Reconstructive Urology

Protective Effect of Wharton’s Jelly-derived Mesenchymal Stem Cells on Renal Fibrosis in Rats with Unilateral Ureteral Obstruction

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Abstract

Background: Renal failure is a global medical problem. The use of mesenchymal stem cells (MSCs) for preservation and regeneration of renal tissue in acute and chronic kidney diseases has recently been the focus of investigation.

Objective: To evaluate the protective effect of MSC injections in a rat model of kidney obstruction.

Design, setting, and participants: We assigned 15 male Wistar rats to three separate groups: the normal group underwent left nephrectomy; the control group underwent laparotomy and left ureter ligation followed by saline injection into the aorta; and the study group received MSCs injected into the aorta inferior to the left renal artery after ligation of the left ureter. Kidneys were harvested 4 wk later and renal parenchyma samples were used for trichrome staining and for expression analyses.

Outcome measurements and statistical analysis: The degree of kidney fibrosis was assessed on pathology. Real-time polymerase chain reaction was used to determine expression levels of VEGF, TNF-α, and E-cadherin, and ΔCT and ΔΔCT values were calculated. Data were analyzed using SPSS v19 with paired t tests and nonparametric independent-sample Kruskal-Wallis tests.

Results and limitations: Fibrosis in the study group decreased from grade 3 or 4 to grade 1. In the control group, TNF-α expression increased and E-cadherin expression decreased. After MSC injection into obstructed kidneys, TNF-α and E-cadherin expression levels decreased and increased respectively, reaching similar levels to those in the normal group. No correlation between tissue regeneration and VEGF levels was observed. More research is needed to focus on other angiogenic factors.

Conclusions: MSC injection could prevent fibrosis in obstructed rat kidney via alterations in TNF-α and E-cadherin expression.

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1. Introduction

Renal failure is a common health problem worldwide [1] and is categorized as acute renal failure (ARF) or chronic renal failure (CRF). ARF accounts for approximately 19.2 out of every 1000 hospitalizations and has high morbidity and mortality. Its prevalence among patients admitted to intensive care units after vessel and abdominal surgery is nearly 20% [2]. In 2017, 1.2 million individuals died due to chronic kidney disease (CKD), contributing disability-adjusted life-years (DALYs) of 35.8 million [3]. In Iran, the most common cause of ARF is glomerulonephritis, followed by diabetes mellitus and hypertension [4]. The most common causes of CRF globally are diabetes mellitus, hypertension, glomerulonephritis, and urologic diseases [5]. Common treatments for CRF include peritoneal dialysis, hemodialysis, and kidney transplantation, each of which has its own side effects and problems [6]. Therefore, recent efforts have focused on new treatment methods that could lead to return of kidney function, decrease morbidity and mortality, and even reduce costs [7]. In the present study we investigated the role of the inflammatory cytokines TNF-α and VEGF and the adhesion molecule E-cadherin in renal fibrosis caused by ureteral obstruction, which leads to renal failure. We also evaluated the effects of mesenchymal stem cells (MSCs) from umbilical-cord Wharton’s jelly in preventing ureteral obstruction–induced renal fibrosis and the expression of inflammatory cytokines.

2. Materials and methods

The study protocol was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences. Anesthesia was in accordance with the institutional animal care protocol (ketamine 75 mg/kg and xylazine 15 mg/kg injected intraperitoneally).

2.1. Animals

Fifteen male Wistar rats, weighing between 250 and 300 g, were randomly divided into three groups (n = 5 per group). Male rats were used to prevent any potential effects of hormonal fluctuation on the results.

2.2. Procedure

The study was carried out from September 2015 to March 2018. Rats in the normal group underwent left nephrectomy and a portion of the renal parenchyma was fixed in formalin for pathology examination. Another specimen from the kidney was kept at −80 °C for expression analyses. Rats in the control group underwent midline laparotomy and the left ureter was identified in the retroperitoneal space and was closed with 4–0 silk sutures. Simultaneously, normal saline was injected into the aorta inferior to the renal artery while pressing the distal part of aorta with a finger and the abdomen was closed with 3–0 Vicryl sutures. Rats in the study group underwent ligation of the left ureter as for the control group, except 10³ MSCs were injected into the aorta inferior to the renal artery instead of saline. After recovery from anesthesia, rats in the control and study groups were sent to the animal center. After 4 wk (approx. 25–30 d), the animals were sacrificed and their left kidneys were removed. Separate kidney samples were fixed in formalin for pathology evaluation and frozen at −80 °C for expression analyses.

2.3. MSC extraction from umbilical cord

Umbilical cords from neonates delivered in hospitals affiliated to Jundishapur University were separated under completely sterile conditions and sent to the Cellular and Molecular Survey Center. The neonates were born to primiparous women who were seronegative for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus, with no underlying disease, without any past operations, and with no previous history of transfusion of blood and/or blood products. MSCs were separated via an enzymatic method by slicing and using collagenase and hyaluronidase enzymes. During culture in growth medium containing nutrients, stem cells started to proliferate and fully expand over the culture medium. The cells were separated and transferred to a second culture (passage). We used passage 2 cells because research has shown that passage 2 cells do not present HLA-2 and do not have issues caused by immunogenicity. To confirm that the cultured cells were indeed pluripotent stem cells, we directed them towards differentiation to specialized cells (bone, cartilage, and muscle) and demonstrated the differentiation using tissue-specific staining [8–12].

2.4. Pathology

Formalin-fixed samples were sent to the pathology laboratory and were stained with trichrome. One pathologist evaluated the slides to determine the degree of fibrosis.

2.5. Gene expression analyses

A parenchymal sample from each kidney was powdered using liquid nitrogen under sterile and RNase-free conditions. RNA was extracted using an RNase Plus Mini kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer’s instruction. cDNA synthesis was performed with First-strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania) was used. GAPDH primers were used for the positive control and for conducting real-time quantitative polymerase chain reaction PCR (qPCR).

Real-time PCR was carried out to amplify DNA using primers for GAPDH, VEGF, TNF-α, and E-cadherin with a SYBR Green PCR Master Mix.

Patient summary: We investigated the effect of stem cell injection in rats with kidney obstruction. The treatment led to changes in the levels of two biomarkers and reduced the amount of kidney fibrosis caused by kidney obstruction.

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kit. Amplifications were carried out in triplicate to ensure reliability, with an extra tube as a negative control.

2.6. Data analysis

Pathology slides were reviewed by a pathologist. Results obtained from qPCR, namely the cycle threshold (CT), melt curve, and amplification curves, were analyzed with SPSS v19 using paired t tests and nonparametric independent-sample Kruskal-Wallis tests.

3. Results

3.1. Macroscopic results

Figure 1 shows the macroscopic kidney appearance for the three groups. Gross morphologic differences between the groups are markedly visible.

3.2. Microscopy results

Pathology slides were evaluated and scored on a scale from 0 to 4 according to the degree of fibrosis, where 0 represents no fibrosis and 4 denotes very extensive fibrosis. As expected, no fibrosis was observed for the normal group, whereas the degree of fibrosis was scored as 3 or 4 in the control group and 1 in the study group (Fig. 2).

3.3. qPCR results

ΔCT values for VEGF, TNF-α, and E-cadherin were calculated with the 95% confidence interval using the formula 

\[ \Delta \text{CT} = \text{CT}_X - \text{CT}_{\text{GAPDH}} \]

where X denotes VEGF, TNF-α, or E-cadherin. We used the Pfaffl formula to calculate \( 2^{-\Delta\text{ACT}} \) for between-group comparisons. The closer the result is to 1, the smaller is the difference between the two groups. For TNF-α, \( 2^{-\Delta\text{ACT}} \) was 1.93 between the control and normal groups, and 0.62 between the study and normal groups. For E-cadherin, \( 2^{-\Delta\text{ACT}} \) was 0.433 between the control and normal groups, and 0.863 between the study and normal groups. For VEGF, \( 2^{-\Delta\text{ACT}} \) was 1.071 between the control and normal groups and 0.909 between the study and normal groups. The \( 2^{-\Delta\text{ACT}} \) values for the group comparisons are shown in Figure 3.

4. Discussion

Urinary obstruction is one of the most common causes of renal failure and its treatment requires timely diagnosis and removal of the obstruction [4]. MSC therapy is a new and promising strategy in treating renal failure, whether acute or chronic. One of the reasons for the effect of MSCs is the mesenchymal origin of nephrons and tubules. MSCs can be isolated from different organs provided they have low immunogenicity. In the present study, we used MSCs obtained from Wharton’s jelly of umbilical cord which has low immunologic effect [13,14]. One of the qualities of mesenchymal stem cells is their ability of gathering in damaged and swollen tissues. Intravascular injection means that MSCs can migrate to glomerules, tubules, periglomerular vessels, and interstitial tissues, whether in acute or chronic conditions [10]. This can also be achieved via intravenous injection, as demonstrated by Huang et al. [15]. It is noteworthy that this migration is mediated by inflammatory cytokines [16].
Studies have shown that stress resulting from urinary obstruction causes a number of changes in inflammatory mediators in kidney. TNF-α is a cytokine that has a role in mediating renal damage; it increases other inflammatory cytokines and extracellular matrix and causes fibrosis [7,17]. VEGF plays a role in matrix remodeling, chemotaxis of monocytes, expression of adhesive molecules, and proliferation of peritubular capillaries, which are necessary for tubular regeneration. Other inflammatory cells that play a role in inflammatory nephropathy include HGF-1, IGF-1, and FGF. E-Cadherin, which is a marker for normal epithelium, decreases in obstructed kidneys [4]. Microscopy revealed that after 48 hr of obstruction, the ureter and pelvis become looser, the renal papilla starts to blunt, and the parenchyma starts to thin. After 12 d, the cortex shallows slightly and pelviccalyceal dilatation continues. After 21–28 d, the kidney returns to its original size but the cortex and medulla become thinner. Finally, 6 wk after ureteral obstruction, the kidney becomes larger and cystic and has a lower weight than the contralateral kidney; nevertheless, it is of note that when the obstruction is incomplete these changes are not as apparent. Interstitial edema develops 48 h after obstruction, and after 7 d severe edema is apparent, with widening of the Bowman space and cellular hyalinization. After 12 d, papillary necrosis, focal tubular destruction, and inflammatory cellular responses occur, with interstitial fibrosis after 16 d. Massive glomerular destruction, tubular atrophy, interstitial fibrosis, and connective tissue proliferation in the collecting system are evident in humans after 5 wk [18].

As shown in Figure 1, renal parenchyma in the control group became thinner, the collecting system widened, and the parenchyma became pale; it can be said that the kidney has essentially become hydronephrotic. Parenchyma in the study group was slightly paler and the collecting system was slightly looser than in the normal group. In comparison to the control group, renal parenchyma in the study group was preserved and there was much less dilatation of the collecting system, demonstrating the protective effect of the MSCs.

There was no kidney fibrosis in the normal group, whereas a fibrosis score of 3 to 4 was assigned for interstitial tissues in obstructed kidneys. This confirms that ureteral obstruction causes deposition of collagen in renal tissue and leads to fibrosis of interstitial tissue. Between-group comparisons revealed that injection of MSCs led to a decrease in the degree of fibrosis in obstructed kidneys, resulting in a score of 1. However, it should be noted that despite this reduction, there was still a certain degree of fibrosis in comparison to the normal group (Fig. 2A–C).

To investigate the effect of ureteral obstruction on renal fibrosis, cytokines, and inflammatory mediators, and whether MSC injection could offer protection against these changes, we measured expression levels of VEGF, TNFα, and E-cadherin in kidney via qPCR. We evaluated the significance of differences between the three groups using CT and $2^{-\Delta CT}$ values, applying Kruskal-Wallis and t tests.

Between-group comparisons of E-cadherin levels using the $2^{-\Delta CT}$ method revealed a value of 0.433 for the control versus the normal group, with a significant difference in expression ($p = 0.001$). The value for the study versus the normal group was 0.863, with no significant difference in expression ($p = 0.191$). The E-cadherin levels significantly differed between the study and control groups ($p = 0.000$). We can conclude that in a kidney with ureteral obstruction, E-cadherin, which reflects the connection between cells of the interstitial tissue, decreases, but MSC injection leads to an increase in E-cadherin expression, reaching the level observed in normal kidney parenchyma.

Docherty et al. [19] showed that although it had been reported that E-cadherin decreased in kidneys with obstructive damage, they found that E-cadherin mRNA increased in a kidney with a ligated ureter in both acute and chronic phases, which puts into question the role of epithelial growth factor. Comparison of our results with the findings reported by Docherty et al. highlights a challenge in elucidating the role of E-cadherin in the pathogenesis of obstructive renal failure.

Between-group comparison of VEGF levels using the $2^{-\Delta CT}$ method revealed significant differences for study
versus normal (p = 0.001) and study versus control (-p = 0.000) groups, but not for the normal group versus the control group (p = 0.256). This indicates a lack of meaningful difference in VEGF expression between obstructed and normal kidneys, but that MSC injection in the study group decreased the level of VEGF in comparison to the other two groups. As VEGF has a key role as an angiogenic factor, this finding requires further investigation. On the basis of our results, we are unable to conclude whether VEGF has any role in kidney remodeling after obstruction and why its level in obstructed kidney is similar to that in normal kidney. It is also unclear why MSC injection induced a decrease in VEGF expression rather than a decrease; this shows that we have yet to fully understand the role of VEGF in kidney obstruction and remodeling.

Comparison of TNF-α levels using the 2^-ΔΔCT method revealed a value of 1.93 for the control group versus the normal group (p = 0.022), indicating a significant difference in TNF-α levels between these groups and higher TNF-α expression in the control group. The value of 0.62 for the study group versus the normal group (p = 0.142) demonstrates that TNF-α levels did not significantly differ between these groups. The difference in TNF-α between the study and control groups was significant (p = 0.001). We can conclude that the increase in TNF-α expression in kidney due to an obstructed ureter can be reversed by MSC injection to reach a level similar to that in normal kidney parenchyma. Between-group t-test results for comparisons for E-cadherin, TNF-α, and VEGF are shown in Table 1.

Using stem cells extracted from human femur and tibia, Qian et al. [8] observed tissue regeneration after renal failure induced by nephrotoxins. The authors reported pathology results similar to our findings, but they used CK18 and AQP1 as epithelial proliferation markers to investigate tissue regeneration rather than TNF-α, E-cadherin, and VEGF.

Verdoorn et al. [6] measured bioactive pattern of lipids to evaluate tissue regeneration in rats whose kidneys were damaged via unilateral ureteral obstruction, but they did not investigate TNF-α, VEGF, and E-cadherin levels. Nevertheless, the lipid patterns demonstrated the effect of stem cells in tissue regeneration after ureteral obstruction [6].

Two separate studies investigated injection of MSCs in rats with CKD, which is similar to Allport’s syndrome in humans [9, 20]. Both groups came to the conclusion that MSCs prevent progression of interstitial fibrosis and glomerular sclerosis, but their role in recovery of kidney function in rats was not confirmed. Results from the present study are congruent with these findings and show the role of stem cells in tissue regeneration. However, these studies investigated CKD resulting from glomerular disease and did not consider obstructed nephropathy as one of the causes of CKD [9, 20].

Semedo et al. [21] investigated the role of stem cells in improving kidney function following acute kidney injury (AKI) and CKD by evaluating TNF-α, α-SMA, FSP-1, TGF-β, and IL-6. Similar to our study, the authors found that TNF-α has an important role in fibrotic renal failure and that stem cells help in retaining renal function by decreasing TNF-α. However, Semedo et al. investigated non-obstructed AKI and CKD, while we examined AKI due to obstructive uropathy. Liu et al. [22] also found that MSCs can reduce α-SMA, TGF-β, and TNF-α, in addition to their ability to decrease malondialdehyde, reactive oxygen species, and glutathione rises induced by unilateral ureteral obstruction. The same group found that human MSC injection reduced not only inflammatory factors but also inflammatory cell infiltration and extracellular matrix deposition [23].

Our study showed that MSCs can have a protective effect on the kidney if they are injected at the beginning of acute renal obstruction. Their role in chronic ureteral obstruction may be different, so further research is required to determine whether this is the case. Considering the lack of satisfactory response of VEGF expression to MSC injection, investigation of the response of other angiogenic factors would be beneficial.

### Table 1 – Between-group t-test results for comparisons for E-cadherin, TNF-α, and VEGF

| E-Cadherin | Mean   | Number | Standard deviation | p value |
|------------|--------|--------|--------------------|---------|
| Normal     | 2.310  | 5      | 0.30402            | 0.191   |
| Test       | 2.542  | 5      |                    |         |
| Normal     | 2.310  | 5      | 0.27115            | 0.001   |
| Control    | 3.522  | 5      |                    |         |
| Test       | 2.524  | 5      | 0.18913            | 0.000   |
| Control    | 3.522  | 5      |                    |         |
| TNF-α      | 1.456  | 5      | 0.84097            | 0.142   |
| Normal     | 2.144  | 5      |                    |         |
| Test       | 1.456  | 5      | 0.58491            | 0.022   |
| Control    | 0.506  | 5      |                    |         |
| Test       | 2.144  | 5      | 0.42020            | 0.001   |
| Control    | 0.506  | 5      |                    |         |
| VEGF       | 2.196  | 5      | 0.10770            | 0.001   |
| Normal     | 2.636  | 5      |                    |         |
| Test       | 2.196  | 5      | 0.16882            | 0.256   |
| Control    | 2.096  | 5      |                    |         |
| Test       | 2.096  | 5      | 0.1158             | 0.000   |

### 5. Conclusions

MSC injection could prevent fibrosis in rats with unilateral ureteral obstruction, in line with the changes in TNF-α and E-cadherin expression observed. No effect on VEGF was observed with regard to tissue repair in renal obstruction.

**Author contributions:** Aryan Kavosh had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** A. Kheradmand.

**Acquisition of data:** A. Kheradmand.

**Analysis and interpretation of data:** P. Kheradmand, Valizadeh.

**Drafting of the manuscript:** Kavosh.

**Critical revision of the manuscript for important intellectual content:** P. Kheradmand.

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References
[1] Hill NR, Fatoba ST, Oke JL, et al. Global prevalence of chronic kidney disease - a systematic review and meta-analysis. PLoS One 2016;11: e0158765.
[2] Liangos O, Wald R, O’Bell JW, Price I, Pereira BJ, Jaber BL. Epidemiology and outcomes of acute renal failure in hospitalized patients: a national survey. Clin J Am Soc Nephrol 2006;1:43–51.
[3] GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease study 2017. Lancet 2020;395:709–33.
[4] Simforooosh N, Nouralizadeh A. Iranian textbook of urology. 1st ed. Tehran: Behineh Publishing Press; 2007. p. 325–33, [in Persian].
[5] Marley J. Campbell–Walsh urology, 5th edition (E-dition) - edited by AJ Wein, LR Kavoussi, AC Novick, AW Partin and CA Peters. Int J Urol Neds 2007;1:94–5.
[6] Verdoorn KS, Lindoso RS, Lowe J, Lara LS, Vieyra A, Einicker-Lamas M. Bone marrow mononuclear cells shift bioactive lipid pattern in injured kidney towards tissue repair in rats with unilateral ureteral obstruction. Nephrol Dial Transplant 2010;25:3867–74.
[7] Brodie J, Humes H. Stem cell approaches for the treatment of renal failure. Pharmacol Rev 2005;57:293–313.
[8] Qian H, Yang H, Xu W, et al. Bone marrow mesenchymal stem cells ameliorate rat acute renal failure by differentiation into renal tubular epithelial-like cells. Int J Mol Med 2008;22:325–32.
[9] Ninichuk V, Gross O, Segerer S, et al. Multipotent mesenchymal stem cells reduce interstitial fibrosis but do not delay progression of chronic kidney disease in collagen4A3-deficient mice. Kidney Int 2006;70:121–9.
[10] Tsai RY, Kittappa R, McKay RD. Plasticity, niches, and the use of stem cells. Dev Cell 2002;2:707–12.
[11] Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–9.
[12] Xiang Y, Zheng Q, Jia B, et al. Ex vivo expansion, adipogenesis and neurogenesis of cryopreserved human bone marrow mesenchymal stem cells. Cell Biol Int 2007;31:444–50.
[13] Brooke G, Cook M, Blair C, et al. Therapeutic applications of mesenchymal stromal cells. Semin Cell Dev Biol 2007;18:846–58.
[14] Le Blanc K. Immunomodulatory effects of fetal and adult mesenchymal stem cells. Cytotherapy 2003;5:485–9.
[15] Huang D, Yi Z, He X, Mo S, Dang X, Wu X. Distribution of infused umbilical cord mesenchymal stem cells in a rat model of renal interstitial fibrosis. Renal Fail 2013;35:1146–50.
[16] Fox JM, Chamberlain G, Ashton BA, Middleton J. Recent advances into the understanding of mesenchymal stem cell trafficking. Br J Haematol 2007;137:491–502.
[17] Meldrum KK, Misseri R, Metcalfe P, Dinarello CA, Hile KL, Meldrum DR. TNF-alpha neutralization ameliorates obstruction-induced renal fibrosis and dysfunction. Am J Physiol Regul Integr Comp Physiol 2007;292:R1456–64.
[18] Chevalier RL, Goyal S, Wolstenholme JT, Thornhill BA. Obstructive nephropathy in the neonatal rat is attenuated by epidermal growth factor. Kidney Int 1998;54:38–47.
[19] Docherty NG, Calvo IF, Quinlan MR, et al. Increased E-cadherin expression in the ligated kidney following unilateral ureteric obstruction. Kidney Int 2009;75:205–13.
[20] Sedrakyan S, Da Sacco S, Milanesi A, et al. Injection of amniotic fluid stem cells delays progression of renal fibrosis. J Am Soc Nephrol 2012;23:661–73.
[21] Semedo P, Correa-Costa M, Antonio Cenedez M, et al. Mesenchymal stem cells attenuate renal fibrosis through immune modulation and remodeling properties in a rat remnant kidney model. Stem Cells 2009;27:3063–73.
[22] Liu B, Ding FX, Liu Y, et al. Human umbilical cord-derived mesenchymal stem cells conditioned medium attenuate interstitial fibrosis and stimulate the repair of tubular epithelial cells in an irreversible model of unilateral ureteral obstruction. Nephrology 2018;23:728–36.
[23] Liu B, Ding F, Hu D, et al. Human umbilical cord mesenchymal stem cell conditioned medium attenuates renal fibrosis by reducing inflammation and epithelial-to-mesenchymal transition via the TLR4/NF-κB signaling pathway in vivo and in vitro. Stem Cell Res Ther 2018;9:7.