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Investigation of the effects of fetal rat kidney-derived mesenchymal stem cells implementation on doxorubicin-induced nephropathy in male Sprague–Dawley rats

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Abstract: The potential protective effects of mesenchymal stem cells (MSCs) on some kidney diseases have been reported. However, the effect of the fetal kidney–derived (FKD)MSCs on doxorubicin-induced nephropathy has not been studied yet. This study aimed to treat rats with doxorubicin-induced kidney injuries by transplantation of –FKD-MSCs. Twenty-four Sprague-Dawley rats were divided into three groups as control, doxorubicin nephropathy (Sham), and doxorubicin + MSC treated group. Serum biochemistry analysis was performed at the beginning and the end of the study. Functional changes in kidneys were evaluated by scintigraphy. In the doxorubicin nephropathy group, histopathological findings such as mesangial cell proliferation, tubular cast, and glomerular hypertrophy were observed, whereas in the MSC group these findings were significantly reduced. CD133 and CD24 positive immunoreactions were the most severe and frequently observed in the MSC group. While positive staining was detected in the tubular epithelium, there was no immunostaining observed in the glomerulus. The results showed that both functional and histological improvements were achieved in the MSC group compared to the Sham group. In conclusion, transplantation of fetal kidney - derived MSCs into patients with renal damage is thought to contribute to the healing of the renal tissue.
Keywords: Doxorubicin, mesenchymal stem cell, nephrotoxicity, rat.

Erkek Sprague-Dawley ratlarda doksorubisin nefropatisinde fetal rat böbreği kökenli mezenkimal kök hücre uygulamasının etkilerinin araştırılması

Özet: Mezenkimal kök hücrelerin (MKH) bazı böbrek hastalıklarındaki potansiyel koruyucu etkileri bildirilmiştir. Bununla birlikte, fetal böbrek kaynaklı (FBK) MKH'ların doksorubisin ile indüklenmiş nefropati üzerindeki etkisi henüz araştırılmamıştır. Bu çalışmanın amacı, doksorubisin kaynaklı böbrek hasarı olan ratlara FBK-MKH'ların transplantasyonu yapılarak hasarın tedavi edilmesidir. Çalışmada yirmi dört adet Sprague–Dawley ırkı rat üç gruba ayrılmıştır. Bunlar: kontrol grubu, doksorubisin nefropatisi (Sham) grubu ve doksorubisin + MKH ile tedavi edilen gruptur. Çalışmanın başında ve sonunda serum biyokimya analizleri gerçekleştirilmiştir. Böbreklerdeki fonksiyonel değişiklikler sintigrafi ile değerlendirilmiştir. Doksorubisin nefropatisi grubunda mezanjiyal hücre proliferasyonu, tübül içi kast birikimi ve glomerüler hipertrofi gibi histopatolojik bulgular gözlenirken, MKH grubunda bu bulgular anlamlı olarak azalmıştır. CD133 ve CD24 pozitif immünreaksiyonlar, en şiddetli ve en sık olarak MKH grubunda gözlenmiştir. Tübüler epitelde pozitif boyanma tespit edilirken glomerulusta immün boyanma gözlenmemiştir. Sonuçlar, Sham grubuna kıyasla MKH grubunda hem fonksiyonel hem de histolojik iyileşmelerin sağlandığını göstermiştir. Sonuç olarak, böbrek hasarı olan hastalara fetal böbrek kaynaklı MKH transplantasyonunun böbrek dokusunun iyileşmesine katkıda bulunduğu düşünülmektedir.
Anahtar sözcükler: Doksorubisin, mezenkimal kök hücre, nefrotoksisite, rat.

Introduction

Chronic renal failure (CRF) can be defined as a chronic and progressive deterioration of metabolic-endocrine functions and adjustment of the fluid-solute balance of the kidney as a result of a decrease in glomerular filtration value. CRF is characterized by the development of tubulointerstitial inflammation and fibrosis in glomerulosclerosis (21).

Doxorubicin (Adriamycin) is an anthracycline and an anti-tumor class drug that has been used for the treatment of several types of cancer (uterine, ovarian, breast, lung) in humans (1, 9, 24) and in animals (4, 12, 16). It induces nephrotoxicity in the cancer patients, thus its clinical applications are limited (24). Doxorubicin is a well-known inducer of kidney injury in rodents and mimics CRF in humans with primary focal segmental glomerulosclerosis. It is mainly metabolized in the liver. It accumulates mainly in the kidney but is also found in the liver, heart, and small intestine (10).

The kidney is defined as an organ with minimal cellular recovery and low regeneration capacity (21). Cellular or regenerative therapies targeting progenitors in the damaged kidney have emerged as an innovative strategy. In stem cell-based therapies, renal tropism, and regenerative capacity can be increased by using the unique characteristics of stem cells and contributing to kidney healing (1, 7, 23).

This study aimed to treat doxorubicin-induced nephropathy in rats by intraperitoneal administration of fetal kidney - derived MSCs (FKD–MSCs). It has been reported in many studies that transplantation of MSCs has the potential to treat many diseases. However, the effect of FKD–MSCs in doxorubicin-induced nephropathy in rats has not been studied previously. It was hypothesized that repeated intraperitoneal administration of FKD–MSCs would result in both structural and functional renal improvement as evaluated by serum biochemistry analysis, dynamic renal scintigraphy, histological, and immunohistochemical examination.

Materials and Methods

Ethical approval: The experimental protocol was approved by the Local Animal Ethics Committee of Dışkapı Yıldırım Beyazıt Training and Research Hospital.

Experimental rats: Twenty - four male, 10- 12 weeks old, conventional Sprague - Dawley rats (200 ± 20 g) were purchased from Dışkapı Training and Research Hospital Laboratory Animal Facility. All rats were kept under standard laboratory conditions (21 ± 2°C, 65% humidity, and 12 h light / 12 h dark). The animals were fed ad libitum with standard rat chow and allowed access to water continuously.

Preparation of the FKD - MSCs: A hysterectomy was performed on 5 pregnant rats (on the 19th day of pregnancy) by median line laparotomy under sterile conditions, and 23 fetuses were taken. The fetuses were anesthetized and euthanized by ether. Forty-six kidney tissues were removed from the fetuses and stored in Dulbecco's Modified Eagle's Medium (DMEM) (Lonza, Belgium).

Fetal kidney tissues were mechanically dissected into small pieces using a sterile scalpel. Then, the tissues obtained by the explant culture method were placed in T25 flasks and incubated at 37°C in a 5% CO_2 humidified atmosphere. 77% DMEM (Lonza, Belgium), 20% fetal bovine serum - FBS (Lonza, Belgium), 2% L - Glutamine (Lonza, Belgium), 1% Penicillin, Streptomycin, Amphotericin (Biological Industries, Israel) were added to the medium. The medium was changed once every 2-3 days to remove non-adherent cells. When about 70% adhesion was present, the adherent MSCs were passaged and aliquoted 1:2 with 0.25% Trypsin in PBS. The cells were grown to the 3rd passage. 10 µl of MSCs prepared for transplantation were taken and mixed by pipetting with 10 µl Trypan Blue. The stained cells were counted using the countess automated cell counting device (Invitrogen, Carlsbad, USA) to determine viability.

Differentiation of the FKD - MSCs: The FKD-MSCs were seeded at a density of $5x10^4$ cells/cm^2 in a six-well culture plate at P3 and differentiated into adipogenic, chondrogenic or osteogenic differentiation medium for 21 days. Von Kossa staining, Oil red staining and Alcian blue staining were used to reveal osteogenic, adipogenic and chondrogenic differentiation, respectively.

Sample size determination and experimental protocol: With a plan to have a continuous response variable from three independent groups and two measurement times, using type-1 (alpha) error rate = 0.05, power (1-beta) = 0.80, effect size: 0.25, the minimum required sample size was determined as 24 animals. Animals were randomly divided into Control, Sham, and MSC groups. To establish nephropathy, the rats in Sham and MSC groups received a single dose tail vein injection of 6 mg/kg / BW doxorubicin (Adriamycin, Adriablastina, Deva Ilaç, Turkey) dissolved in 0.9% NaCl. 7 days after these injections, animals were kept in a metabolic cage for a day to collect 24 h urine. Glucose, bilirubin, urobilinogen, ketone, density, pH, erythrocyte, protein, nitrite, ascorbic acid, leukocyte levels in urine were evaluated by urinalysis device (LabU Reader Plus, Hungary) using a strip (Lab Strip U11 Plus, Germany). The protein level above 25 mg in the 24 h urine of the rats in the Sham and MSC groups showed that the animal model was established. BUN, creatinine, albumin, total protein, triglycerides, Na, Cl, K, cholesterol levels were measured using AU5800 chemical autoanalyzer (Beckman Coulter, CA, USA) from the blood collected at the beginning and end of the study.

The following treatments were applied to the groups:

Group 1 (n = 8): Control group. No medication was applied to these animals.

Group 2 (n = 8): Sham group. 0.9% NaCl (1 ml) were administered intraperitoneally 3 times in one-week intervals.

Group 3 (n = 8): MSC group. 2 x 10^6 FKD - MSCs (1 ml) were administered intraperitoneally 3 times in one-week intervals. All rats were monitored for 5 weeks.

***Dynamic renal scintigraphy*:** After 5 weeks of observation, 10 mg/kg xylazine (Xylazinbio 2%, Bioveta, Ivanovicena Hane, Czech Republic) and 75 mg/kg ketamine (Ketasol 10%, Richter Pharma AG, Wels, Austria) were administered intraperitoneally to provide general anesthesia. Renal scintigraphy was performed to observe functional recovery. For this purpose, commercially available MAG3 (TechneScan, Nepha, Ankara, Turkey) labeled with 99 mTc, was administered to rats from the tail vein in a volume of 0.2 ml at a dose of 2 mCi. Conventional double-headed wide-angle gamma camera (ECAM, Siemens, Illinois, USA) and parallel collimator were used for imaging. Animals were placed in the prone position, 7-8 cm away from the collimator. Dynamic images were obtained simultaneously with the drug application. In the perfusion phase, the data were collected at the end of 60 seconds in the form of 1-second windows and the function phase as 10-second windows for 20 minutes. All images were obtained in a 256 x 256 matrix and 3.20 magnification.

Image and data analysis: Renogram curves were achieved from all groups. Quantitative parameters obtained from renogram curves were selected based on statistical significance according to the previous renal animal study findings (5). These are peak counting (C_{max}), normalized residual activity (NORA), renal retention (RR), and split renal function (SRF). RR is the ratio of minimal transit time to the mean transit time. This parameter, as an index to calculate renal retention function, should be set between 0.1 and 1.0. On the other hand, NORA is the ratio of a 1– minute renal activity at 20 minutes of the renogram to the renal activity at the first 1 to 2– minutes interval of the renogram.

Euthanasia of animals: Forty-eight hours after scintigraphy, rats were euthanized by decapitation under the xylazine/ketamine general anesthesia. Blood samples and kidneys were obtained, and histopathologic examination and immunohistochemistry were performed.

Histological and immunohistochemical study: The tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. The tissues were cut into 4 µm thick sections and stained with hematoxylin and eosin (H&E). The samples were examined under a light microscope to evaluate the general histomorphological structure of the kidneys. A blinded and semi-quantitative analysis was used to quantify the level of kidney injury. Three randomly selected areas were evaluated in each kidney section. The cases were classified as mild, moderate, and severe based on the assessment of levels of glomerular hypertrophy, congestion, mesangial cell proliferation, dilatation, and hyaline cylinder deposits in tubules.

CD24 and CD133 positive cells were investigated by using specific antibodies in renal tissue samples of rats. Immunohistochemical staining results were evaluated semi-quantitatively together with the intensity and percentage of staining of the positive cells. Positive staining changed from light yellow to brown. The staining severity score was scored as no color 0, light yellow 1, yellow 2, and brown 3. The percentage of positive cells was scored as 0 for 0 - 5%, 1 for 6 - 25%, 2 for 26 - 50%, 3 for 51 - 75%, and 4 for > 75%. Both data were considered during the evaluation. Five randomly selected sites (x 400) were evaluated in each preparation.

Statistical analysis: Descriptive statistics of the variables included in the study were shown as "Arithmetic Mean ± Standard Error" or "Median (Min-Max)" considering the distribution of data. Kruskal-Wallis test was used to examine the difference between pathological scores and urinalysis results between the groups. In cases where the differences between the groups were found to be significant, the Dunn-Bonferroni test was used as the post-hoc test. Blood analysis and renogram data were subjected to two-way mixed ANOVA using the general linear modeling procedure for repeated measures to analyze the differences between groups and time or laterality, where necessary. The model included the main effects of Group *(Control, Sham, MSC)*, Time *(t^1, t^2)* (or side *(right, left)*) and Time*Group *(or Side*Group)* interaction term. In cases where interaction terms were not significant, the Tukey test was used as post-hoc procedure to analyze main effects. The simple effects analysis was used by applying Bonferroni correction in cases where interaction terms were significant. Data were analyzed using SPSS 14.01 (SPSS Inc, USA). The $P<0.05$ criteria were used for all statistical evaluations.

Results

Biochemical study: It was observed that blood urea levels were increased in the Sham and MSC groups compared to the Control group. However, there was no significant difference between urea levels between MSC and Sham groups. Albumin levels were decreased in Sham and MCS groups when compared to the Control group. Cholesterol levels were increased in Sham and MSC groups when compared to the Control group. Creatin levels were increased in the Sham group, but there was no significant difference between MSC and Control groups.

Triglyceride values were significantly increased in the Sham group when compared to the Control and MSC group. Sodium levels were increased in Sham and MCS groups when compared to the Control group. Chloride levels were increased in the MCS group, but there was no significant difference between the Sham and Control groups. When total protein and potassium levels were evaluated, no significant difference was found between the groups. Biochemical findings are presented collectively in Table 1.

Urine analysis: When the 24 h urine samples were examined, bilirubin, urobilinogen, ketone, ascorbic acid, leukocyte levels were not different between the groups, but glucose and nitrite were positive and protein levels were above 500 mg/dl in the Sham and MSC group compared to the Control group. The pH of the urine was significantly different between the groups (P<0.05). The pH levels were elevated the most in the Control group, then the Sham, and MSC group, respectively. Urine density was found to be significantly different between the groups (P<0.05). As the highest in the MSC group (1.025), then the Sham group (1.020), then the Control group (1.010).

Table 1. Biochemical findings of the rats in all study groups.

Biochemical assays	Rat Groups	t^1	t^2	P		
		Mean ± SEM	Mean ± SEM	Time	Group	Time*Group
ALB	Control	3.09 ± 0.04 A,a	2.72 ± 0.06 B,a			
	Sham	2.99 ± 0.03 A,a	1.59 ± 0.14 B,b	<0.001	<0.001	<0.001
	MSC	2.85 ± 0.03 A,b	1.59 ± 0.16 B,b			
TP	Control	5.86 ± 0.1 A,a	5.44 ± 0.09 B,a			
	Sham	5.44 ± 0.07 A,a	5.16 ± 0.37 B,a	0.008	<0.001	0.492
	MSC	5.06 ± 0.05 A,b	4.31 ± 0.22 B,b			
UREA	Control	47.25 ± 2.14 A,ab	48.25 ± 1.26 A,a			
	Sham	50.75 ± 1.42 B,a	107.88 ± 23.23 A,b	<0.001	0.036	0.027
	MSC	42.25 ± 0.9 B,b	10.13 ± 17.59 A,b			
K	Control	4.71 ± 0.15 B	5.72 ± 0.42 A			
	Sham	5.71 ± 0.09 B	6.06 ± 0.53 A	0.018	0.184	0.572
	MSC	5.26 ± 0.23 B	5.84 ± 0.32 A			
CREA	Control	0.42 ± 0.01 A,a	0.46 ± 0.02 A,b			
	Sham	0.39 ± 0.01 B,b	0.58 ± 0.04 A,a	<0.001	0.001	0.003
	MSC	0.34 ± 0.01 B,c	0.45 ± 0.02 A,b			
CHOL	Control	67.75 ± 2.99 A,a	68.25 ± 3.48 A,c			
	Sham	75 ± 3.58 B,a	541.13 ± 49.26 A,a	<0.001	<0.001	<0.001
	MSC	76.38 ± 1.21 B,a	237.25 ± 35.74 A,b			
TRIG	Control	55.13 ± 3.18 A,a	66.63 ± 5.47 A,b			
	Sham	50.75 ± 4.62 B,a	258.25 ± 55.94 A,a	0.001	0.003	0.001
	MSC	58.13 ± 7.06 A,a	66.88 ± 28.59 A,b			
Na	Control	139.75 ± 0.37 A,a	136.63 ± 1.12 A,b			
	Sham	135.63 ± 0.38 B,b	141 ± 0.6 A,a	0.006	0.923	<0.001
	MSC	136 ± 0.65 B,b	141 ± 1.52 A,a			
Cl	Control	101.38 ± 0.53 A,a	101 ± 0.27 A,b			
	Sham	97.38 ± 0.56 B,b	101.38 ± 1.49 A,b	<0.001	<0.001	0.003
	MSC	99.63 ± 0.73 B,ab	107.25 ± 0.98 A,a			

ALB: Albumin, TP: Total Protein, UREA: Urea, K: Potassium, CREA: Creatinine, CHOL: Cholesterol, TRIG: Trigylceride, Na: Sodium, Cl: Chlorine.
t^1: Beginning of the study, t^2: End of the study.
A,B: Values in the same row with different superscripts show the statistical difference (P<0.05).
a,b,c: Values in the same column with different superscripts show the statistical difference for each parameter (P<0.05).

Scintigraphic evaluation: As a result of the scintigraphic evaluation, peak count (C_{max}) values were significantly low whereas renal retention (RR) was increased in the Sham group. Normalized residual activity (NORA) was increased in the MSC and the Sham groups compared to the Control group (P<0.05). There was no significant difference between the groups in terms of separated renal function (SRF) (Table 2).

Histopathology results: Renal pathology was evaluated (Figure 1) as normal in the Control group (Figure 1A - 1D). In the Sham group, glomerular hypertrophy, congestion of glomerular capillaries, mild mesangial proliferation, intratubular cast, and tubular vacuolization were observed (Figure 1B - 1E). In the MSC group, all these changes were significantly alleviated/regressed (Figure 1C - 1F).

Immunohistochemical results: The staining intensity and percentage of CD24 and CD133 were significantly higher in the MSC group (P<0.05) (Table 3) (Figure 2). The staining intensity and percentage of CD24 and the staining intensity of CD133 were not significantly different in the Control and the Sham groups (Figure 2C - 2F). The staining percentage of CD133 was found to be higher in the MSC than the Control (Figure 2A - 2D) and the Sham groups, respectively (Figure 2B - 2E). Positive staining was mostly observed in tubules epithelia. No staining was observed in the glomerulus and intertubular areas.

Table 2. Distribution of the peak counting, normalized residual activity, renal retention, split renal function and time to peak counting values according to the rat groups.

Renogram Parameters	Group	Right	Left	P		
		Mean ± SEM	Mean ± SEM	Side	Group	Side*Group
C_{max}	Control	554.81 ± 6.8 a	535.14 ± 8.06 a	0.191	<0.001	0.433
	Sham	289.27 ± 12.32 c	291.27 ± 10.52 c			
	MSC	437.2 ± 17.55 b	424.6 ± 36.28 b			
NORA	Control	0.4 ± 0.01 c	0.41 ± 0.01 c	0.319	<0.001	0.962
	Sham	0.87 ± 0.01 a	0.88 ± 0.01 a			
	MSC	0.63 ± 0.02 b	0.64 ± 0.02 b			
RR	Control	0.33 ± 0.01 c	0.32 ± 0.01 c	0.367	<0.001	0.375
	Sham	0.63 ± 0.01 a	0.64 ± 0.01 a			
	MSC	0.39 ± 0.001 b	0.4 ± 0.001 b			
SFR	Control	51.21 ± 0.52	48.79 ± 0.52	0.117	0.445	0.205
	Sham	49.71 ± 0.52	50.04 ± 0.49			
	MSC	50.59 ± 0.73	49.43 ± 0.73			

C_{max}: peak count, NORA: normalized residual activity, RR: renal retention, SRF: separated renal function
a,b,c: Values in the same column with different superscripts show the statistical difference for each parameter (P<0.05).

Table 3. The staining intensity and percentage of CD24 and CD133 in the rat groups.

	Groups	n	Mean ± SEM	Median (Min-Max)	P
CD24 staining intensity	Control	8	1.75 ± 0.16	2 (1 - 2) b	<0.001
	Sham	8	1.88 ± 0.12	2 (1 - 2) b	
	MSC	8	2.87 ± 0.12	3 (2 - 3) a	
CD24 staining percentage	Control	8	1.88 ± 0.12	2 (1 - 2) b	<0.001
	Sham	8	2.25 ± 0.16	2 (2 - 3) b	
	MSC	8	3.75 ± 0.16	4 (3 - 4) a	
CD133 staining intensity	Control	8	1.88 ± 0.12	2 (1 - 2) b	<0.001
	Sham	8	1.75 ± 0.16	2 (1 - 2) b	
	MSC	8	3 ± 0	3 (3 - 3) a	
CD133 staining percentage	Control	8	2.38 ± 0.18	2 (2 - 3) b	<0.001
	Sham	8	1.38 ± 0.18	1 (1 - 2) c	
	MSC	8	3.5 ± 0.19	3,5 (3 - 4) a	

a,b: Values in the same column with different superscripts show the statistical difference for each parameter (P<0.05).

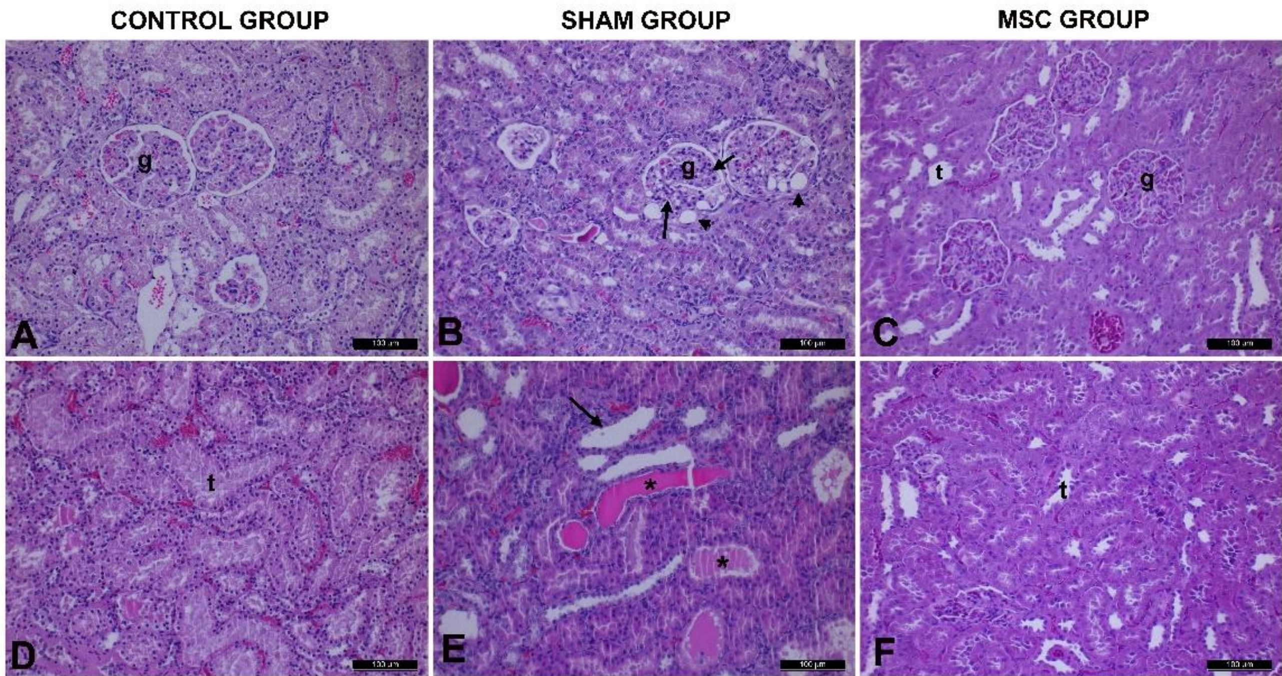

Figure 1. Histopathological appearances of the groups.
A. Control Group. Normal histological view of the glomerulus (g). HE. Bar: 100µm. **B.** Sham Group. Proliferations of mesangial cells (arrows) and enlargement of glomerular capillaries (arrowheads) in the glomerulus (g). HE. Bar: 100µm. **C.** MSC Group. Slightly hyperemic in glomerular capillaries (g) and tubular epithelia in normal view (t). HE. Bar: 100µm. **D.** Control Group. Normal histological view of the tubules (t). HE. Bar: 100µm. **E.** Sham Group. Dilated tubule (arrow) and hyaline cylinder accumulations in the lumen of the tubules (asterisks). HE. Bar: 100µm. **F.** MSC Group. Lesions in the tubules (t) are regressed. HE. Bar: 100µm.

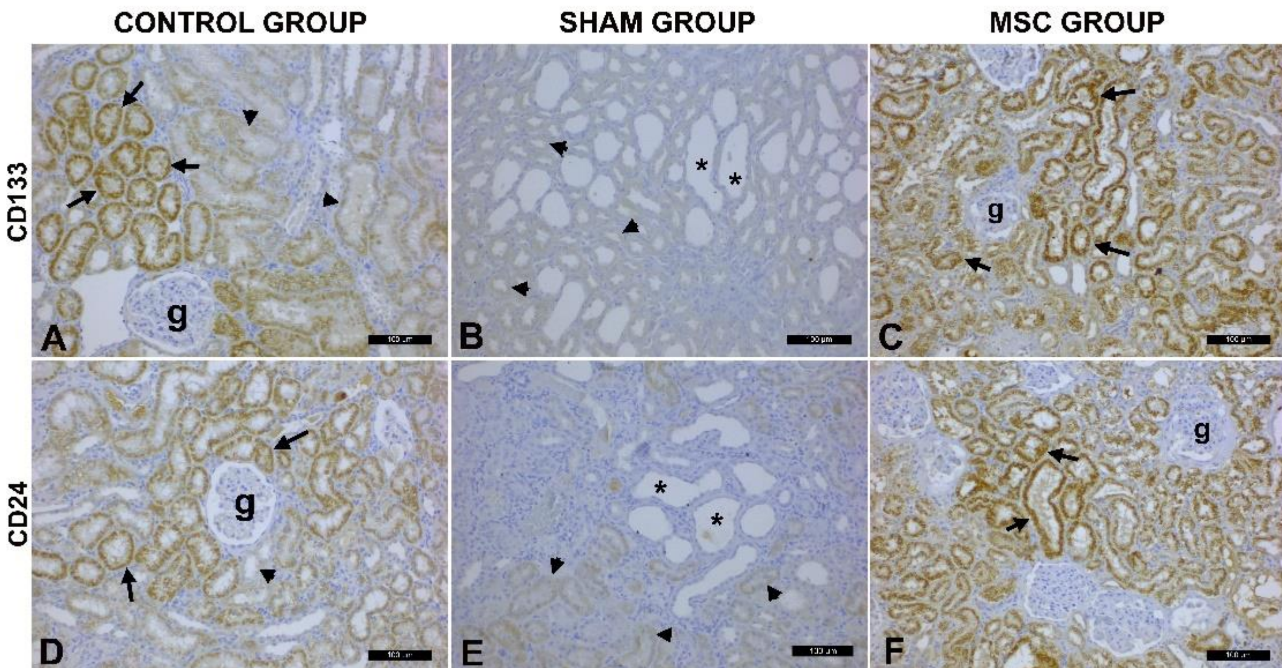

Figure 2. Immunohistochemical staining views of CD133 (A-C) and CD24 (D-F) in the groups.
A. Control Group. Light yellow (+1) (arrowheads) and brown (+3) (arrows) CD133 positive immunoreactions in the tubular epithelia. DAB. Bar: 100µm. **B.** Sham Group. Light yellow (+1) (arrows) CD133 positive staining in tubules epithelia and no positive immunoreactions in the dilated tubules (asterisks). DAB. Bar: 100µm. **C.** MSC Group. Brown (+3) CD133 positive immunostaining in the epithelia of the tubules (arrows). DAB. Bar: 100µm. **D.** Control Group. Light yellow (+1) (arrowheads) and yellow (+2) (arrows) CD24 positive immunostaining in the tubules. DAB. Bar: 100µm. **E.** Sham Group. A small part of light yellow (+1) CD24 positive staining areas (arrowheads) and immunonegative reactions in the dilated tubules (asterisks). DAB. Bar: 100µm. **F.** MSC Group. Brown (+3) (arrows) CD24 positive staining in most tubules in the microscope field (arrows). Glomeruli (g) are negative for CD133 and CD24 antibodies. DAB. Bar: 100µm.

Discussion and Conclusion

Many researchers have studied the benefit of stem cells in the treatment of kidney diseases (1, 3, 11, 15, 21, 25). Human umbilical cord-derived mesenchymal stem cells were transplanted to the rats with doxorubicin-induced nephropathy from the tail vein. Serum interleukin-6, tumor necrosis factor-α, and prostaglandin E_2 levels of the treatment group were decreased for 4 weeks, and interleukin-10 levels were increased. According to these results, it was concluded that MSCs treated doxorubicin-induced kidney damage and inflammation and may play a potential role in the clinical treatment of renal diseases (11).

It has been reported that MSCs are administered to mice with acute renal injury to assist both structural and functional renal repair, which is achieved by trans differentiation of MSCs to the tubular epithelium. However, only 2 to 2.5% of these injected MSCs have been engrafted (18). In another study, intraarterial administration of MSCs reduced necrosis, improved renal function, and increased α -smooth muscle actin (α-SMA) expression, however, no MSC was engrafted in the renal tissue (23). In a clinical study of the CRF model, significant differences in serum creatinine and creatinine clearance levels were detected before and after treatment when 1 x 10^6 MSCs / kg were administered twice intravenously (3). In a study published in 2014, bone marrow-derived MSCs were administered intravenously at a dose of 2 x 10^6 in doxorubicin -induced nephropathy model, and urinary protein, blood creatinine, and triglyceride levels were improved in the treatment group (6).

According to the results of a meta-analysis study, the administration of cellular therapy directly via the renal artery or via tail vein injection (intravenously), resulted in functional and histological improvement in experimental CKD animal models (17). The same study concluded that intraperitoneal administration of cell-based therapy has not showed any significantly improved outcome. However, this conclusion was based on a very limited number of studies.

Studies have shown that intravenously infused human cord-derived MSCs were homed to the renal tubular and renal interstitial area, but intraperitoneally transplanted human cord-derived MSCs were not found in the kidneys of the rats. However no significant differences were found between the groups in terms of renal morphology (11). The intravenous transplantation requires general anesthesia. The injection on the renal artery not only requires general anesthesia but is an invasive method. After evaluating the results of the previous studies, we preferred transplanting the FKD-MSCs intraperitoneally, a less invasive and non-anesthesia requiring method.

According to results of a study conducted in 2019, it was concluded that human umbilical cord blood-derived MSCs attenuated cisplatin-induced nephrotoxicity in mice (20). No significant differences regarding different delivery routes (intravenous and intraperitoneal) were determined when structural injuries and renal function was evaluated . Based on the results of this previous study, we decided to administer FKD- MSCs intraperitoneally at a dose of 2 x 10^6.

The sources from which the stem cells are derived are known to have different effects in treatment (15). For this reason, many researchers are trying to find the best treatment option using different cell sources. In a study conducted by Morigi and Benigni in 2013, the therapeutic properties of stem cells obtained from different sources in acute renal failure (ARF) were compared. Accordingly, bone marrow-derived, cord blood-derived and amniotic fluid-derived stem cells were transplanted into mice, and the results showed that renal histology was maintained in all groups and a decrease in BUN was observed. Survival time was evaluated by looking at the sources from which the stem cells were obtained, and the survival rate was increased in the cord blood derived MSC transplanted group (13). Another study reported that adipose stem cells contribute to treatment in an animal model of CRF (22). In a study conducted in eight cats with CKD, repeated intravenous transplantation of allogeneic adipose derived-MSCs was performed. As a result, no statistically significant difference was present between MSC and placebo groups (19). However, this conclusion was based on a very limited of patients. In our study repeated intraperitoneal transplantation of FKD–MSCs resulted in renal improvement in the MSC group when compared to the sham group.

The most important biochemical finding in CRF is increased BUN levels. Increased proteinuria, serum creatinine, and BUN have been reported in rats treated with doxorubicin intravenously to form a CRF model (11). In our study, it was observed that BUN levels were increased in the Sham and the MSC group compared to the Control group. The application of MSCs did not affect BUN levels but caused a decrease in cholesterol and triglyceride levels. A decrease in BUN levels after MSC transplantation was observed in the ARF model (13). In our study, the CRF model was used, and as irreversible damage occurs in CRF, it is thought that the MSC application did not affect BUN levels. It is known that MSCs that are transplanted systemically without specific targeting migrate through cytokines to other places where inflammation of the organism occurs and ameliorates the damage (13, 14). In our study, the decrease in cholesterol and triglyceride levels in the MSC group, suggests that the stem cells were also located in other organs. Our study focuses on how the kidneys are affected by MSCs.

Therefore, the effects of MSCs on the other organs are beyond the scope of this paper.

Urine analysis plays an important role in renal diseases. Protein levels above 500 mg/dl in doxorubicin induced CRF model, indicates kidney damage (11). In the present study, it was found that protein value was high in the Sham and the MSC groups due to doxorubicin injection. Since kidney damage has become chronic, MSC transplantation has not prevented protein excretion in the urine.

Dynamic renal imaging using MAG3 labeled with 99mTc shows tubular uptake and release at a given time interval. 99mTc-MAG3 can be evaluated using activity curves over some time and quantitative results are obtained from the renogram. In human studies, NORA and transit time have been used to evaluate extraction/excretory function in the kidneys. Besides, when large databases are studied, NORA is an important criterion for the assessment of renal drainage (5). According to the results of this study, NORA and RR values are important in assessing renal dysfunction. In our study, the NORA values of the Sham and MSC groups were the same, but the RR value was higher in the Sham group than the MSC group. When these two parameters are interpreted together, it has been shown that MSCs have a positive effect on healing by decreasing retention, proving that MSC transplantation affects functional recovery.

In CRF, the number of nephrons gradually decreases as fibrous tissue replaces the kidney tissue. After some time, varying according to the rate of progression of the underlying disease, the kidneys no longer meet the needs of the body and uremic syndrome occurs (2). After the kidneys have been damaged to a certain extent and a critical amount of parenchyma has been lost in CRF, even if the primary disease is completely cured, progression to end-stage renal failure cannot be prevented (25). That means terminal renal failure is inevitable after irreversibly decreasing renal function to a critical level. Histopathological examination of kidneys in this period has many common findings: Glomeruli become sclerotic, fibrous tissue develops in renal interstitium, and chronic inflammation of lymphocytes and macrophages occurs (2, 9). Most of the tubules become atrophic and dilated (22). In our study, the renal injury was manifested by glomerular hypertrophy, mesangial proliferation, and tubular vacuolization in the Sham group, whereas these changes were significantly alleviated in the MSC group. These histopathologic results showed that MSCs are effective in kidney healing.

CD133 + and CD24 + cells are renal stem / progenitor cells. Renal CD133 + and CD24 + cells are the cells that determine the potential for self–regeneration, and differentiation of both podocytes and tubular cells. Increased expression of Osr1, Nanog, HGF, BMP - 7, WT - 1, and Pax2 in rat kidneys with CRF is correlated with CD133 + and CD24 + renal stem/progenitor cells. However, this increase reduces TGF-β1 expression and prevents interstitial fibrosis (8). When the data obtained in our study were evaluated, while the number of positive cells was reduced in the Control and the Sham groups; it was found to be higher in the MSC group. CD24 and CD133 staining intensity and percentage were higher in the MSC group compared to the other groups. According to these results, intraperitoneally administered FKD-MSCs reached the damaged kidney tissues and proliferated to treat the tissue. When the findings were evaluated, it was observed that both functional and histological improvements were achieved in the MSC group compared to the Sham group.

In conclusion, transplantation of FKD-MSCs into patients with renal damage is thought to contribute to the healing of the renal tissue. To investigate this functional and histological result in detail, the effective pathways of transplanted MSCs should be investigated in detail with other studies.

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Ethical Statement

This study was approved by the Local Animal Ethics Committee of Dışkapı Yıldırım Beyazıt Training and Research Hospital (Approval number: 2014 / 55).

Conflict of Interest

The authors declared that there is no conflict of interest.

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