275-83.
18. Changizi-Ashtiyani C, Najafi H, Firouzifar MR, et al. Grape seed extract for reduction of renal disturbances following reperfusion in rats. Iranian journal of kidney diseases. 2013;7:28-35.
19. Changizi-Ashtiyani S, Alizadeh M, Najafi H, et al. Physalis alkekengi and Alhagi maurorum ameliorate the side effect of cisplatin-induced nephrotoxicity. Cancer Gene Ther. 2016;23:235-40.
20. Erpicum P, Detry O, Weekers L, et al. Mesenchymal stromal cell therapy in conditions of renal ischaemia/reperfusion. Nephrology Dialysis Transplantation. 2014;29:1487-93.
21. Andreoli SP. Acute kidney injury in children. Pediatric nephrology. 2009;24:253.
22. Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. Comprehensive Physiology. 2012;2:1303.
23. Moosavi SM, Changizi-Ashtiyani S, Hosseinkhani S, et al. Comparison of the effects of L: -carnitine and alpha-tocopherol on acute ureteral obstruction-induced renal oxidative imbalance and altered energy metabolism in rats. Urol Res. 2010;38:187-94.
24. Sheashaa H, Lotfy A, Elhusseini F, et al. Protective effect of adipose-derived mesenchymal stem cells against acute kidney injury induced by ischemia-reperfusion in Sprague-Dawley rats. Experimental and therapeutic medicine. 2016;11:1573-80.
25. Lehnhardt A, Kemper MJ. Pathogenesis, diagnosis and management of hyperkalemia. Pediatric nephrology. 2011;26:377-84.
26. Seguro AC, Shimizu MHM, Monteiro JL, et al. Effect of potassium depletion on ischemic renal failure. Nephron. 1989;51:350-4.
27. Steenhard BM, Isom KS, Cazcarro P, et al. Integration of embryonic stem cells in metanephric kidney organ culture. J Am Soc Nephrol. 2005;16:1623-31.
28. Najafi H, Firouzifar MR, Shafaat O, et al. Protective effects of Tribulus terrestris L extract against acute kidney injury induced by reperfusion injury in rats. Iranian journal of kidney diseases. 2014;8:292.
29. Franchi F, Peterson KM, Xu R, et al. Mesenchymal stromal cells improve renovascular function in polycystic kidney disease. Cell transplantation. 2015;24:1687-98.
30. Kosieradzki M, Rowiński W. Ischemia/reperfusion injury in kidney transplantation: mechanisms and prevention. Transplant Proc. 2008;40:3279-88.
31. Rowart P, Erpicum P, Detry O, et al. Mesenchymal stromal cell therapy in ischemia/reperfusion injury. J Immunol Res. 2015;2015:602597
32. de Almeida DC, Donizetti-Oliveira C, Barbosa-Costa P, et al. In search of mechanisms associated with mesenchymal stem cell-based therapies for acute kidney injury. Clin Biochem Rev. 2013;34:131-44.
33. Vanella L, Sanford C, Kim DH, et al. Oxidative stress and heme oxygenase-1 regulated human mesenchymal stem cells differentiation. Int J Hypertens. 2012;2012:890671.
34. Liu H, McTaggart SJ, Johnson DW, et al. Original article anti-oxidant pathways are stimulated by mesenchymal stromal cells in renal repair after ischemic injury. Cytotherapy. 2012;14:162-72.
35. Zhuo W, Liao L, Xu T, et al. Mesenchymal stem cells ameliorate ischemia-reperfusion-induced renal dysfunction by improving the antioxidant/oxidant balance in the ischemic kidney. Urologia internationalis. 2011;86:191-6.
36. Zhang G, Zou X, Huang Y, et al. Mesenchymal stromal cell-derived extracellular vesicles protect against acute kidney injury through anti-oxidation by enhancing Nrf2/ARE activation in rats. Kidney and Blood Pressure Research. 2016;41:119-28.
37. Lombardo E, DelaRosa O, Mancheno-Corvo P, et al. Toll-like receptor–mediated signaling in human adipose-derived stem cells: implications for immunogenicity and immunosuppressive potential. Tissue Engineering Part A. 2008;15:1579-89.
38. Gregorini M, Bosio F, Rocca C, et al. Mesenchymal stromal cells reset the scatter factor system and cytokine network in experimental kidney transplantation. BMC immunology. 2014;15:44.
39. Mizuno S, Nakamura T. Prevention of neutrophil extravasation by hepatocyte growth factor leads to attenuations of tubular apoptosis and renal dysfunction in mouse ischemic kidneys. The American journal of pathology. 2005;166:1895-905.

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