Stem Cells International

Stem Cell Applications in Regenerative Medicine for Kidney Diseases

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Editorial

Stem Cell Applications in Regenerative Medicine for Kidney Diseases

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Broad clinical conditions can lead to kidney disease, which is a worldwide public health problem that affects millions of people from all sexes, ages, and racial groups. Acute kidney injury (AKI) may be secondary to kidney hypoperfusion, direct kidney damage due to toxins, sepsis, or immune-mediated, and postobstructive. AKI occurs not only in patients without previous kidney impairment but also in a manner of superimposed on the chronic kidney disease (CKD) settings. Overall, CKD is mainly caused by diabetic kidney disease (DKD), hypertension, and glomerulonephritis. Therefore, the search for nonpharmacological strategies for controlling kidney diseases is of utmost importance.

The present special issue has been designed to stimulate continuing efforts to develop novel therapeutic strategies to accelerate AKI recovery and curtail CKD progression. It includes five review articles and two original papers from leading and emerging scientists with diverse expertise and covers three thematic areas: (i) the therapeutic potential of mesenchymal stem cells (MSCs) in acute and chronic kidney diseases; in particular, AKI caused by toxicants and acute ischemia-reperfusion, and CKD secondary to hypertension, systemic lupus erythematosus (SLE), and diabetes mellitus (DM); (ii) therapeutic perspectives of MSCs in peritoneal fibrosis, a clinical condition that can be found in end-stage kidney disease (ESKD); (iii) and the emerging studies of inducible pluripotent stem cells (iPSCs) and their differentiation into kidney cells.

In the paper of the present special issue entitled "Clinical Efficacy and Safety of Mesenchymal Stem Cells for Systemic Lupus Erythematosus," T. Zhou et al. performed a meta-analysis comprising prospective and retrospective case series and randomized controlled trials of MSC-based therapy in SLE, in particular, lupus nephritis. That condition may progress to CKD. Importantly, their main findings included that MSCs promoted a decrease in proteinuria at 3 and 6 months and SLE activity, as assessed by a lower score of SLEDAI (at 2 and 6 months); although, that therapy did not yield a reduction in serum creatinine. Regarding key aspects of MSC-based therapy, in eight studies, the MSC source was the umbilical cord (UC), whereas two studies included a combination of UC and bone marrow- (BM-) derived MSC injection. The number of injected cells diverged in the studies, from 1×10^6/kg or 5×10^7 to 2×10^8 cells per infusion, as well as the number of injections (1 to 5 times). Except for one study that injected MSCs via the renal artery, the other studies used the intravenous route for cell injection. MSC efficacy may be affected by several conditions not only related to their administration (source, dose, frequency, and route) but also by their heterogeneity due to culture conditions. Therefore, the International Society for Cell Therapy proposed the assessment of MSC functionality based on assays that demonstrate the secretion of trophic factors, modulation of immune cells, and differentiation capacity [1]. Thus, injecting less heterogenic and more functional MSCs will improve clinical outcomes.

In line with the benefits of MSC in CKD setting, a study by E. C. Costalonga et al. entitled "Adipose-Derived Mesenchymal Stem Cells Modulate Fibrosis and Inflammation in the Peritoneal Fibrosis Model Developed in Uremic Rats" described a model of CKD (induced by 0.75% adenine-containing diet for 30 days) and peritoneal fibrosis (induced by chlorhexidine gluconate) in a preclinical model. In ESKD,

patients under peritoneal dialysis are at high risk of developing peritoneal fibrosis and losing the efficacy of their treatment [2]. The authors documented that adipose-tissue-derived MSC (1×10^6 cells, 2 times, and intravenous route) abrogated peritoneal fibrosis and fostered antifibrotic (decrease in TGF-β, fibronectin, and collagen) and anti-inflammatory (decrease in IL-1β, TNF-α, and IL-6) responses. Therefore, the results obtained from this study contribute to set the basis for establishing further investigation of the therapeutic potential of MSCs for peritoneal fibrosis treatment in both preclinical and clinical studies.

There is compelling evidence of the therapeutic potential of MSCs for kidney disease, but whether microvesicles (MVs) and exosomes (EXs) released by these cells hold similar efficacy is still a subject of debate in the literature. In the preclinical study by C. S. R. A. Ishiy et al. entitled "Comparison of the Effects of Mesenchymal Stem Cells with Their Extracellular Vesicles on the Treatment of Kidney Damage Induced by Chronic Renal Artery Stenosis," the authors developed a model of renovascular hypertension (2 kidneys, 1 clip) in rats and compared the outcomes after adipose tissue-derived MSC injection, and MV and EX treatment. All three treatments (adipose tissue-derived MSCs, MVs, and EXs) reduced blood pressure, proteinuria, and the expression of collagen type I and TGF-ß in kidney cortex and medulla when compared to the stenotic kidney and sham, yet not preventing the increase in heart weight, a finding that is secondary to systemic arterial hypertension. In addition, IL-10 levels increased in all three treatments. Of note, adipose tissue-derived MSC and MVs contributed to lower proteinuria values more than the treatment with EXs, whereas whole cells were more effective in reducing the inflammatory cytokine IL-1β. The results of this study shed light on the importance of selecting the most appropriate therapy in accordance with the main target of a specific disease.

To further substantiate the investigation of the impact of MSC on functional and structural results in preclinical models using small and large animals and the translational approach to clinical studies, the review of C. Sávio-Silva et al. entitled "Mesenchymal Stem Cell Therapy for Diabetic Kidney Disease: A Review of the Studies Using Syngeneic, Autologous, Allogeneic, and Xenogeneic Cells" addressed the main issues of MSC-based therapy for diabetic kidney disease (DKD). The authors highlighted the importance of recapitulating DKD microenvironment and the interaction of cell-cell and cell-matrix interactions in vitro for evaluating the therapeutic potential of MSCs. They also critically analyzed the main challenges of evaluating MSC efficacy in preclinical models, such as MSC phenotype, source, route of delivery, and homing. Notably, there are several rodent models of type 1 and type 2 DM that can be used to verify the MSC therapeutic potential and assessment of functional and structural outcomes in kidney disease. Taking a step forward, the authors documented the main findings of clinical trials using autologous or allogenic-derived MSCs for type 1 and type 2 diabetic individuals. Noteworthily, MSC-based preclinical and phase I/II clinical data encourage the design of future large-scale controlled clinical trials that evaluate DKD response to MSC therapy; although, type 2 DM-derived MSCs raised some concerns about their efficacy due to changes in their microenvironment imposed by hyperglycaemia. Importantly, assessing donor-to-donor MSC heterogeneity, the potential immunogenicity of allogenic-derived MSCs, and their differentiation state should be taken into account in future studies [3, 4].

In the manuscript entitled "The Efficacy of Mesenchymal Stem Cells in Therapy of Acute Kidney Injury Induced by Ischemia-Reperfusion in Animal Models," T. Zhou et al. discussed at length the main findings of MSC-based therapy in AKI setting. In their meta-analysis comprising acute ischemia-reperfusion injury, MSC promoted a decrease in creatinine levels at short- (1 day) and long- (>7 days-) terms, as well as in proteinuria. Increased creatinine and proteinuria are two crucial hallmarks of kidney dysfunction and may predict progression to CKD [5]. Importantly, MSC treatment specifically decreased AKI-related targets, such as markers of oxidative stress, inflammation, and fibrosis, which ultimately lead to structural damage amelioration. Therefore, we can anticipate that MSC-based therapy may prevent AKI progression to CKD and may mitigate the damage of AKI superimposed on CKD.

In line with the findings of MSC-based therapy in AKI, S. Lin et al. performed a meta-analysis entitled "Nephroprotective Effect of Mesenchymal Stem Cell-Based Therapy of Kidney Disease Induced by Toxicants" and verified the efficacy of MSC-based treatment in AKI injury secondary to diverse toxicants (glycerol, cisplatin, adriamycin, methotrexate, streptozotocin, cadmium, rifampicin, gentamicin, and aristolochic acid). Despite different sources (bone marrow, adipose tissue, umbilical cord, amniotic fluid, and embryonic stem cells), routes (intravenous, intraperitoneal, intra-aorta, subcapsular, intrarenal parenchyma, and subcutaneous), and doses ($2 \times 10^5 - 5 \times 10^6$) that were analyzed, MSC treatment decreased serum creatinine, serum blood urea nitrogen, and albuminuria levels, as well as restored the imbalance of prooxidants and antioxidants within the kidneys, and ameliorated inflammation and fibrosis. These findings paved the way for the development of clinical trials in a broad AKI setting.

To gain further insight into the novel strategies to repair kidney damage and improve our understanding of signaling pathways involved in renal homeostasis and injury, in the manuscript entitled "Differentiating Induced Pluripotent Stem Cells into Renal Cells: A New Approach to Treat Kidney Diseases," P. de Carvalho Ribeiro et al. discussed the main protocols available in the literature for the differentiation of IPSCs into renal cells from human and rodent sources. These protocols may take from 4 to 26 days to be performed and set the basis for fostering the development of renal progenitors and podocyte or tubular-like cells that can be injected into animals to repair kidney damage. Notably, this knowledge also contributed to the advancement of developing kidney organoids for regenerative medicine.

In conclusion, the bench to bedside pathway has been constructed for MSC-based treatment in the kidney disease setting. Experimental animal models indicated that MSCs

are effective for treating acute and chronic kidney diseases. MSCs demonstrated efficacy in controlling several biological processes, such as oxidative stress, inflammation, and fibrosis, as well as in ameliorating renal functional and structural parameters. Therefore, it is important to comprehend and interpret these experimental data and equally important to critically review clinical studies. Despite the data encouraging the design of controlled randomized clinical trials to evaluate acute and chronic kidney disease response to MSC-based therapy, rigorous reporting of safety and efficacy is still needed.

Conflicts of Interest

The editors declare that they have no conflicts of interest regarding the publication of this special issue.

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Research Article

Nephroprotective Effect of Mesenchymal Stem Cell-Based Therapy of Kidney Disease Induced by Toxicants

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Background. Renal damage caused by drug toxicity is becoming increasingly common in the clinic. Preventing and treating kidney damage caused by drug toxicity are essential to maintain patient health and reduce the social and economic burden. In this study, we performed a meta-analysis to assess the nephroprotective effect of mesenchymal stem cells (MSCs) in the treatment of kidney disease induced by toxicants. *Methods*. The Cochrane Library, Embase, ISI Web of Science, and PubMed databases were searched up to December 31, 2019, to identify studies and extract data to assess the efficacy of MSCs treatment of kidney disease induced by toxicants using Cochrane Review Manager Version 5.3. A total of 27 studies were eligible and selected for this meta-analysis. *Results*. The results showed that a difference in serum creatinine levels between the MSC treatment group and control group was observed for 2, 4, 5, 6-8, 10-15, 28-30, and ≥42 days (2 days: WMD = −0.88, 95% CI: -1.34, -0.42, $P = 0.0002$; 4 days: WMD = −0.74, 95% CI: -0.95, -0.54, $P < 0.00001$; 5 days: WMD = −0.46, 95% CI: -0.67, -0.25, $P < 0.0001$; 6-8 days: WMD = −0.55, 95% CI: -0.84, -0.26, $P = 0.0002$; 10-15 days: WMD = −0.37, 95% CI: -0.53, -0.20, $P < 0.0001$; 28-30 days: WMD = −0.53, 95% CI: -1.04, -0.02, $P = 0.04$; ≥42 days: WMD = −0.22, 95% CI: -0.39, -0.06, $P = 0.007$). Furthermore, a difference in blood urea nitrogen levels between the MSC treatment group and control group was observed for 2-3, 4-5, 6-8, and ≥28 days. The results also indicate that MSC treatment alleviated inflammatory cells, necrotic tubules, regenerative tubules, and renal interstitial fibrosis in kidney disease induced by toxicants. *Conclusion*. MSCs may be a promising therapeutic agent for kidney disease induced by toxicants.

1. Introduction

Kidney injury occurs during acute kidney injury (AKI) and chronic kidney disease (CKD), and it is a common condition associated with the morbidity and mortality of patients. A total of 80% of patients who survive an AKI episode completely recover kidney function, and recovered AKI patients present an almost 9-fold increase in risk for CKD development [1]. Toxicant-induced kidney injury is one of the most common causes of kidney disease, causing substantial morbidity and hampering drug development [2]. At present, renal damage caused by drug toxicity is becoming increasingly common in the clinic. Preventing and treating kidney damage caused by drug toxicity is essential to maintain patient health and reduce the social and economic burden.

Mesenchymal stem cells (MSCs), which are multipotent mesenchymal cells present in various tissues, have multilineage differentiation ability under appropriate conditions and are easy to obtain. They are a promising therapeutic option for some diseases because of their unique property of releasing some important bioactive factors [3–5]. Drug toxicity can induce renal tubular epithelial cell damage or death and can lead to renal interstitial inflammation, which eventually develops into renal interstitial fibrosis and renal loss. Previous studies have shown that MSCs can play a protective role against injury of renal tubular epithelial cells and prevent renal interstitial fibrosis [6–10]. Before clinical application, animal experiments in vivo are generally required to confirm the effectiveness of MSCs. Furthermore, there are few clinical trials of MSCs on kidney disease induced by toxicants. Therefore, in this study, we performed a meta-analysis to assess the

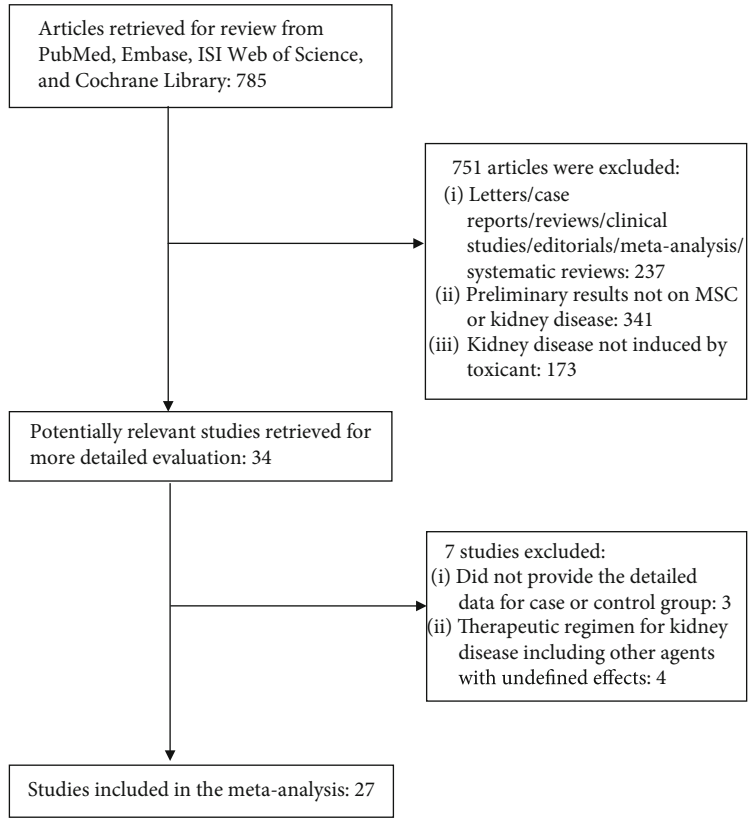


Figure 1: Flow diagram of the selection process.

nephroprotective effect of MSCs in the treatment of kidney disease induced by toxicants in animals.

2. Materials and Methods

2.1. Search Strategy. We searched databases (Cochrane Library, Embase, ISI Web of Science, and PubMed) up to Dec 31, 2019, using the following search terms: (mesenchymal stem cells OR MSC OR MSCs OR multipotent stromal cells OR mesenchymal stromal cells OR mesenchymal progenitor cells OR stem cells) AND (gentamicin OR aristolochic acid OR cisplatin OR adriamycin OR cadmium chloride OR methotrexate OR rifampicin OR glycerol OR streptozocin) AND (kidney injury OR renal failure OR kidney disease). The search was confined to English-language literature. An additional search was conducted among the eligible manual references of the cited articles.

2.2. Inclusion and Exclusion Criteria. Our meta-analysis included studies analyzing the efficacy of MSC treatment in mice or rats with kidney disease. The following studies were excluded from the analysis: (1) letters, case reports, reviews, clinical studies, editorials, meta-analysis, and systematic reviews; (2) studies lacking the targeted indicators or number of case or control groups and were conducted in humans; (3) studies of kidney disease that was not induced by toxicants; and (4) studies with therapeutic regimen for kidney disease that included other agents with undefined effects.

2.3. Outcome Measures. We filtered the following outcomes associated with the efficacy of MSC treatment from the recruited studies: serum creatinine (Scr), blood urea nitrogen (BUN), urinary albumin excretion (UAE), malondialdehyde (MDA), L-glutathione (GSH), superoxide dismutase (SOD), and renal pathology. In addition, we conducted a mutual consensus when met with disagreements.

2.4. Quality Assessment. Two investigators independently evaluated the methodological quality using the Cochrane Handbook for Interventions. We assessed the following sections of every investigation: selection bias, attrition bias, performance bias, detection bias, reporting bias, and other bias. Each item was classified as unclear, high risk, or low risk.

2.5. Statistical Analysis. Review Manager Version 5.3 was applied to explore whether MSC treatment achieved a good efficacy in kidney disease induced by toxicants, and STATA 12.0 was used to test the publication bias. Heterogeneity of variation among individual studies was quantified and described using I^2. The fixed effects model was used if the P value of the heterogeneity test was ≥ 0.1. Otherwise, the random effects model was applied to pool the outcomes. In addition, to compute continuous variables, we analyzed weighted mean differences (WMDs) for the mean values. We also calculated 95% confidence intervals (95% CI) for the included studies using the Mantel-Haenszel (M-H) method. Additionally, we evaluated the publication bias using Begg's rank

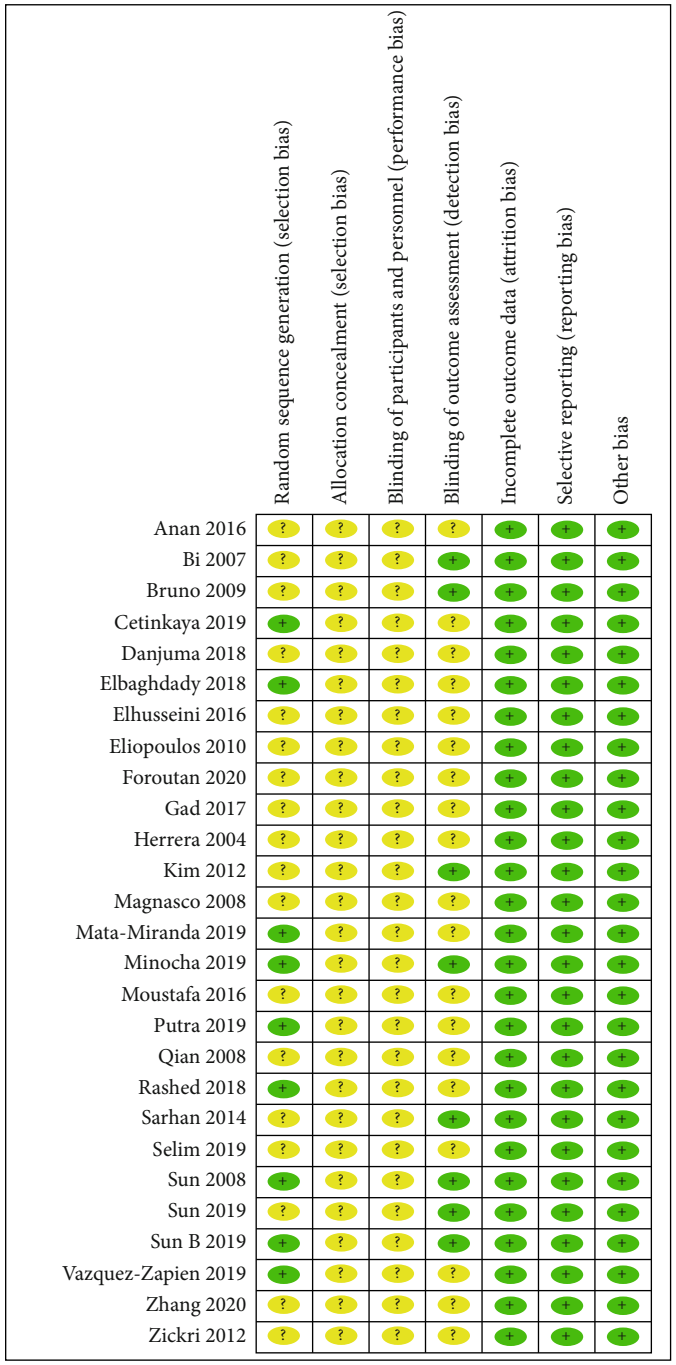
Table 1: Characteristics of the studies included in this meta-analysis.

Author, year	*n*	Type of animal	Type of injury	MSC type	Number of MSC	Route of delivery	Endpoints for this meta-analysis
Herrera 2004	24	Mice	Glycerol-induced	BM-MSCs	1×10^6	Intravenous injection	Scr
Bi 2007	12	Mice	Cisplatin-induced	BM-MSCs	2×10^5	Intravenous injection or intraperitoneal injection	Scr, BUN
Sun 2008	40	Rat	Glycerol-induced	BM-MSCs	2×10^6	Abdominal aorta injection	Scr, BUN
Qian 2008	6	Rat	Glycerol-induced	BM-MSCs	1×10^4	Intravenous injection	Scr
Magnasco 2008	22	Rat	Adriamycin-induced	BM-MSCs	3×10^6	Intravenous injection	Scr, BUN, UAE, renal damage score
Bruno 2009	16	Mice	Glycerol-induced	BM-MSCs	—	Intravenous injection	Scr, BUN, MDA, GSH, SOD, renal damage score
Eliopoulos 2010	10	Mice	Cisplatin-induced	BM-MSCs	5×10^6	Intraperitoneal injection	Scr, BUN
Kim 2012	17	Rat	Cisplatin-induced	AD-MSCs	5×10^5	Intravenous injection	Scr, BUN
Zickri 2012	30	Rat	Adriamycin-induced	hUC-MSCs	5×10^5	Intravenous injection	Scr
Sarhan 2014	19	Rat	Adriamycin-induced	BM-MSCs	4×10^6	Intravenous injection	Scr, BUN, UAE, renal pathology, MDA, GSH
Moustafa 2016	80	Rat	Cisplatin-induced	BM-MSCs	5×10^6	Intravenous, intraarterial or kidney subcapsular injection	Scr, MDA, GSH, SOD
Elhusseini 2016	40	Rat	Cisplatin-induced	AD-MSCs	5×10^6	Intravenous injection	Scr, BUN, Ccr, renal pathology, MDA, GSH, SOD
Anan 2016	13	Rat	Adriamycin-induced	BM-MSCs	1×10^6	Intravenous injection	Scr, BUN, SOD
Gad 2017	24	Rat	Methotrexate-induced	BM-MSCs	2×10^6	Intraperitoneal injection	Scr, BUN, MDA, GSH
Rashed 2018	20	Rat	Streptozotocin -induced	BM-MSCs	1×10^6	Intravenous injection	Scr, BUN, UAE, Ccr
Elbaghdady 2018	20	Rat	Cadmium chloride-induced	BM-MSCs	2×10^6	Intravenous injection	Scr
Danjuma 2018	16	Rat	Rifampicin-induced	BM-MSCs	2.5×10^5	Intravenous injection	Scr, BUN
Putra 2019	10	Rat	Gentamicin induced	hUC-MSCs	1×10^6	Intraperitoneal injection	Scr, BUN, renal pathology
Cetinkaya 2019	17	Rat	Aristolochic acid induced	hAMSC	6×10^5	Intravenous injection	Scr, BUN
Selim 2019	70	Rat	Cisplatin-induced	AD-MSCs; BM-MSCs	4×10^6	Intravenous injection	Scr, BUN
Mata-Miranda 2019	10	Mice	Cisplatin-induced	mESCs	1×10^6	Intraperitoneal injection	Scr
Vazquez-Zapien 2019	19	Mice	Cisplatin-induced	mESCs	1×10^6	Intraperitoneal injection	Scr
Minocha 2019	3	Rat	Cisplatin-induced	AFSC	2×10^6	Intravenous injection	Scr, BUN

Table 1: Continued.

Author, year	*n*	Type of animal	Type of injury	MSC type	Number of MSC	Route of delivery	Endpoints for this meta-analysis
Sun B 2019	10	Rat	Cisplatin-induced	USCs	2×10^6	Intravenous injection	Scr
Sun 2019	6	Rat	Cisplatin-induced	BM-MSCs	—	Renal parenchyma injection	Scr, BUN
Zhang 2020	9	Rat	Cisplatin-induced	USCs	5×10^6	Subcutaneous injection	Scr, Ccr, renal pathology
Foroutan 2020	6	Rat	Cisplatin-induced	BM-MSCs	—	Intraperitoneal injection	Scr, BUN

Note: BM-MSCs: bone marrow mesenchymal stem cells; hAMSCs: human amnion-derived mesenchymal stem cells; hUC-MSCs: human umbilical cord-mesenchymal stem cells; AD-MSCs: adipose tissue-derived mesenchymal stem cells; mESCs: mouse embryonic stem cells; AFSCs: amniotic fluid stem cells; USCs: urine-derived stem cells; Scr: serum creatinine; BUN: blood urea nitrogen; UAE: urinary albumin excretion; Ccr: creatinine clearance rate; MDA: malondialdehyde; GSH: L-glutathione; SOD: superoxide dismutase.



(a)

Figure 2: Continued.

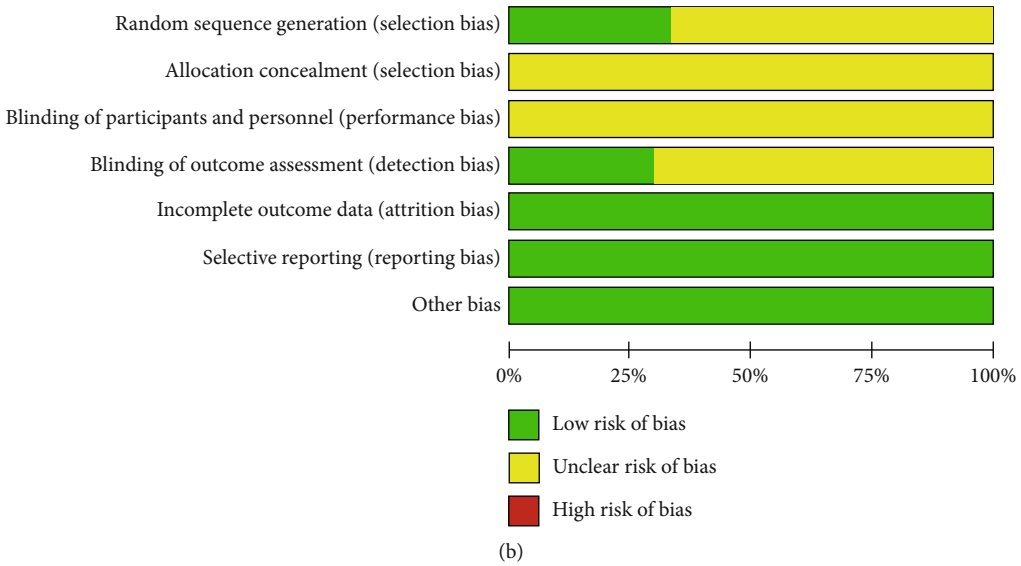


Figure 2: (a) Aggregate Risk of bias graph for each experimental animal studies; "?": Unclear risk; "+": Low risk. (b) Risk of bias summary.

correlation test as well as Egger's linear regression method among the studies. A P value < 0.05 was considered of statistical significance.

3. Results

3.1. Search Results. The databases mentioned above were searched, and only studies in mice or rats that evaluated the therapeutic efficacy of MSC treatment on kidney disease induced by toxicants were selected. Twenty-seven studies [11–37] were eligible and selected for this meta-analysis, and a flowchart of inclusion of studies is presented in Figure 1. Study characteristics are shown in Table 1.

3.2. Quality Assessment of Included Studies. The methodological quality of the selected studies was considered acceptable because most study domains were ranked as unclear risk or low risk of bias. Unclear risk of bias was mostly detected in performance and selection bias. Low risk of bias mostly occurred in detection, reporting, and attrition bias. Figure 2 shows a summary of the risk of biases of the selected studies.

3.3. Scr. A total of 27 studies [11–37] were selected to assess the effect of MSCs on Scr, and the results show that a difference between the MSC treatment and control groups was observed for 2, 4, 5, 6-8, 10-15, 28-30 days, and ≥42 days (2 days: WMD = −0.88, 95% CI: -1.34, -0.42, $P = 0.0002$; 4 days: WMD = −0.74, 95% CI: -0.95, -0.54, $P < 0.00001$; 5 days: WMD = −0.46, 95% CI: -0.67, -0.25, $P < 0.0001$; 6-8 days: WMD = −0.55, 95% CI: -0.84, -0.26, $P = 0.0002$; 10-15 days: WMD = −0.37, 95% CI: -0.53, -0.20, $P < 0.0001$; 28-30 days: WMD = −0.53, 95% CI: -1.04, -0.02, $P = 0.04$; ≥42 days: WMD = −0.22, 95% CI: -0.39, -0.06, $P = 0.007$; Figure 3 and Table 2). However, no difference was observed between the MSC treatment and control groups for 3 days (3 days: WMD = −0.09, 95% CI: -0.25, -0.06, $P = 0.24$; Figure 3 and Table 2).

3.4. BUN. A total of 18 studies [11–15, 17–19, 21, 22, 24, 26–29, 32–34, 36, 37] were selected to assess the effect of MSCs on BUN, and the results indicate that the difference between the MSC treatment and control groups was observed for 2-3, 4-5, 6-8, and ≥28 days (2-3 days: WMD = −25.08, 95% CI: -37.49, -12.67, $P < 0.0001$; 4-5 days: WMD = −45.71, 95% CI: -59.36, -32.05, $P < 0.00001$; 6-8 days: WMD = −57.55, 95% CI: -99.19, -15.91, $P = 0.007$; ≥28 days: WMD = −23.39 , 95% CI: -36.39, -10.40, $P = 0.0004$; Figure 4 and Table 2). However, no difference was observed between the MSC treatment and control groups for 13-15 days (WMD = −13.40, 95% CI: -32.34, 5.54, $P = 0.17$; Figure 4 and Table 2).

3.5. Urinary Albumin Excretion. Three studies [22, 26, 27] were selected in the meta-analysis for the assessment of MSCs on UAE. The results show that the MSC group had a lower UAE than the control group (WMD = −22.66, 95% CI: -26.41, -18.90, $P < 0.00001$; Table 2).

3.6. Oxidative Stress. Four studies [17, 19, 23, 27] were selected for the assessment of MDA, four [17, 19, 23, 27] for GSH, and three [11, 17, 23] for SOD. The results indicate that a difference between the MSC treatment and control groups was observed for MDA, GSH, and SOD (MDA: WMD = −17.21, 95% CI: -20.38, -14.04, $P < 0.00001$; GSH: WMD = 4.62, 95% CI: 2.74, 6.50, $P < 0.00001$; SOD: WMD = 5.42, 95% CI: 2.92, 7.93, $P < 0.0001$; Table 2).

3.7. Assessment of Renal Pathology. Four studies [17, 24, 27, 35] for inflammatory cells, two studies [17, 27] for necrotic tubules, two studies [17, 27] for regenerative tubules, and three studies [17, 27, 35] for renal interstitial fibrosis were included in this meta-analysis. The results indicate that the difference in inflammatory cells, necrotic tubules,

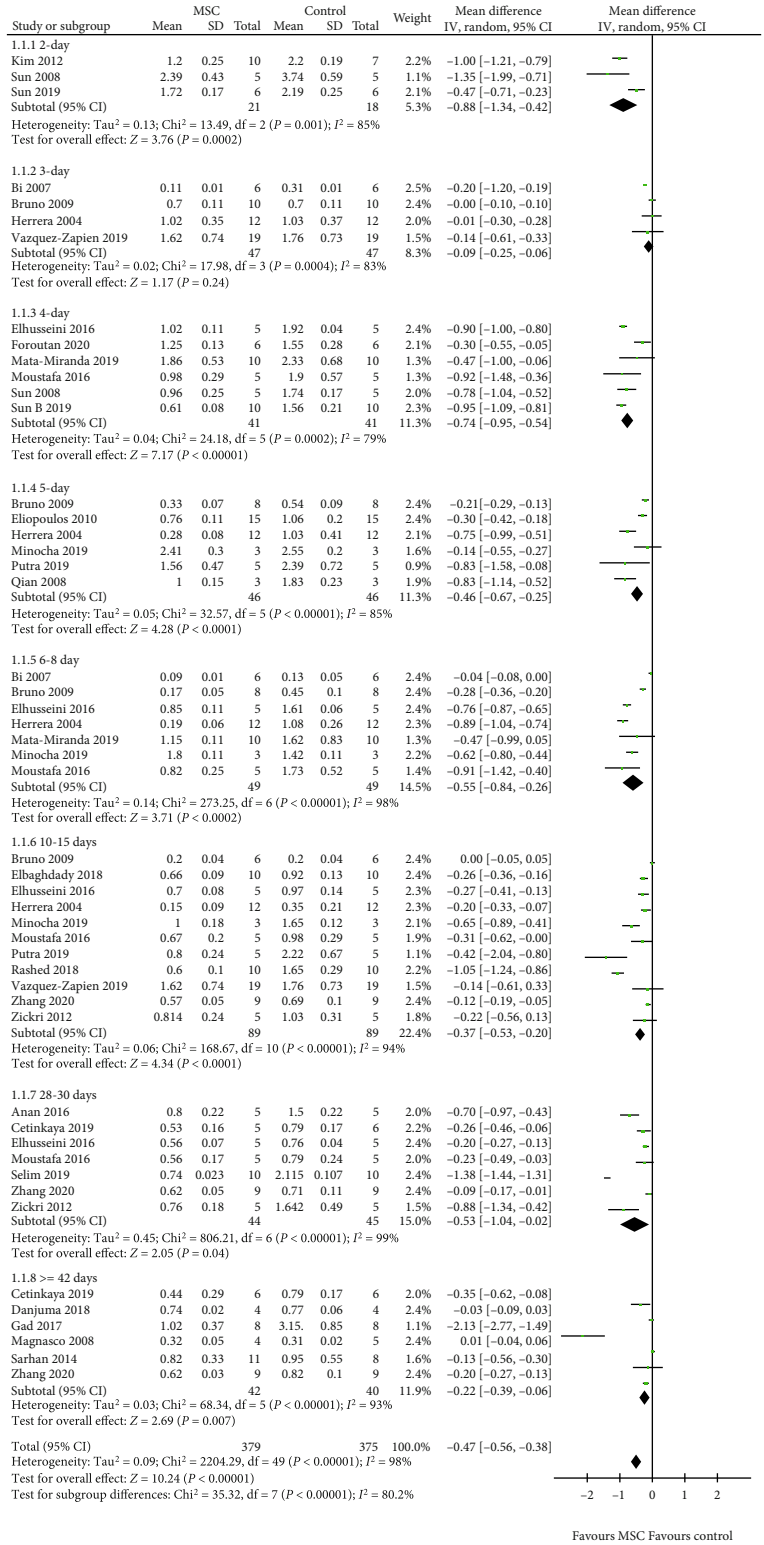


Figure 3: Effect of MSC on Scr.

Table 2: Meta-analysis of the efficacy of MSC in therapy of renal injury induced by toxicant.

Indicators	Time point	Studies number	Q test P value	Model selected	OR/WMD (95% CI)	P
Scr	2 days	3	0.001	Random	-0.88 (-1.34, -0.42)	0.0002
	3 days	4	0.0004	Random	-0.09 (-0.25, 0.06)	0.24
	4 days	6	0.0002	Random	-0.74 (-0.95, -0.54)	<0.00001
	5 days	6	<0.00001	Random	-0.46 (-0.67, -0.25)	<0.0001
	6-8 days	7	<0.00001	Random	-0.55 (-0.84, -0.26)	0.0002
	10-15 days	11	<0.00001	Random	-0.37 (-0.53, -0.20)	<0.0001
	28-30 days	7	<0.00001	Random	-0.53 (-1.04, -0.02)	0.04
	≥42 days	6	<0.00001	Random	-0.22 (-0.39, -0.06)	0.007
BUN	2-3 days	6	<0.00001	Random	-25.08 (-37.49, -12.67)	<0.0001
	4-5 days	8	<0.00001	Random	-45.71 (-59.36, -32.05)	<0.00001
	6-8 days	5	<0.00001	Random	-57.55 (-99.19, -15.91)	0.007
	13-15 days	4	<0.00001	Random	-13.40 (-32.34, 5.54)	0.17
	≥28 days	8	<0.00001	Random	-23.39 (-36.39, -10.40)	0.0004
UAE	—	3	0.72	Fixed	-22.66 (-26.41, -18.90)	<0.00001
MDA	—	4	0.41	Fixed	-17.21 (-20.38, -14.04)	<0.00001
GSH	—	4	<0.00001	Random	4.62 (2.74, 6.50)	<0.00001
SOD	—	3	<0.00001	Random	5.42 (2.92, 7.93)	<0.0001
Renal pathology						
Inflammatory cells	—	4	<0.00001	Random	-2.66 (-3.83, -1.49)	<0.00001
Necrotic tubule	—	2	<0.00001	Random	-2.58 (-4.75, -0.40)	0.02
Regenerative tubules	—	2	—	Fixed	6.00 (3.45, 8.55)	<0.00001
Renal interstitial fibrosis	—	3	<0.00001	Random	-5.82 (-7.41, -4.23)	<0.00001

Note: Scr: serum creatinine; BUN: blood urea nitrogen; UAE: urinary albumin excretion; Ccr: creatinine clearance rate; MDA: malondialdehyde; GSH: L-glutathione; SOD: superoxide dismutase.

regenerative tubules, and renal interstitial fibrosis between the MSC treatment and control groups was significant (inflammatory cells: WMD = −2.66, 95% CI: -3.83, -1.49, $P < 0.00001$; necrotic tubules: WMD = −2.58, 95% CI: -4.75, -0.40, $P = 0.02$; regenerative tubules: WMD = 6.00, 95% CI: 3.45, 8.55, $P < 0.00001$; renal interstitial fibrosis: WMD = −5.82, 95% CI: -7.41, -4.23, $P < 0.00001$; Table 2).

3.8. Publication Bias. Publication bias was tested in this meta-analysis, and a funnel plot generated using STATA 12.0 for the primary outcome. Begg's test and Egger's test results suggest that publication bias was present ($P \leq 0.01$ and $P \leq 0.01$, respectively; Figure 5).

4. Discussion

We reviewed all the selected studies and evaluated the Scr, BUN, UAE, oxidative stress, and renal pathology results to assess the nephroprotective effect of MSCs in the treatment of kidney disease induced by toxicants. We found that MSC treatment reduced Scr levels at 2, 4, 5, 6-8, 10-15, 28-30, and ≥42 days and reduced BUN levels at 2-3, 4-5, 6-8, and ≥28 days. We also found that the MSC group had a lower UAE than the control group. It has been previously shown that MSC treatment reduces the levels of Scr, BUN, and proteinuria in lupus nephritis in mice [38]. Chen et al. [39] found that MSC ameliorates ischemia/reperfusion injury-induced acute kidney injury in rats and reduces Scr levels. Xiu et al. [40] found that MSC transplantation significantly reduces the concentration of BUN and Scr, prevents tissue injury, and reduces mortality after lipopolysaccharide-induced acute kidney injury. Clinical trials also supported that MSC injection decreases rejection after transplantation. Tan et al. [41] found that the therapy with MSCs achieve better renal function and lower incidence of acute rejection at 1 year compared with the anti-IL-2 receptor antibody induction. Vanikar et al. [42] demonstrated that infusion of MSCs as well as hematopoietic stem cells eases immunosuppression in living donor renal transplantation. Our previous meta-analysis also found that MSCs reduce Scr levels, BUN levels, and proteinuria, as well as alleviate renal damage in animal models of AKI [43]. Lower proteinuria was also found in patients with SLE after MSC therapy [44].

The MSC treatment group had a higher level of GSH, SOD, and a lower level of MDA when compared with the control group. El-Metwaly et al. [45] found that MSCs increase GSH levels and reduce MDA levels in lung tissue of rats subjected to acute lung injury. Li et al. [46] reported that MSCs can restore the levels of GSH and MDA in rats with chronic alcoholism, and its effects on repairing sciatic nerve were obvious. Liu et al. [47] reported that MSCs significantly increase the activity of glutathione (GSH) and

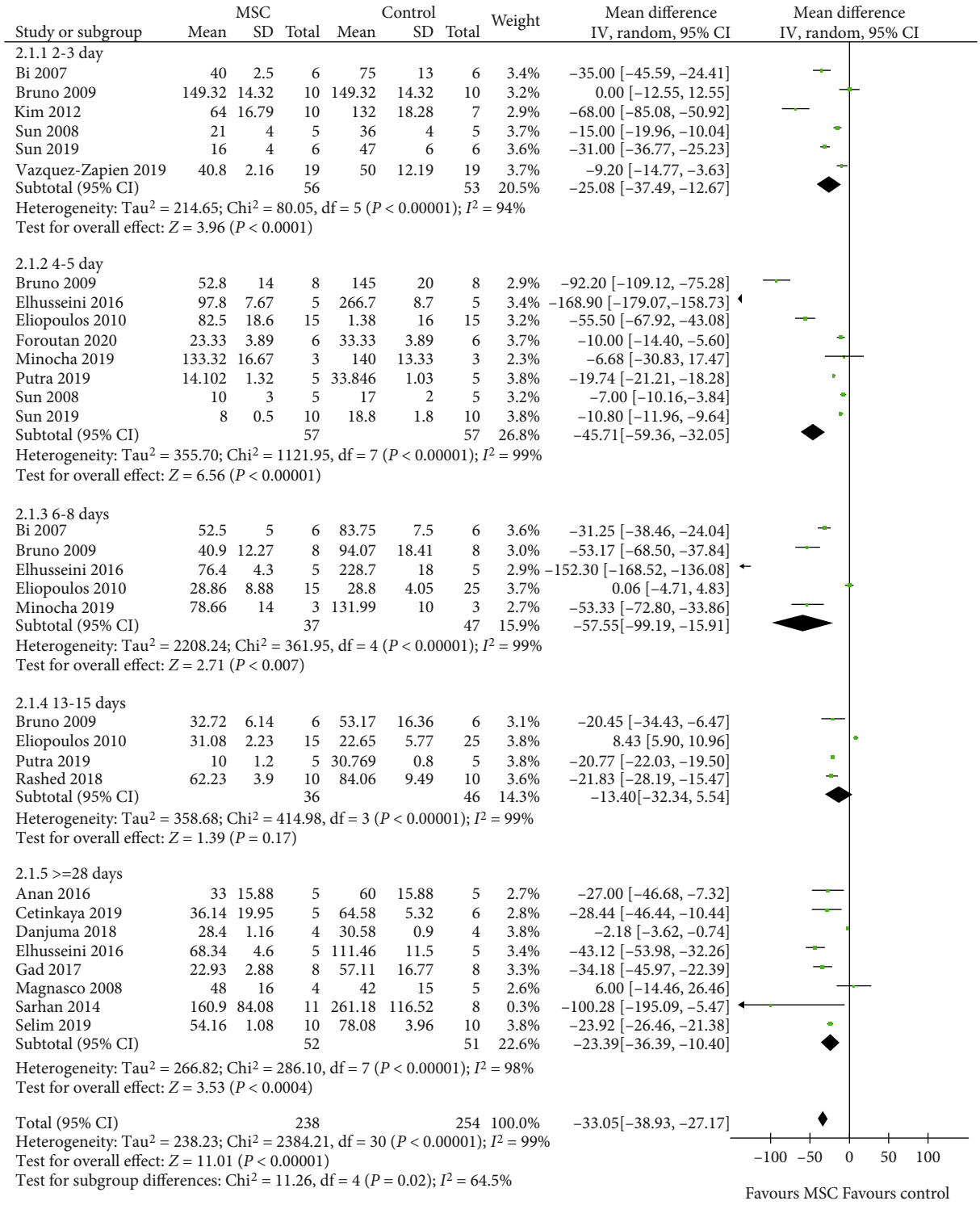


Figure 4: Effect of MSC on BUN.

reduce the levels of MDA in rats induced by unilateral ureteral obstruction.

The mechanism by which MSCs repair injured kidneys may be complex. After kidney injury, VCAM-1, GFP, SDF -1/CXCR4, and CD44 are upregulated in the injured tissue, which may play important roles in the migration of MSCs to the damaged area. These substances may be partly secreted by the MSCs themselves [20, 48, 49]. The presence of MSCs may limit the injury and repair the ischemic tubular damage to maintain the glomerular filtration rate and downregulate BUN [50]. In addition, MSCs lower the expression of several proinflammatory cytokines such as TNF-α, IL-1β, and IFN-γ

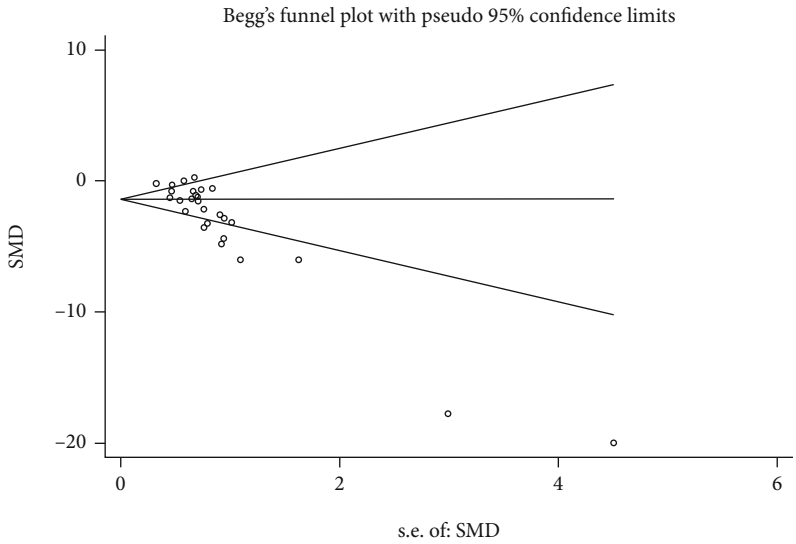


Figure 5: Publication bias.

as well as increase anti-inflammatory cytokines such as IL-1, IL-10, Bcl-2, TNF-α, bFGF, and prostaglandin E2 [49, 51]. Another possibility is that MSCs may restore damaged cells and prevent apoptosis by secreting microvesicles, which contain microRNAs, mRNAs, or proteins [49]. To conclude, MSCs can migrate to the damaged tissue, promote the recovery of renal function, enhance proliferation, and reduce fibrosis and inflammation.

Furthermore, our study indicates that MSC treatment can alleviate inflammatory cells, necrotic tubules, regenerative tubules, and renal interstitial fibrosis in kidney disease induced by toxicants. Some previous studies indicated that MSC treatment can alleviate renal pathological changes in unilateral ureteral obstruction rat or mice [9, 10, 52].

However, this meta-analysis also has some limitations. First, a small sample size was found for the recruited studies. The administered dose and the type of MSCs were not exactly the same. Publication bias was found in this meta-analysis, and the results should be reassessed in the future. Furthermore, the studies frequently had different animal models (mouse or rat), toxin doses, and administration routes for renal injury. These limitations may affect the robustness of our results.

5. Conclusions

The MSC treatment reduced Scr levels after 2, 4, 5, 6-8, 10-15, 28-30, and ≥42 days and reduced BUN levels after 2-3, 4-5, 6-8, and ≥28 days. The results also indicate that MSC treatment alleviated the inflammatory cells, necrotic tubules, regenerative tubules, and renal interstitial fibrosis in kidney disease induced by toxicants.

Abbreviations

MSCs: Mesenchymal stem cells
Scr: Serum creatinine
BUN: Blood urea nitrogen
UAE: Urinary albumin excretion
MDA: Malondialdehyde
GSH: L-glutathione
SOD: Superoxide dismutase
WMDs: Weighted mean differences
CI: Confidence intervals
M-H: Mantel-Haenszel.

Data Availability

The data supporting this meta-analysis are from previously reported studies and datasets, which have been cited. The processed data are available from the corresponding author upon request.

Consent

There are no human subjects in this article and informed consent is not applicable.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

TBZ contributed to the conception and design of the study. TBZ and SJL were responsible for collection of data and performing the statistical analysis and manuscript preparation. WSL and CLL were responsible for checking the data. All authors were responsible for drafting the manuscript, read, and approved the final version.

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Review Article

Mesenchymal Stem Cell Therapy for Diabetic Kidney Disease: A Review of the Studies Using Syngeneic, Autologous, Allogeneic, and Xenogeneic Cells

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Diabetic kidney disease (DKD) is a microvascular complication of diabetes mellitus (DM) and comprises multifactorial pathophysiologic mechanisms. Despite current treatment, around 30-40% of individuals with type 1 and type 2 DM (DM1 and DM2) have progressive DKD, which is the most common cause of end-stage chronic kidney disease worldwide. Mesenchymal stem cell- (MSC-) based therapy has important biological and therapeutic implications for curtailing DKD progression. As a chronic disease, DM may impair MSC microenvironment, but there is compelling evidence that MSC derived from DM1 individuals maintain their cardinal properties, such as potency, secretion of trophic factors, and modulation of immune cells, so that both autologous and allogeneic MSCs are safe and effective. Conversely, MSCs derived from DM2 individuals are usually dysfunctional, exhibiting higher rates of senescence and apoptosis and a decrease in clonogenicity, proliferation, and angiogenesis potential. Therefore, more studies in humans are needed to reach a conclusion if autologous MSCs from DM2 individuals are effective for treatment of DM-related complications. Importantly, the bench to bedside pathway has been constructed in the last decade for assessing the therapeutic potential of MSCs in the DM setting. Laboratory research set the basis for establishing further translation research including preclinical development and proof of concept in model systems. Phase I clinical trials have evaluated the safety profile of MSC-based therapy in humans, and phase II clinical trials (proof of concept in trial participants) still need to answer important questions for treating DKD, yet metabolic control has already been documented. Therefore, randomized and controlled trials considering the source, optimal cell number, and route of delivery in DM patients are further required to advance MSC-based therapy. Future directions include strategies to reduce MSC heterogeneity, standardized protocols for isolation and expansion of those cells, and the development of well-designed large-scale trials to show significant efficacy during a long follow-up, mainly in individuals with DKD.

1. Introduction

1.1. Epidemiology. The global diabetes mellitus (DM) prevalence in 2019 was estimated at 9.3% (463 million) in adults aged 20-79 years, rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1]. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income (4.0%) countries. Of importance, one in two (50.1%) people living with DM does not know that they have DM. Therefore, almost half a billion people are living with diabetes worldwide, and the number is projected to increase by 25% in 2030 and 51% in 2045. Likewise, the global prevalence of impaired glucose tolerance is estimated to be 7.5% (374 million) in 2019 and projected to reach 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045 [1].

Using the WHO (World Health Organization) database, the International Diabetes Federation documented that 8.4% of all-cause deaths were attributable to DM in adults aged 20–79 years, almost 5.1 million deaths [2]. A sensitivity analysis adjusting relative risks by 20% found that the estimate of DM-attributable mortality lies between 5.1% of total mortality (3.3 million deaths) and 10.1% of total mortality (6.6 million deaths) [2]. Overall, 1 in 12 global all-cause deaths was estimated to be attributable to DM in adults [2].

Diabetic kidney disease (DKD) is a microvascular complication of DM and the most common cause of end-stage kidney disease (ESKD) worldwide, with approximately 30% of patients with type 1 DM (DM1) and approximately 40% of patients with type 2 DM (DM2) developing DKD, as reviewed elsewhere [3]. DKD accounts for cardiovascular complications and the high mortality rate of patients with DM. In the United States, the unadjusted prevalence of CKD stages 1-5 (not including ESKD) was estimated to be 14.8% (from 2011 through 2014), with stage 3 being the most prevalent stage [4]. There is an increase of 1.1% per year of new cases of ESKD, and the active waiting list is 2.8 times larger than the availability of donor kidneys.

1.2. Pathophysiology of DKD. Natural history of DKD comprises hyperfiltration, progressive albuminuria, decrease in eGFR (estimated glomerular filtration rate), and, ultimately, ESRD. Yet, albuminuria is a continuum; eGFR deterioration can start to decline before progression to overt nephropathy, which can be explained by other risk factors, such as obesity, hypertriglyceridaemia, hypertension, and glomerular hyperfiltration [5]. Thus, albuminuria and eGFR predict the progression of renal impairment in DM1 and DM2 individuals with DKD. Classification of DKD is summarized as follows: (i) stage 1 (prenephropathy): normoalbuminuria (<30 g/g Cr) and eGFR $\geq$ 30 ml/min/1.73 m^2, (ii) stage 2 (incipient nephropathy): microalbuminuria (30-299 g/g Cr) and eGFR $\geq$ 30 ml/min/1.73 m^2, (iii) stage 3 (overt proteinuria): macroalbuminuria ($\geq$300 g/g Cr) or persistent proteinuria ($\geq$0.5) and eGFR $\geq$ 30 ml/min/1.73 m^2, (iv) stage 4 (kidney failure): any albuminuria status and eGFR < 30 ml/min/1.73 m^2, and (v) stage 5 (renal replacement therapy): any status on continued dialysis therapy [6].

Histologically, metabolic changes associated with DM lead to glomerular hypertrophy, glomerulosclerosis, arteriolar hyalinosis, arteriosclerosis, tubule-interstitial inflammation, and fibrosis. The main glomerular changes consist of thickening of the glomerular basement membrane (GBM), expansion of the mesangial matrix, atrophy and loss of podocyte pedicels associated with effacement, and diffuse or nodular intercapillary glomerulosclerosis (Kimmelstiel-Wilson lesion) [3].

Systemic inflammatory milieu due to metabolic dysregulation (hyperglycemia, hyperlipidaemia, insulin resistance, and β-cell dysfunction) and haemodynamic changes (systemic hypertension) characterizes DKD pathophysiology. In addition, DKD is associated with endothelial dysfunction; activation of RAAS (renin-angiotensin-aldosterone system); increase in AGEs (advanced glycation end products); elevation of NADPH oxidase; upregulation of GLUT1; generation of reactive oxygen species (ROS); upregulation of growth factors, such as VEGF (vascular endothelial growth factor) and TGF-β (transforming growth factor-β); activation of aldose reductase and the polyol pathways; mitochondrial dysfunction; downregulation of adiponectin; and nitric oxide (NO) loss, as reviewed elsewhere [7, 8]. Those derangements entail adverse effects on the renal system, such as oxidative stress; apoptosis; autophagy dysfunction; intracellular signaling cascade activation, such as protein kinase C (PKC)/mitogen-associated protein kinase (MAPK) and subsequent NF-κB; and inflammation, which is associated with inflammatory interleukins (IL), cytokines, and chemokines (IL-1, IL-6, IL-18, TNF-α (tumor necrosis factor-α), CSF-1 (colony stimulating factor-1), MCP-1 (monocyte chemoattractant protein-1), and MIF (macrophage inflammatory factor)). Exacerbated production of profibrotic cytokines (CTGF (connective tissue growth factor) and TGF-β) associated with fibrosis is also involved in DKD. Collectively, all those mechanisms contribute to DKD progression and to both functional (declining eGFR and proteinuria) and structural (fibroblast accumulation, mesangial cell expansion and proliferation, extracellular matrix accumulation, GBM thickening, podocyte loss/dysfunction, tubule-interstitial dysfunction, and endothelial dysfunction) kidney damage, which lead ultimately to systemic complications (ESKD, cardiocerebrovascular events, vascular events, neuropathy, and death).

1.3. Treatment. Due to DM prevalence worldwide, it is crucial to develop cost-effective strategies at every step: (1) prevention of obesity, (2) screening for and prevention of diabetes in an at-risk population, (3) glycemic control once diabetes develops, (4) blood pressure (BP) control once hypertension develops, (5) screening for diabetic chronic kidney disease (CKD), (6) RAAS inhibition/blockade in those with diabetic CKD, and (7) control of other cardiovascular (CV) risk factors such as management of low-density lipoprotein cholesterol (LDL-C) [9, 10].

Despite diabetic patients being treated with angiotensin-receptor-blockers (ARBs), renal disease progression risk over 2 years increases with increasing proteinuria and albuminuria and decreasing eGFR [11]. To note, RAAS inhibition possesses remarkable renoprotective effect when used in earlier stages of renal disease, whereas in late stages, that approach has less efficacy [12]. Yet, the combination of ARBs and angiotensin-converting-enzyme (ACE) inhibitors is a robust approach to block RAAS; it was associated with an increased risk of adverse events, such as acute kidney injury and hyperkalemia [13].

Novel drugs have been recently associated with clinical benefit profiles, which should be considered in the decision-making process when treating patients with DM2. Glucagon-like peptide 1 receptor agonists (GLP1-RA) and sodium-glucose cotransporter-2 inhibitors (SGLT2i) reduce atherosclerotic major adverse cardiovascular (CVs) events to a similar degree in patients with established atherosclerotic CV disease, whereas SGLT2i have a more marked effect on preventing hospitalization for heart failure and progression of DKD [14, 15].

In the DKD treatment setting with drugs and lifestyle changes, novel approaches are further required to halt the progression of DKD or regenerate the damaged tissue, such as cell therapy [16]. In this review, we will focus on both *in vitro* and *in vivo* studies using syngeneic, autologous, allogeneic, or xenogeneic mesenchymal stem cells (MSCs) for treating DKD. We will describe the main findings of MSC-based therapy in preclinical and clinical studies and discuss the benefits, outcomes, and challenges of that therapy for halting DKD progression.

2. Mesenchymal Stem Cells (MSCs)

MSCs, commonly referred to as mesenchymal stem cells or mesenchymal stromal cells, are a diverse population of cells with a wide range of potential therapeutic applications for different organs and tissues. MSCs can be obtained from many tissue sources, consistent with their broad, possibly ubiquitous distribution.

Historically, MSCs were isolated from bone marrow (BM-MSC) and spleen from guinea pigs by Friedenstein et al. [17]. They observed that BM-MSCs were plastic adherent cells and were capable of forming single-cell colonies. When BM-MSCs are expanded in culture, round-shaped colonies resembling fibroblastic cells are formed and subsequently identified by a Colony Forming Unit-fibroblast (CFU-f) assay. They were the first to demonstrate that BM-MSCs exhibited multipotential capacity to differentiate into mesoderm-derived tissues.

BM-MSCs can be isolated by (a) using gradient centrifugation (Ficoll or Percoll) to separate nonnucleated red blood cells from nucleated cells, (b) taking advantage of their ability to adhere to plastic, (c) taking advantage of the ability of monocytes to be separated from BM-MSCs by trypsinization [18].

During the 1980s, BM-MSCs were found to be able to differentiate into osteoblasts, chondrocytes, adipocytes, and muscle tissue [19]. In the 1990s, BM-MSCs were shown to differentiate into ectodermal-derived tissue [20, 21]. During the early 21st century, *in vivo* studies documented that human BM-MSCs differentiated into endodermal-derived cells [22, 23], cardiomyocytes [24], and renal mesangial and epithelial tubular cells [25, 26]. However, their efficiency to differentiate into other tissues is extremely low *in vivo* and therefore is not the main mechanism of tissue repair and regeneration.

More recently, BM-MSC secretome has demonstrated potential clinical applications and includes both soluble proteins (cytokines, chemokines, growth factors, and proteases) and factors released in extracellular vesicles, for example, microvesicles (size 100-1000 nm) and exosomes (EXOs; size 40-100 nm) [27]. These extracellular vesicles contain proteins, lipids, mRNA, and miRNA and rarely DNA [28]. Mitochondria or mitochondrial DNA can also be transferred by extracellular vesicles or nanotubes built between cells that are regulated by dynamin-related proteins Miro-1 and Miro-2 [29]. Therefore, BM-MSC secretome is involved in cell survival and growth, immune modulation, and attenuation of fibrosis. High-resolution proteomic and lipidomic analyses have shown that key regulators of some pathways are enriched in both microvesicles and EXOs, including GTPase activity, translation, vesicle/membrane, and glycolysis, whereas other pathways are enriched more in microvesicles (cell motion, mitochondria, endoplasmic reticulum, and proteasome) and others in EXOs (extracellular matrix, binding, immune response, and cell adhesion) [30].

Of importance, MSCs possess ubiquitous distribution in perivascular niches and can be derived and propagated *in vitro* from different organs and tissues (AT, amniotic fluid, BM, brain, cord blood, dental pulp, kidney, liver, lung, muscle, pancreas, placental membranes, spleen, thymus, and large vessels, such as aorta artery and vena cava) [31, 32]. Most frequent sources of MSC isolation include BM, adipose tissue (AT-MSC), and umbilical cord blood (UCB-MSC). In BM, one in 10,000 nucleated cells is a MSC. To note, 1.0 g of aspirated AT yields approximately 3.5×10^5-1×10^6 AT-MSCs. This is compared to 5×10^2-5×10^4 of BM-MSCs isolated from 1.0 g of BM aspirate [33].

Isolation of MSCs from AT is based on mincing fat tissue, followed by several washings in order to remove contaminating hematopoietic cells, incubation of tissue fragments with collagenase, and centrifugation of the digest, thereby separating the floating population of mature adipocytes from the pelleted stromal vascular fraction [34]. UCB-MSC is also a straightforward protocol and consists in carefully dissecting the UC into two regions, e.g., the cord lining and Wharton's jelly. After cutting the UCB longitudinally, it is necessary to scrape Wharton's jelly away from the blood vessels and inner epithelium and then remove the blood vessels. After collecting any remaining perivascular Wharton's jelly tissue under and around the blood vessels, which represents the cord lining, the digestion of that tissue with trypsin will allow the adherence of tissue pieces and the egression of MSCs in 2-3 days, as briefly described elsewhere [35].

MSC populations originating from different tissues and organs exhibit similar morphology and, to a certain extent, surface marker profile [31]. On the other hand, differentiation assays indicate some variation among cultures in the frequency of cells that possess the capacity to differentiate into osteogenic or adipogenic lineages. For example, vena cava-derived MSCs were very efficient at depositing a mineralized matrix, whereas muscle-derived MSCs showed little efficiency for osteogenic differentiation, as opposed to an inverse capacity of adipocyte differentiation of these cells [31]. Conversely, adipogenic differentiation observed in lung-, brain-, and kidney-derived MSCs seemed to be less efficient. Likewise, UCB-MSCs exhibit significantly stronger osteogenic capacity but lower capacity for adipogenic differentiation in comparison to BM-MSCs [36]. Of importance, AT-MSCs exhibit similar capacity of differentiation when compared to BM-MSCs [37].

The International Society for Cell Therapy (ISCT) established the characteristics of MSCs from all sources, either autologous or allogeneic: (1) adherence to plastic under standard culture conditions; (2) expression of CD73, CD90, and CD105 surface molecules in the absence of CD34, CD45, HLA-DR, CD14 or CD11b, CD79, or CD19 surface molecules, as assessed by flow cytometry analysis; and (3) differentiation capacity for osteoblasts, adipocytes, and chondroblasts *in vitro* [38]. In comparison to fibroblasts, both cells express CD44 and CD49b, whereas CD20, CD31,

CD33, CD117, and CD133 are negative in both cells. Some markers are only expressed in MSCs (CD10, CD26, CD54, CD106, CD146, and ITGA11), as well as the potential of colony forming [39].

A recent update from ISCT includes analyses that mitigate the heterogeneity of MSCs, such as assays that demonstrate the secretion of trophic factors, the modulation of immune cells, and other relevant functional properties, such as angiogenesis [40]. The ISCT MSC committee recommended that the studies should describe (i) tissue source origin of MSCs, which would highlight tissue-specific properties; (ii) the stemness properties described by both *in vitro* and *in vivo* data; and (iii) a robust matrix of functional assays to demonstrate the properties of these cells associated with the intended therapeutic mode of actions. In addition, basic assays for MSC-based products comprise donor screening, viability test, purity test (residual contaminant tests and pyrogenic/endotoxin tests), safety test (bacterial, fungal, mycoplasma, viral tests, and tumorigenicity assays), identity tests (immunophenotypic profiles), and potency tests (multilineage differentiation, secretion profiles, CFU-f assay, and immunosuppressive assay). All of these procedures should be done in a Good Manufacturing Practice (GMP) facility.

To assess MSC self-renewal capacity, doubling time and CFU-f are broadly used. In a particular DM setting, immunological assays can be based on activation protocols that discriminate between TLR- (Toll-like receptor-) 4-dependent phenotype MSC-1 and TLR3-dependent MSC-2 phenotype [41]. That polarization may be achieved with short-term incubation (1 h) with LPS (10 ng/ml) or poly(I:C) (poly-deoxy-inosinic-deoxy-cytidylic acid) (1 mg/ml), respectively, followed by incubation for 24 to 48 h in growth medium, since LPS acts as an agonist for TLR4 and poly(I:C) acts as an agonist for TLR3. Another approach for assessing MSC-based immunomodulatory properties would be based on the coculture of MSCs with cells of the immune system by the (a) stimulation of MSCs with IFN-γ (IFN-γ primed MSCs) and subsequent analysis of various ribonucleic acids (IDO, CXCL9, CXCL10, CXCL11, CIITA, HLAD, and PDL1 or CD274, ICAM-1 or CD54, TLR3, TRAIL, and CCL5) and (b) coculture of MSCs with human peripheral blood mononuclear cells (PBMCs) and analysis of the signature of the secretome in relation to cytokine/chemokine secretion and T cell proliferation [42]. Coculture of MSCs and B-lymphocytes and NK cells may also be a useful strategy to assess MSC-based immunomodulatory properties. To note, such assays are important in addressing MSCs before and after freezing. It is also worth mentioning that PBMCs should be used from donors that show a normal pattern of proliferation and without much variability.

2.1. In Vitro Studies: Recapitulation of DKD Microenvironment for Evaluating the Therapeutic Potential of MSCs. To recapitulate, the *in vitro* DKD milieu is challenging since cell-cell and cell-matrix interaction is severely affected during disease progression. The most frequent approach is to evaluate the cells under normal glucose medium (5.5 mmol/l) and high glucose (25-30 mmol/l or less frequent 40 mmol/l). Mannitol (20 mmol/l) associated with normal glucose (5.5 mmol/l) is used as a control of osmolality. Peroxide hydrogen and TNF-α may be added to the medium as inducers of oxidative stress [43] and inflammation [44], respectively. The coculture of MSCs, MSC-conditioned medium or EXOs, and different types of renal cells represents a platform in which the DKD microenvironment may be recapitulated. The most appropriate approach to recreate DKD *in vitro* (high glucose, peroxide hydrogen, and TNF-α), the amount of cells (ratio of MSCs and renal cells), the type of cell interaction (direct versus indirect, e.g., using a Transwell® chamber), and duration of the coculture (6 h, 12 h, 24 h, 48 h, 72 h, or 96 h) were broadly tested in the literature.

Immortalized mouse podocytes cultured in high glucose medium and cocultured directly with BM-MSC transfected with miR124a, for 24 h, exhibited increased viability and decreased apoptosis (decrease in caspase-3 and Bax gene expression and increase in Bcl2 gene expression) [45]. Mouse podocytes (MPC5 cells) treated with high glucose medium and cocultured with AT-MSC-derived EXOs, for 24 h, 48 h, 72 h, and 96 h, exhibited less apoptosis in concentration- and time-dependent manners [46]. Mechanistically, AT-MSC-derived EXOs enhanced autophagy flux and reduced podocyte injury by inhibiting the activation of mTOR/SMAD1 signaling and increasing miR-486 expression.

For glomerular mesangial cells (GMCs) cultured in high glucose medium, direct coculture with BM-MSC (ratio 10 : 1) or MSC-conditioned medium for 72 h decreased equally TGF-β and phosphorylated SMAD2/3 proteins, which were abrogated by BMP-7 antibody [47]. Likewise, GMC cultured in a high glucose medium and cocultured with BM-MSC (4×10^5 cells/well) in a Transwell® chamber for 72 h led to an increase in lipoxin A4, a key lipid involved in inflammation resolution [48].

For renal tubular epithelial cells (TECs) cultured in high glucose medium, the coculture for 24 h with AT-MSC (1×10^5 cells/well) using a Transwell® chamber inhibited apoptosis of those cells, induced klotho expression, and downregulated the Wnt/β-catenin signaling pathway [49]. In addition, high glucose medium supplemented with TNF-α may also mimic the DKD microenvironment [44]. In that study, proximal TECs (HK2) were cocultured with UCB-MSC in a Transwell® chamber at a 5 : 1 ratio, for 72 h, in high glucose medium and TNF-α. UCB-MSC increased cell viability, ATP production, and E-cadherin expression, as opposed to a decrease in fibronectin, SGLT2, pNF-κB p65, and MCP-1.

Not only MSCs but also EXOs cocultured for 96 h with TECs in primary renal cell culture of streptozotocin- (STZ-) induced diabetic rats entailed in antiapoptotic and antidegenerative effects (increase in ZO-1 and lectin expression and decrease in TGF-β1) [50].

Endothelium may also be damaged during DM progression. Thus, the murine islet microvascular endothelium cell line experienced apoptosis and endothelial cell activation (increase in VCAM (vascular cell adhesion molecule) expression and reduction in eNOS (endothelial nitric oxide synthase) phosphorylation) upon H_2O_2 conditioning, which

was abrogated by MSC treatment and activation of the β-catenin-dependent Wnt signaling pathway [51].

Therapeutic potential of MSCs can also be verified in a coculture platform with other cells that play a role in DKD progression, such as macrophages. Indirect coculture of BM-MSC (3×10^4 cells/well) with LPS-treated macrophages (rat peritoneum; 1.5×10^5 cells/well), at a 1 : 5 ratio for 6 h, led to a decrease in IL-1β, IL-6, MCP-1, and TNF-α expression [52]. Coculture of immortalized macrophage cell line (RAW264.7) with human UBC-MSCs (at a 2 : 1 ratio), for 24 h, suppressed LPS-induced M1 macrophage polarization (decrease in inflammatory proteins, such as IL-1β, TNF-α, IL-6, and iNOS (inducible nitric oxide synthase)), which was mediated by the increase in arginase 1 production [53]. To note, iNOS metabolizes arginine to nitric oxide and citrulline, whereas arginase (M2-macrophage) hydrolyzes arginine to ornithine and urea. Therefore, the arginase pathway limits arginine availability for nitric oxide synthesis, and ornithine itself can further lead to polyamine and proline synthesis, which have important biological implications for proliferation and tissue repair. In addition, MSC-conditioned medium reversed cytokine-mediated mitochondrial dysfunction in HK2 cells (TECs) by increasing mitochondria mass and biogenesis and decreasing ROS production [53].

Aging has also an adverse impact on MSC function and possesses biological and therapeutic implications. Moreover, CKD and DM are linked to accelerated aging. The p66 protein is related to aging and controls cellular response to oxidative stress, senescence, and apoptosis. Renal-derived Sca-1^+ MSCs from p66 knockout mice and cultured in high glucose medium exhibited higher rates of proliferation; decreased senescent proteins (p53, p21, and $p16^{INK4a}$); higher levels of IGF-1 (insulin growth factor-1), HGF (hepatocyte growth factor), and VEGF; and upregulation of β-catenin signaling when compared to renal-derived Sca-1^+ MSC from wild-type mice [54].

2.2. Preclinical Studies: Small and Large Animals. MSC-based therapy is a promising strategy for accelerating kidney recovery, repairing and regenerating tissue damage after acute injury following ischemia-reperfusion, kidney transplant, and drug-mediated toxicity, as reviewed elsewhere [55]. In a meta-analysis including MSC from rat and mice (~200 animals treated) and different types of acute and chronic kidney injury (but not DKD), routes of delivery (intravenous, intrarenal, intraperitoneal, and intra-arterial), and MSC number (range, 7.5×10^4-3.0×10^6), the beneficial outcomes for kidney recovery favored MSC treatment [56].

Of importance, MSC efficacy is challenged by several factors, such as viability, cell source, MSC phenotype, homing capacity, route of delivery, site of infusion, number of infusions, cell passage, cell potency, severity of condition, and target impact [57]. In the sensitivity analysis of that meta-analysis, there was a trend toward greater reduction in serum creatinine of the MSC-treated group when compared with the control group regarding the MSC number (>106), arterial route (versus intravenous route), model of injury (ischemia-reperfusion injury versus toxic and chronic injury), and late administration (>1 day after injury) [56]. Thus, these data provided insightful information in terms of MSC efficacy and safety in preclinical models and paved the way for studies in other kidney diseases, such as DKD.

Next, we discuss some key aspects of MSC-based cell therapy in preclinical studies.

2.2.1. MSC Phenotype. Emerging concepts indicate that MSCs may function as sensors and switchers of inflammation, which may explain their immunomodulatory properties [58, 59]. In an inflammatory environment associated with high levels of IFN-γ (interferon-γ) and TNF-α, MSCs acquire an immunosuppressive phenotype (MSC2) and through Toll-like receptor- (TLR-) 3 lead to an increase in production of TGF-β, IDO (indoleamine 2,3-dioxygenase), NO (nitric oxide), and PGE2 (prostaglandin E2). These events stimulate the amount of $CD4^+CD25^+FoxP3^+$ T regulatory cells. Conversely, in the absence of an inflammatory environment (low levels of IFN-γ and TNF-α), MSCs acquire a proinflammatory phenotype, and through TLR4, LPS (lipopolysaccharide), and high levels of chemokine C-X-C motif ligand (CXCL)9, CXCL10, MIP- (macrophage inflammatory protein-) 1α, MIP-1β, and CCL5/RANTES (regulated on activation, normal T cell expressed and secreted), but low levels of IDO, NO, and PGE2, activation of cytotoxic T lymphocytes is triggered.

Interaction of MSCs and monocytes play also a key role in our understanding of mechanisms of MSC-mediated tissue regeneration [58, 59]. When MSCs acquire an immunosuppressive phenotype (high levels of IDO and PGE2) in the presence of IL-6, there is a polarization from monocytes (M0) to macrophage anti-inflammatory phenotype (M2 macrophages; CD206 and CD163 expression; production of high levels of IL-6 and IL-10). On the other hand, proinflammatory MSC-induced phenotype may lead to polarization from M0 to proinflammatory macrophage (M1 macrophage; CD86 expression; production of high levels of IFN-γ and TNF-α).

However, further investigation is warranted to verify whether MSC phenotype changes in accordance with DKD progression. In other settings, such as kidney transplant, MSC infusion posttransplant allowed their preferential recruitment in the inflammatory milieu of the graft created by ischemia/reperfusion injury, and once in that environment, MSC contributed to upregulation of inflammation, thereby causing premature graft dysfunction [60]. By contrast, autologous BM-derived MSC infusion induced a significant prolongation of kidney graft survival by a T cell regulatory-dependent mechanism when a protocol biopsy showed signs of subclinical rejection and/or an increase in interstitial fibrosis/tubular atrophy 4 weeks or 6 months posttransplantation [61]. Additionally, autologous BM-derived MSC, when injected before living-related kidney transplant, led to a decrease in the circulating memory $CD8^+$ T lymphocytes and donor-specific $CD8^+$ T lymphocyte cytolytic response [62] and might induce tolerance [63].

2.2.2. Routes of MSC Delivery. Stem cell route delivery (intravenous, intra-arterial, or intraparenchymal) may affect MSC efficiency for kidney repair and regeneration in different

models of acute and chronic kidney injury. The intravenous route is the route used most often, to inject not only MSCs [64–67] but also different kidney-derived progenitor/stem cells [68, 69] in several models of acute and chronic kidney injury in rodents. To note, MSCs, BM-derived mononuclear cells (BM-MNCs), and other kidney progenitors are initially trapped inside the pulmonary microvasculature following intravenous administration [70]. In line with these findings, the number of cells, multiple intravenous injections, and cell size increase the chance of pulmonary trapping, as murine MSCs measure 15-19 μm [70, 71]. Similar observations were reported in nonhuman primates when MSCs were injected intravenously [72, 73]. Sodium nitroprusside pretreatment, a vasodilator, may reduce mouse MSC trapping in the lungs [71] and require further analyses of its efficiency in larger animals.

However, infused human MSCs are able to migrate beyond the lungs after intravenous administration in a rodent model of cisplatin-induced acute kidney injury and may be detected in peritubular areas, where they ameliorated renal cell apoptosis and increased cell proliferation [74].

Intra-arterial routes for delivering progenitor/stem cells include intracarotid [75], intracardiac [76], or intra-aorta [77–81]. When the intra-aorta route is employed, the clamps can be applied above and below the renal arteries [77, 78] or only below the renal arteries [79–81], which can be challenging in small animals [82]. Bioluminescence analyses supported a distinct localization of MSCs in the murine kidneys submitted to ischemia-reperfusion injury when these cells were injected in the suprarenal aorta (intracarotid), in contrast to intrajugular vein injection, which was associated with predominant accumulation of cells in both lungs [83]. In larger animals (ovine), autologous MSCs delivered through renal arteries were also effective in reducing tubular injury after ischemia-reperfusion injury [84].

Although intraparenchymal (under renal capsule) administration of progenitor/stem cells or MSCs has beneficial effect on kidney repair [79, 85–89], this route is less practical for clinical application, especially when the renal disease is diffuse and technical issues limit a broader use, such as haemorrhage. However, the bioengineering field has undergone considerable evolution, so that MSC sheets may be transplanted directly into the kidneys and suppress the progression of DKD in rats [90].

2.2.3. MSC Homing (CXCR4 and SDF-1 Axis). Stromal-derived factor-1 (SDF-1), also known as CXCL12, and its receptor C-X-C chemokine receptor 4 (CXCR4) axis is a crucial key pathway in cell trafficking.

After acute kidney injury, the levels of SDF-1 mRNA levels increase more than 2.5-fold and remain high as ~2.0-fold after 24 h within kidney cortex tissue [91]. That increase leads to homing and migration of CXCR4-expressing cells in the injured kidneys. However, MSCs, which express CXCR4, migrate to damaged tissues with limited efficiency. Therefore, CXCR4 gene-modified BM-MSCs lead to accumulation of these cells in the injured kidney and activation of PI3K/AKT and MAPK signaling pathway [92], which represents a promising strategy for advancing MSC-based therapy.

2.2.4. Animal Models of Diabetic Kidney Disease. There are several animal models of DKD in rodents, which mimics DM in humans either DM1 or DM2. Therefore, DM and subsequent DKD can be obtained by genetic manipulation, induced by drugs (streptozotocin or STZ) or high-fat diet, or even a combination of approaches, including uninephrectomy to accelerate DKD progression, as reviewed elsewhere [16].

Thus, pharmacologic induction of DKD with STZ, with or without accelerating factors, such as high-fat diet, uninephrectomy, or use of the nonobese diabetic (NOD) strain, has been the most common rodent model of DKD to study the potential therapy of MSCs [7].

The Animal Models of Diabetic Complications Consortium (AMDCC) defined the following criteria for validating a progressive mouse model of DKD [93]: (i) greater than 50% decline in GFR over the lifetime of the animal; (ii) greater than 10-fold increase in albuminuria compared to controls for the strain at the same age and gender; and (iii) kidney-specific histopathology induced by DM: advanced mesangial matrix expansion ± nodular sclerosis and mesangiolysis, any degree of arteriolar hyalinosis, and GBM thickening by >50% over baseline tubule-interstitial fibrosis.

Recent models of DM1 (E1-DKD; expression of a kinase-negative epidermal growth factor receptor in pancreatic islet cells e) and DM2 (BTBR$^{ob/ob}$; knockout for leptin) that reflect human DKD [94, 95] may represent promising models to verify not only stem cell-based therapy but also drug, gene, nanoparticle, and other approaches to halt DKD progression [16]. E1-DKD and BTBR$^{ob/ob}$ models develop proteinuria in a time-dependent manner, mesangial expansion, thickening of GBM, widening of podocyte foot process, podocyte apoptosis, glomerular sclerosis, and reduction of podocyte genes and protein. Notably, BTBR$^{ob/ob}$ mice comprise a reversible model of DM upon leptin administration [96], which indicates, therefore, a robust model to test MSC therapeutic potential.

NOD mice develop autoimmune insulitis caused by polygenes including specific MHC class II alleles and many non-MHC loci, mimicking DM1 [97]. NOD mice develop albuminuria associated with enlarged glomeruli and mesangial sclerosis. An insulin-2 Akita mouse exhibits an autosomal dominant mutation in the Ins-2 gene that causes misfolding of insulin protein [97]. These mice develop increased mesangial matrix and GBM thickening, but no mesangiolysis or widespread marked or nodular mesangial sclerosis. Similarly, the *db*/*db* mouse is a model of DM2, which develops hyperglycemia, obesity, and albuminuria due to a G-to-T mutation in the gene coding the leptin receptor (*db*/*db*) [97]. They develop glomerular hypertrophy, mesangial matrix expansion, and GBM thickening, but no mesangiolysis or nodular mesangial sclerosis. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat model of hyperphagia-induced obesity due to a spontaneous lack of CCK_1 (cholecystokinin) receptors represents a broadly established model of DM2, which develops proliferation of the mesangial matrix, GBM thickening, diffuse glomerulosclerosis, nodular lesions, tubular atrophy associated with mononuclear cell infiltration, and fibrosis [97]. Other rodent models of DM2 and DKD include GK rat, NZO mouse, KK-Ay mouse, and ZDF rat, as reviewed elsewhere [97].

In Table 1, we document the preclinical studies, including the MSC source, number of cells and injections, route of delivery, and outcomes in the DKD setting [44, 45, 47, 48, 49, 50, 52, 90, 98–118]. The majority of the studies comprised syngeneic MSCs obtained from BM, single-dose injection via an intravenous route, and successful outcomes for halting DKD progression.

Briefly, these studies provided evidences that MSC-based therapy may decrease fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) in either DM1 or DM2 animals, and in DM1 animals, plasmatic insulin levels increased or exogenous insulin requirement decreased. Likewise, MSC-based therapy has important therapeutic implications in the DKD setting, providing insights into cellular and molecular mechanisms. Therefore, MSCs contributed to improving functional parameters, such as the increase in glomerular filtration and the decrease in albuminuria and structural parameters. The studies indicated an improvement in renal histology and the curtailing of biological processes of inflammation, cell death (apoptosis and necrosis), oxidative stress, and fibrosis. In addition, MSC-based therapy promoted preservation of renal mass, upregulation of tubular epithelial and podocyte genes, augmentation in growth factors within the kidneys, decreasing endothelium damage, amelioration of tubular glucotoxicity by decreasing cellular glucose uptake in the kidneys, and increasing the antiaging klotho protein.

Differentiated BM-MSCs to insulin-secreting β-cells may also represent a promising strategy to treat DM and clinical complications, as documented by the amelioration of endothelium activation by decreasing fibrinogen levels, blood pressure, cytoplasmic calcium, and apoptosis (p53 and Bax), as well as by improving cardiac parameters in STZ-induced diabetic rats [119].

Likewise, secretome of BM-MSC obtained from Zucker DM2 fatty rats improved endothelial cell function by increasing ~3-fold the formation of tubule-like structures and migration of these cells, which was mediated by IGF-1, LTBP-1 (latent TGF-β binding protein), and LTBP-2, as well as by promoting vascular formation *in vivo* [120]. In addition, diabetic secretome exhibited increased expression of proangiogenic genes (ANPEP, MCP-1, MIP-2, HIF-2, IGF-1, IL-6, PLAU, TIE1, and TNF-α) and reduced antiangiogenic genes (COL18A1, COL4A3, F2, IFN-γ, and TGF-β1/3). Extracellular matrix-related proteins (FMOD, OSTP, and COBA1) were also higher in diabetic secretome. These data indicate that BM-MSCs from DM2 rats have a unique secretome with distinct angiogenic properties and provide new insights into the role of BM-MSCs in aberrant angiogenesis in the diabetic milieu.

The hyperglycemic milieu may also adversely impact MSC functionality. Therefore, AT-MSC extracted from Zucker diabetic fatty rats exhibited downregulation of markers of pluripotency (lower capacity of osteogenic and endothelial differentiation *in vitro*) and self-renewal, which may compromise the efficiency of direct self-repair and autologous cell therapy [121]. In addition, these cells exhibited loss of viability, impairment of capillary-like tube formation in Matrigel, decreased expression of stemness genes, signaling pathways important for stem cell maintenance (Nocth1, Notch2, Wnt1, and Dhh) genes, and cell trafficking (CXCL2 and CXCR4) genes, as well as decreased angiogenesis *in vivo* [121].

Likewise, MSCs extracted from rodents with DM2 or large animals with metabolic syndrome have morphological abnormalities (larger number of degenerated mitochondria and marked expansion of endoplasmic reticulum), less proliferative potential associated with an increase in doubling time, alteration in gene expression (downregulation of growth factors IGF-1 and EGF, and angiogenic factors TBX1 and TBX5, and upregulation of proinflammatory genes IFN-γ and IL-1β. IL-2, regulated on activation normal T cell expressed and secreted (RANTES), TNF-α, as well as alpha muscle actin, which represents the stress fiber, and XBP-1, which represents endoplasmic reticulum stress), greater senescence, lower viability and homing capacity, increased apoptosis, and a reduction in clonogenic and multidifferentiation potentials [115, 122, 123]. Conversely, BM-MSCs from diabetic rodents may preserve their multipotent capacity when compared to nondiabetic animals [124].

Of importance, studies with longer duration are required to improve our understanding on the safety profile of MSC-based therapy, such as the cytogenetic aberrations observed during the propagation of these cells in culture. In MSCs derived from mice (C57BL/6 and BALB/c), such aberrations were observed after several passages *in vitro* [125], as well as their malignant transformation *in vivo*, either after injection [126] or promoting the growth of a preexisting tumor [127]. The injection of human (xenogeneic) MSCs in murine models may be associated with the formation of tumors in these animals, as well as with other structural changes, such as chronic jejunitis and villous atrophy, during a three-month follow-up period [128].

2.3. Autologous-Derived MSC for Halting the Progression of DKD in Humans: Advantages and Drawbacks. BM-MSCs are the main source of autologous cell transplantation for various diseases including DM-related micro- and macrovascular complications [129]. Therapy with autologous MSCs is of great interest and has advantages for the patient, as these cells are readily available. MSC-based therapy is based on the extraction of these cells from the patient, expansion *in vitro*, and injection back into the patient, thus avoiding complications resulting from graft rejection and/or the need for an immunosuppressive regimen. Therefore, while patient-derived (autologous) MSC may be the safer choice in terms of avoiding unwanted immune response, factors including donor comorbidities (DM, chronic kidney disease, hypertension, and others) and aging may preclude those cells from use.

Notwithstanding recent promising results with MSC therapy in several diseases, moving the concept forward toward the DKD setting should be critically assessed by looking for intrinsic MSC abnormalities caused by the hyperglycemic milieu, which may adversely affect their therapeutic potential in diabetic patients. Thus, AT-MSCs extracted from diabetic individuals have a greater capacity for adipogenic differentiation, but less chondrogenic and osteogenic differentiation [130, 131]. Conversely, BM-MSCs from diabetic

Table 1: Preclinical studies in small and large animals to verify the therapeutic potential of MSCs in DKD.

MSC type	MSC source	Model of DKD and groups	Number of injections/route of delivery	Number of cells injected	Results	Ref
Xenogeneic	h-BM	STZ-induced DM1 in NOD/*scid* mice: control, DKD, DKD+hMSC	Single dose, intracardiac	2.5×10^6	DKD+hMSC versus DKD: ↑ Pancreatic insulin content and islet cell number ↓ Renal macrophage infiltration Improvement in renal histology	[98]
Syngeneic	BM	STZ-induced DM1 in C57BL/6 mice: DKD+vehicle and DKD +MSC	Single dose, IV	0.5×10^6	DKD+MSCs versus DKD: ↓ FBG ↓ Albuminuria and glycosuria Improvement in renal and β-cell histology	[99]
Syngeneic	BM	STZ-induced DM1 in C57BL/6 mice: control, DKD+vehicle, DKD+MSC	Two doses (20 days apart), IV	0.5×10^6	DKD+MSCs versus DKD: ↓ Albuminuria Improvement in renal histology No improvement in β-cell function and histology	[100]
Syngeneic	BM	STZ-induced DM1 in Sprague-Dawley rats: DKD, DKD +MSC, DKD+CSA, DKD +MSC+CSA (MSCA)	Single dose, intracardiac	2×10^6	MSCA group versus DKD: ↓ FBG ↓ Albuminuria Improvement in renal mass index	[101]
Autologous	AT	STZ-induced DM1 in diabetes Sprague-Dawley rats: control, DKD+vehicle, DKD+AT-MSC	Single dose, IV	1×10^7	DKD+AT-MSCs versus DKD: ↓ Renal p-p-38, p-ERK, and p-JNK ↓ Renal MDA and carbonyl protein ↓ Renal TNF-α, IL-1β, IL-6 ↓ Renal MnSOD and CuZn-SOD	[102]
Xenogeneic	h-UCB	STZ-induced DM1 in Sprague-Dawley rats: control, DKD, DKD+h-UCB-SC	Single dose, IV	1×10^6	DKD+h-UCB-SCs versus DKD: ↓ FBG ↓ Albuminuria ↓ Renal fibronectin, α-SMA ↑ Renal E-cadherin	[103]
Xenogeneic	h-UCB	STZ-induced DM1 in Sprague-Dawley rats: control, DKD, DKD+h-UCB-SC	Single dose, IV	5×10^5	DKD+h-UCB-SCs versus DKD: ↔ FBG ↔ Albuminuria Improvement in renal histology ↓ Renal TGF-β1, α-SMA ↑ Renal E-cadherin, BMP-7	[104]
Syngeneic	BM	STZ-induced DM1 in Sprague-Dawley rats: control, DKD +MSC, DKD+medium	Single dose, left renal artery	2×10^6	DKD+MSCs versus DKD +medium: ↔ FBG ↓ Kidney weight, kidney/body weight, creatinine clearance ↓ Albuminuria Improvement in renal histology ↑ Renal nephrin, podocin, VEGF, BMP-7	[105]
	BM		Single dose, IV			[106]

Table 1: Continued.

MSC type	MSC source	Model of DKD and groups	Number of injections/route of delivery	Number of cells injected	Results	Ref
Syngeneic, UTDM		STZ-induced DM1 in Sprague-Dawley rats: control, DKD +vehicle, DKD+UTMD, DKD +MSC, DKD+MSC+UTMD		1×10^6	DKD+MSC and DKD+MSC +UTMD versus DKD+vehicle and DKD+UTMD: ↓ FBG ↑ Plasma insulin Attenuated β-cell damage ↓ Albuminuria ↓ Renal TGF-β1 ↑ Renal synaptopodin, IL-10 *After UTMD: MSC homing was increased to kidneys (~2x)	
Syngeneic	BM	STZ-induced DM1 in Wistar rats: control, DKD+vehicle, DKD+MSC	2 doses (1 week apart), IV	2×10^6	DKD+MSCs versus DKD: ↓ FBG ↓ Albuminuria ↓ Creatinine clearance Improvement in renal histology ↓ Renal MCP-1, ED-1, IL-1β, IL-6, TNF-α ↑ Renal HGF	[107]
Syngeneic	BM	STZ-induced DM1 in Wistar rats: DKD, DKD+MSC, DKD +insulin, DKD+probucol	2 doses (1 week apart), IV	2×10^6	DKD+MSCs versus DKD: ↓ FBG ↓ Albuminuria ↓ Creatinine clearance ↓ Kidney/body weight Improvement in renal histology ↓ Renal fibronectin, collagen I, TGF-β1, MDA content, ROS fluorescence ↑ Renal SOD activity ↓ Cellular glucose uptake mediated by GLUT1 in kidneys	[108]
Syngeneic	BM	STZ-induced DM1 in albino rats: control, DKD, DKD +vehicle, DKD+MSC	Single dose, IV	1×10^6	DKD+MSCs versus DKD: ↓ FBG ↓ Albuminuria ↓ Body weight ↓ Serum creatinine and urea ↑ Renal VEGF and antiapoptotic bcl2 ↓ Renal TNF-α, proapoptotic Bax, TGF-β Improvement in renal histology	[109]
Syngeneic	BM	STZ-induced DM1 in Wistar rats: control, DKD+vehicle, DKD+MSC	2 doses (1 week apart), IV	2×10^6	DKD+MSCs versus DKD: ↓ FBG ↓ Albuminuria ↓ Kidney/body weight ↓ Creatinine clearance Improvement in renal histology ↓ Renal collagen I, collagen IV, α-SMA, TGF-β, P-smad3/smad2/3 ↑ Renal E-cadherin, BMP-7	[47]

Table 1: Continued.

MSC type	MSC source	Model of DKD and groups	Number of injections/route of delivery	Number of cells injected	Results	Ref
Syngeneic	BM *SDF-1-loaded microbubbles	STZ-induced DM1 in Sprague-Dawley rats: DKD+vehicle, DKD+UTMD, DKD+UTMD +MSC-SDF-1	Single dose, IV	1×10^6	DKD+UTMD+MSC-SDF-1 versus DKD: Improvement in renal histology ↑ MSC engraftment with SDF-1 (7-fold versus control and 1.6-fold versus UTDM)	[110]
Syngeneic	BM	STZ-induced DM1 in C57BL/6 mice: DKD+vehicle, DKD +MSC	Single dose, IV	0.5×10^6	DKD+MSCs versus DKD: ↓ Kidney ↓ Kidney/body weight ↓ Serum creatinine, urea, and plasma cystatin C ↓ Renal collagen I and fibronectin ↓ Renal tubular apoptotic index, ROS total, lipid peroxidation, oxidative protein damage, F4/80-positive cells ↑ Renal nephrin, tubular Ki67 proliferation index ↑ Plasma bFGF, EGF, HGF, IL-6, and IL-10 Improvement in renal histology	[111]
Syngeneic	AT	STZ-induced DM1 in Sprague-Dawley rats: control, DKD, DKD+vehicle, DKD+MSC	Single dose, IV	1×10^7	DKD+MSCs versus DKD: Improvement in renal histology ↓ Kidney apoptosis (TUNEL, ↓ Bax and ↑ Bcl2), expression of Wnt1, Wnt3a, Snail, active β-catenin ↑ Renal klotho	[49]
Syngeneic	BM	STZ-induced DM1 in Sprague-Dawley rats: control, DKD, DKD+MSC	Single dose, IV	2×10^6	DKD+MSC versus DKD: ↔ FBG ↓ Albuminuria ↓ Kidney weight ↓ Serum creatinine ↓ Renal PAI-1, TGF-β1, Smad3	[112]
Xenogeneic	h-BM (DM1 and normal individuals)	STZ-induced DM1 in C57BL/6 mice: DKD+DM1-MSC, DKD +control MSC, DKD+vehicle	Single dose, intrasplenic	1×10^6	DKD+MSC versus DKD: ↓ FBG (~70% of mice) ↑ Serum insulin Improvement in glucose tolerance test Improvement in pancreatic inflammation (↓ IL-2 and INF-γ) and β-cell function	[113]
Xenogeneic (Lewis and SD-Tg rats -> C57BL/6J and C57BL/6-Tg mice)	BM	STZ-induced DM1 and HFD-induced DM2 in C57BL/6J and C57BL/6-Tg mice: Control, STZ+vehicle, STZ +MSC, STZ+MSC-CM Control, HFD+vehicle, HFD +MSC, HFD+MSC-CM	STZ model: 2 doses (4 weeks apart) HFD model: 4 doses (2 weeks apart) IV	1×10^4 MSC/body weight	STZ model: STZ+MSC and STZ+CM-MSC versus STZ +vehicle Improvement in renal histology ↓ FBG: all groups versus control ↓ Renal TNF-α, ICAM-1, p-p38-MAPK	[50]

Table 1: Continued.

MSC type	MSC source	Model of DKD and groups	Number of injections/route of delivery	Number of cells injected	Results	Ref
Syngeneic	BM	STZ-induced DM1 in albino Wistar rats: control, DM, DKD, DM+MSC, DKD+MSC	Single dose, IV	1×10^6	↑ Renal ZO-1, megalin HFD model: HFD+MSC and HFD+CM-MSC versus HFD +vehicle Improvement in renal histology ↓FBG: all groups versus control; HFD-MSC versus HFD-vehicle ↓ Renal TNF-α, ICAM-1, TGF-β ↑ Renal ZO-1, megalin MSC-treated versus nontreated: ↓ Serum creatinine, urea, uric acid ↓FBG ↑ Serum insulin ↓ Albuminuria ↓ Serum TGF-β, FGF-2, PDGF ↔ Serum AGEs ↑ Serum HO-1 activity ↓ Renal IL-8, MCP-1	[114]
Syngeneic (from each model of diabetic and control rats)	BM+treatment with UCB extracts preinfusion	STZ-induced DM1 in Sprague-Dawley rats and C57BL/6 mice; DM2 in OLETF diabetic rats: control, STZ or OLEFT, STZ +MSC, OLEFT+MSC	Four doses (2 weeks apart), IV	1×10^4 MSC/body weight	MSC-treated versus nontreated: ↔ FBG ↔ Albuminuria ↔ Renal histology MSC+UCB extract-treated versus nontreated: ↔ FBG ↓ Albuminuria Improvement in renal histology	[115]
Syngeneic	BM+treatment with melatonin preinfusion	STZ-induced DM1 in Wistar rats: control, DKD, DKD +MSC, DKD+MSC+melatonin	Single dose, IV	1×10^6	DKD+MSCs versus DKD (effects intensified with melatonin): ↑ Renal SOD, Beclin-1 ↓ Renal TGF-β	[116]
Syngeneic	BM	STZ-induced DM1 in Sprague-Dawley rats: control, DKD +vehicle, DKD+MSC	Four doses (1-2 weeks apart), IV	5×10^6	DKD+MSCs versus DKD: ↑ Rat survival ↓ Serum urea ↓ Albuminuria ↓ Renal TGF-β1, fibronectin, ICAM-1, MCP-1, CD68, TNF-α, IL-6, IL-1β ↓ Serum IL-1α, IL-1β, IL-6, IFNγ Improvement in renal histology	[52]
Syngeneic	BM +transfection with miR-124a	STZ-induced DM1 in Sprague-Dawley rats: control, MSC, DKD, DKD+MSC with miR124a mimics, inhibitors, and negative control	Single dose, IV	3×10^6	DKD+MSCs versus DKD: ↔ FBG ↔ Albuminuria MSC+miR124a: ↑ Renal nephrin, podocin, CD2AP, Bcl-2	[45]

Table 1: Continued.

MSC type	MSC source	Model of DKD and groups	Number of injections/route of delivery	Number of cells injected	Results	Ref
Xenogeneic (human -> monkeys)	BM	STZ-induced DM1 in cynomolgus monkeys (*Macaca fascicularis*) treated with insulin glargine and glulisine +acute ischemia-reperfusion injury: control, DKD, DKD +MSC	Single dose; intra-arterial (suprarenal aorta)	5×10^6 cells/kg	↓ Renal TGF-β1, collagen I and III, caspase-3, Bax DKD+MSCs versus DKD: ↔ Serum creatinine, urea, TNF-α, IFN-γ ↔ Albuminuria ↔ Urinary NGAL, GST-α, and TIMP-1 Improvement in renal histology (↓ necrosis)	[117]
Syngeneic	Amniotic liquid (adenovirus SIRT3 overexpression)	db/db mice: wild type, control, DKD+adenovirus control, DKD+adenovirus-SIRT3	Single dose, intraparenchymal	3×10^6	DKD versus DKD+SIRT3: ↓ Body weight ↓ FBG, serum insulin, C-peptide, glucagon, HbA1c ↓ Serum creatinine, urea ↓ Serum TNF-α, IL-6, MCP-1 ↓ Systolic blood pressure ↓ Albuminuria ↓ Kidney weight, oxidative stress, collagen I/III/IV deposition, MMP9, TGF-β Improvement in renal histology	[118]
Syngeneic	BM	STZ-induced DM1 in Sprague-Dawley rats: control, DKD +vehicle, DKD+MSC, DKD +MSC+WRW4 (1 mg/kg), DKD+LXA4 (10 mg/kg), DKD +LXA4+WRW4	Two doses (1 week apart), IV	5×10^6	DKD+MSCs versus DKD: ↑ Rat survival ↔ FBG ↓ Serum creatinine and urea ↓ Glycosuria, albuminuria ↑ Renal LXA4 ↓ Renal TGF-β1, p-SMAD2/3 ↓ Serum TNF-α, IL-6, IL-8, IFN-γ (LXA4 treatment exhibited similar findings when compared to MSC, which was abrogated by WRW4 treatment)	[48]
Allogeneic (from CAG-EGFP.SD-Tg rats)	AT	Spontaneously diabetic Torii (SDT) fatty rats (SDT.Cg-Lepfa/JttJcl)+unilateral nephrectomy: control; DKD +MSC suspension via IV route; DKD+MSC sheets transplanted directly into the kidney	Single dose, IV or cell sheets transplanted directly into the kidney	6×10^6/ml via IV route and cell sheets	DKD+cell sheets versus DKD +MSC via IV route and DKD: ↓ Albuminuria, proteinuria, and urinary L-FABP, KIM-1, IL-6 Improvement in renal histology DKD+cell sheets and DKD +MSC via IV route versus DKD: ↓ Urinary podocalyxin and TNF-α	[90]
Xenogeneic (human -> macaques)	UCB	STZ-induced DM1 in rhesus macaques+high-fat and high-salt diet (for 2 years): control; DKD; DKD+MSC	4 doses (2 weeks apart), IV	2×10^6/kg	DKD+MSCs versus DKD: ↓ FBG, insulin requirement ↓ Serum creatinine and BUN ↑ eGFR ↓ Albuminuria ↓ Renal IL-1β, IL-16, TNF-α, CTGF, SGLT2 ↑ Renal IL-6 ↓ Serum IFN-γ, TNF-α, IL-1β,	[44]

Table 1: Continued.

MSC type	MSC source	Model of DKD and groups	Number of injections/route of delivery	Number of cells injected	Results	Ref
Xenogeneic (human -> mice)	UCB	Unilateral nephrectomy+STZ-induced DM1 in CD1 mice	3 doses (4 weeks apart), IV	5×10^5	IL-5, IL-12p70, IL-15, IL-16 Improvement in renal histology DKD+MSCs versus DKD: ↔ Serum glucose ↓ Serum creatinine and BUN ↓ Albuminuria ↓ Renal mRNA desmim, α-SMA, Fn1, Kim-1, NGAL, MCP-1, VCAM-1, ICAM-1, IL-1b, TNF-α, IL-6, iNOS ↑ Renal mRNA arginine 1 Improvement in renal histology	[53]

MSCs: mesenchymal stem cells; BM-MSC: bone marrow-derived MSCs; h-BM-MSC: human bone marrow-derived MSC; AT-MSC: adipose tissue-derived MSCs; h-UCB-SCs: human umbilical cord blood-derived stem cells; MSC-CM: MSC-conditioned medium; DM: diabetes mellitus; DKD: diabetic kidney disease; AGEs: advanced glycation end products; BMP-7: bone morphogenic protein-7; CSA: cyclosporine; EGF: epidermal growth factor; FBG: fasting blood glucose; bFGF: basic fibroblast growth factor; Fn1: fibronectin-1; GST-α: glutathione S-transferase-α; HFD: high-fat diet; HGF: hepatocyte growth factor; HO-1: heme-oxygenase-1; ICAM-1: intercellular adhesion molecule-1; iNOS: inducible nitric oxide synthase; IL: interleukin; IFN-γ: interferon-γ; IV: intravenous; KIM-1: kidney injury molecule-1; LETO: Long-Evans Tokushima Otsuka rats; L-FABP: liver-type fatty acid binding protein; LXA4: lipoxin A4; MDA: malondialdehyde; miR: microRNA; MCP-1: monocyte chemoattractant protein-1; MMP: matrix metalloproteinase; NGAL: neutrophil-gelatinase associated lipocalin; OLETF: Otsuka Long-Evans Tokushima Fatty diabetic rats; PAI-1: plasminogen activator inhibitor-1; PDGF: platelet-derived growth factor; ROS: reactive oxygen species; SDF-1: stromal-derived factor-1; SIRT3: sirtuin 3; SOD: superoxide dismutase; α-SMA: α-smooth muscle actin; STZ: streptozotocin; TGF-α: transforming growth factor α; TGF-β1: transforming growth factor β1; TIMP-1: tissue inhibitor metalloproteinase-1; TNF-α: tumor necrosis factor-α; UTMD: ultrasound-targeted microbubble destruction; VCAM-1: vascular cell adhesion molecule-1; VEGF: vascular endothelial growth factor; ZO-1: *zonula occludens*-1.

individuals preserve their multipotent capacity [113]. Therefore, the source of MSC may play a critical role in decision-making for treating diabetic individuals. AT-MSCs isolated from the ischemic limb of diabetic patients seem to be less potent when compared phenotypically and functionally to control nondiabetic counterparts with no signs of limb ischemia [132]. To note, 40% of diabetic and 20% of nondiabetic AT-MSC samples displayed high expressions of fibroblast marker, which inversely correlated with the expression of CD105. In diabetic patients, significantly decreased expression of VEGF and CXCR4 was verified in fibroblast-positive AT-MSCs when compared to their fibroblast-negative counterparts, which may negatively affect angiogenic and homing capacity mediated by AT-MSCs, respectively [132]. Reduced osteogenic differentiation and the downregulation of chemokine CXCL12 were also observed in fibroblast-negative diabetic AT-MSCs. Both diabetic and nondiabetic AT-MSCs were able to differentiate into adipocytes and chondrocytes, yet not exhibiting islet-like cell differentiation in that study [132]. Importantly, *in vitro* studies documented the differentiation potential of human AT-MSCs into islet-like cells when these cells were obtained from healthy individuals who underwent abdominoplasty or liposuction [133–135]. Transdifferentiated cells exhibit positive staining for dithizone, increased expression of islet cell-related genes (Pdx-1, Isl1, Ngn3, NeuroD1, Pax4, and GLUT2), and insulin secretion when these cells were challenged with high concentrations of glucose.

Not only the source of MSCs but also the type of DM may affect the therapeutic potential of MSCs. MSCs extracted from DM1 individuals exhibited preserved morphology, growth kinetics, multipotency, and proliferative, immunomodulatory, immunosuppressive, and migratory capacities [113, 136].

In contrast, MSCs extracted from individuals with DM2 have greater senescence, lower viability, increased apoptosis (increased proapoptotic gene expression, such as p53, caspase 9, and BAX, and low antiapoptotic gene expression, such as Bcl-2), less proliferative potential associated with increased doubling time, and a reduction in angiogenic potential [130, 137].

CD105 (endoglin) is associated with angiogenesis [138], and its positivity in AT-MSC leads to higher rates of proliferation [139]. Therefore, reduced CD105 expression and proliferation of AT-MSC in DM2 individuals indicate an impairment of angiogenesis of these cells [137]. Conversely, CD105 negativity in human AT-MSC indicates a more efficient immunomodulatory capacity when compared to CD105-positive cells [140].

In line with the derangement observed in MSC-induced angiogenesis of rodents, AT-MSCs extracted from DM2 individuals with critical limb ischemia are dysfunctional, e.g., they exhibited a reduction in fibrinolytic activity and an increase in prothrombotic activity and PAI- levels. Those cells also possess lower efficiency of proliferation, migration, and CFU-f assay, as well as derangement in the PDGF (platelet-derived growth factor) signaling pathway [131]. PDGF

signaling is known to modulate essential MSC processes, such as differentiation, migration, and proliferation, as well as coagulation and fibrinolysis systems. In addition, AT-MSC obtained from diabetic patients exhibited a decrease in angiogenic potential (lower level of VEGF expression and cell proliferation) when compared to healthy donors in a murine model of an ischemic flap [141]. Notably, VEGF and HGF secretion, tubulogenesis, and cell proliferation in diabetic conditioned media were increased in response to hypoxic stimuli, and it was similar to those of control cells. These findings may be important in the context of future study of autologous cell-based therapy in diabetic patients and indicate that hypoxia-mediated preconditioning may be a useful strategy for increasing the therapeutic potential of diabetic MSCs.

The change in the secretome of diabetic MSCs grown even under normoglycemic conditions is related to the development of metabolic memory, a process in which hypomethylation in gene promoters leads to dysregulation of gene expression and implies the persistence of DM-related complications even when glucose returns to normal levels. That effect is supported by studies that show changes in glucose metabolism in diabetic MSCs and by the fact that their functional capacities were not altered by normalization of glucose levels *in vitro* [120, 122].

Therefore, serum obtained from DM2 individuals may increase the BM-MSC proliferation *in vitro* rate, and HbA1c levels may play a role in that effect, indicating that higher rates of proliferation occur when HbA1c levels were 8-10% (versus HbA1c < 6.5%), yet serum derived from individuals with HbA1c > 10% exhibited a decrease in MSC proliferation [142]. On the other hand, diabetic serum decreased osteogenic differentiation in a concentration-dependent manner of HbA1c levels. These findings indicate the impact of the hyperglycemia control on MSC function and suggest that diabetic-derived MSC may be adversely affected in the diabetic milieu. A key aspect in that setting includes the adequate treatment of DM in order to support a better therapeutic potential of MSCs. Not only DM but also other chronic diseases, such as CKD, may impair MSC functionality. Autologous AT-MSCs obtained from CKD individuals (stages 3 and 4), when injected intravenously (1×10^6/kg), exhibited a safety profile and contributed to decreasing proteinuria, yet not modifying eGFR in six patients [143]. Other progenitor cells, such as endothelial progenitor cells, are affected by uremia regardless of the presence of DM [144].

Notably, BM-MSC of newly diagnosed (<6 weeks) DM1 individuals (all males, 23.2 ± 2.9 years) presented similar morphology, immunophenotype, differentiation potential, gene expression of immunomodulatory molecules, and *in vitro* immunosuppressive capacity when compared to normal individuals [113]. However, the HGF gene was significantly downregulated in DM1-derived MSC. When injected into STZ-induced diabetic mice, both DM1 and control MSCs lead to improvement in serum glucose and insulin and in pancreatic histology.

In line with these findings, Davies et al. compared BM-MSC from individuals with newly diagnosed (<6 weeks) DM1 ($n = 10$; mean age 22 years, range 18-35 years; 9 males), late stage of DM1 with severe renal failure ($n = 12$, mean age 42 years, range 31-62 years; 7 males), and healthy BM donors ($n = 19$, mean age 37 years, range 21-70 years; 13 males) [136]. They found that gene expression was different between healthy controls and late DM1 in relation to cytokine secretion, immunomodulatory activity, and wound healing potential. Despite these difference between BM-MSC, DM1-derived MSCs did not demonstrate a significant difference from healthy controls in growth characteristics (CFU-f and doubling time), immunosuppressive activity, migratory capacity, or trophic properties at baseline and after exposure to pro-inflammatory cytokines IFN-γ and TNF-α (similar activity of IDO and upregulation of IL-6, CXCL1, and CXCL6).

To further substantiate the benefits of autologous MSC-based therapy, preconditioning strategies are key aspects to preserve MSC function, such as hypoxia culture, as previously described [141]. In addition, antioxidant pretreatment (N-acetylcysteine and ascorbic acid 2-phosphate) of BM-MSC from obese diabetic, B6.Cg-Lep^{ob}/J mice significantly reduced the excessive TNF-α response observed in diabetic mice and improved IL-10 secretion [145]. Iron chelator deferoxamine pretreatment of human AT-MSCs increases hypoxia inducible factor 1-α (HIF-1α), which led to an upregulation of angiogenic factors (VEGF and angiopoietin-1), neuroprotective factors (nerve growth factor, glial cell-derived neurotrophic factor, and neurotrophin-3), and cytokines with anti-inflammatory activity (IL-4 and IL-5) [146]. Deferoxamine pretreatment also promoted the increase in the capacity of MSC secretome *in vitro*, which was associated with a decrease in neuron death. PDGF pretreatment of human AT-MSCs extracted from DM2 individuals rescued these cells from the diabetic phenotype by improving the proliferation, migration, and the capacity of clot lysing and repairing skin wound in an animal model [131].

Another approach to decrease abnormalities of BM-MSC obtained from DM1 and DM2 animals is the coculture with human umbilical cord extracts (Wharton's jelly extract supernatant). Therefore, Wharton's jelly extract supernatant represents a cocktail of growth factors (IGF-1, EGF, PDGF-AB, and b-FGF); components of extracellular matrixes (hyaluronic acid, collagen, and MUC-1), L-glutamate, and EXOs may also ameliorate proliferative capacity, motility, mitochondrial degeneration, endoplasmic reticular functions, and EXO secretion in both DM1- and DM2-derived BM-MSC, since that supernatant provide the physiological environment to preserve MSC properties and functionality [115]. These findings highlight the importance of seeking potential preconditioning approaches in the clinical setting. In addition, adenoviral transfection of Sirtuin3 in amniotic fluid stem cells protected these cells from high glucose-induced apoptosis by preserving mitochondrial function (increase in mitophagy, mitochondrial potential, respiratory function, and ATP levels, as well as a decrease in ROS, cytochrome c, and caspase activity) and ameliorating cell proliferation [118].

In conclusion, despite the fact that autologous MSC-based therapy has already been reported to ameliorate kidney injury, many difficulties must be overcome to successfully implement that therapy for treating DKD. Key aspects include the type of diabetes, time elapsed since the diagnosis

due to cellular metabolic memory, and cell source, which may impair MSC functional properties.

In addition, some points are beyond the fact of choosing autologous or allogeneic MSCs for treating individuals with DM1 and DM2. Due to the expressive quantity of MSCs required to form a biobank and provide them to immediately infuse into patients, MSC expansion is a key aspect of cell therapy preparation. Both autologous and allogeneic MSCs cultured for a prolonged period may be affected by disturbance in the cellular structure and function. Chromosomal instability and aberrations have been shown in AT-MSCs after prolonged time *in vitro* [147], which leads to their discard. In contrast to these evidences, other researchers indicated MSC genetic stability during several passages in culture [148, 149]. Likewise, cell viability is another important characteristic to be assessed before administration, especially to avoid senescent cell infusion. Senescent cells have major alterations in the overall secretome components, leading to a switch from beneficial to a harmful profile [150].

Another important aspect that must be taken into account in cell therapy with MSCs is the fact that their beneficial effect can be neglected by the occurrence of adipogenic differentiation during long-term follow-up, which can contribute to glomerulosclerosis [78].

The malignant transformation of MSCs has not been described in clinical trials [151, 152]. As reviewed elsewhere, there are controversial data regarding protumorigenic effect of MSC on preclinical models. Some authors argued that MSCs are mobilized into the circulation with further migration and incorporation into the tumor microenvironment [153]. In that setting, MSC may contribute either to enhance tumor growth by decreasing apoptosis and promoting angiogenesis or to inhibit tumor growth in both in vitro and in vivo studies. Importantly, allogenic-derived MSC obtained from different sources and injected through different pathways for the treatment of broad clinical conditions, including graft-versus-host disease and cardiovascular and neurological diseases, was not associated with tumor development throughout a follow-up of 30 days to 6.8 years [153].

2.4. Clinical Studies. We have consulted the *Clinical Trials* web portal (*clinicaltrials.gov*, access in January 2020) with the keywords "mesenchymal stem cell" or "mesenchymal stromal cell" and "diabetes". We defined inclusion criteria as completed studies that have reported results on PubMed. These studies were mainly single-center prospective phase I/II clinical trials, which evaluated safety and tolerability and explored the therapeutic effects of MSCs on beta-cell regeneration and the impact on fasting plasma glucose (FBG), HbA1c, endogenous insulin, and C-peptide increment and the reduction of daily insulin requirement $\geq 50\%$, which reached the efficacy level. A dose-escalating (0.3×10^6/kg, 1.0×10^6/kg, or 2.0×10^6/kg) randomized-controlled trial assessing one intravenous infusion of MPCs (rexlemestrocel-L) in DM2 individuals without DKD documented safety and efficacy of cell therapy [154]. In patients treated with the highest dose, there was a significant decrease in HbA1c at 8 weeks with 33% of patients achieving the clinical target HbA1c < 7%.

To note, there was only one multicentric study, which also included individuals with DKD [155]. In that randomized (1 : 1 : 1), double-blind, sequential, dose-escalating (150×10^6 or 300×10^6, single intravenous dose), multicenter, and placebo-controlled trial, safety and efficacy of adult allogeneic BM-derived MPCs (MPCs, rexlemestrocel-L) were evaluated in type 2 diabetic individuals with DKD (eGFR 20-50 ml/min/1.73 m^2). In terms of safety, no patients exhibit treatment-related severe adverse events and only one patient developed antibody specific to the donor HLA (antibody specificity to donor antigen (class I) B40; mean fluorescence intensity 530) at week 4 that were undetectable at week 12. The primary exploratory efficacy parameter comprised eGFR, so that the placebo-adjusted least square mean change in eGFR at week 12 was 4.4 ± 2.2 ($p = 0.05$) and 1.6 ± 2.2 ml/min/1.73 m^2 ($p = 0.47$) for the 150×10^6 and 300×10^6 groups, respectively. Relative to placebo, there was a suggestion of stabilization of eGFR in the rexlemestrocel-L 150×10^6 group, most notably at the 12-week primary endpoint. Importantly, when subgroup analyses were performed (GFR ≤ 30 or >30 ml/min/1.73 m^2), the subgroup with eGFR > 30 ml/min/1.73 m^2 treated with 150×10^6 cells manifested a lower decrease in eGFR when compared to the control group at 12 weeks ($p = 0.04$). In addition, there was a statistically significant decrease in the median IL-6 values for the 300×10^6 group compared to placebo at week 12, but not for other markers (HbA1c, TNF-α, and C-reactive protein).

We observed a balanced distribution between allogeneic-MSCs and autologous-MSC-based studies for both DM1 and DM2 individuals. MSCs have also shown beneficial effects on glycemic control when combined to hematopoietic stem cells (HSCs) or BM-MNCs. However, studies have still not been able to establish insulin-free status in this group of patients, even by differentiation of human AT-MSC into insulin-secreting MSCs (AT-ISC-MSC) [156–158]. That approach is based on growing MSCs with growth factors and serum with supplements, such as nicotinamide, activin A, exendin, pentagastrin, HGF, B-27, N2, and antibiotics for 4 days [158]. After that, these cells secrete C-peptide and insulin *in vitro* and express genes responsible for insulin secretion (pax-6, pdx1, and isl-1).

Likewise, a nonmyeloablative low-intensity conditioning regimen combined to MSC therapy failed to demonstrate insulin independence [156–158]. The objective of the treatment is to stop autoimmune destruction of β-cells with high-dose immunosuppressive drugs. A similar approach was also attempted to reset the deleterious immunologic system with a reconstituted one originated from autologous hematopoietic stem cells [159]. The rationale is to preserve residual β-cell mass and facilitate endogenous mechanisms of β-cell regeneration. For example, a nonmyeloablative low-intensity conditioning regimen combined to autologous AT-IS-MSC and HSC was based on rabbit antithymocyte globulin, methylprednisolone, and bortezomib [158]. For allogeneic AT-ISC-MSC associated with HSC infusion, nonmyeloablative low-intensity conditioning included target specific irradiation to subdiaphragmatic lymph nodes, spleen, part of pelvic bones, and lumbar vertebrae before cell

infusion [156]. In addition, anti-T cell antibody (rabbit antithymocyte globulin) and anti-B cell antibody (ABA) were administered intravenously to prevent rejection and facilitate grafting of transplanted cells. Of importance, no immunosuppressive medication was required posttransplant. To note, the outcomes in β-cell function from those studies should also be analyzed in light of the use of the immunosuppressive regimen *per se*.

MSC-based therapy was considered a safe procedure in all studies that verified the therapeutic potential of these cells. In a systematic review and meta-analysis of clinical trials that evaluated MSC safety in more than a thousand individuals diagnosed with other clinical conditions, a significant association between MSC infusion and fever was shown [160]. However, no other immediate event (acute infusion toxicity), organ system complications, infection, and long-term adverse events (death, malignancy) were documented.

In terms of efficacy, both autologous- [157, 158, 161–164] and allogeneic- [155, 156, 165–168] derived MSCs accomplished the major secondary endpoints, as effective in changing metabolic hallmarks of DM, such as C-peptide synthesis and reducing exogenous insulin requirement, FBG, and HbA1c, as described in Tables 2 and 3, respectively. In the same way, allogeneic MSCs were effective as autologous MSCs in improving the final diastolic volume and left ventricular ejection fraction of patients with ischemic cardiomyopathy [169]. Notably, alloimmune reactions in those patients receiving allogeneic MSCs were very low (3.7%). In renal transplant patients, the infusion of both autologous [170] and allogeneic [171] MSCs was considered safe and effective. These data suggest the possibility of developing a biobank of allogeneic MSCs for therapeutic purposes in several pathologies, since these cells lack the expression of class II MHC (Major Histocompatibility Complex) antigens and costimulatory molecules (CD80/B7.1 and CD86/B7.2) [39]. Noteworthily, the potential impact of donor-to-donor heterogeneity and the potential immunogenicity of allogeneic cells, depending on the culturing conditions and passages, the microenvironment, and the differentiation state, may alter the immunogenic phenotype, as recently reviewed [172].

Importantly, MSC differentiation, when exposed to a proinflammatory microenvironment, may result not only in upregulation of cell surface immunogenic molecules but also in a decrease in immunoregulatory or immunosuppressive molecule secretion, such as PGE2, as reviewed elsewhere [173]. On the other hand, when MSCs differentiate into chondrocytes, they may not impair the production of immunomodulatory molecules. Therefore, some strategies may overcome immunogenicity, such as using 3D cell culture conditions and gene therapy [173]. Notwithstanding that controversial data, the formation of donor-specific antibodies after allogeneic MSC injection occurs eventually is not sustained and does not adversely affect the benefits of cell therapy in clinical practice [154, 155, 169, 173, 174]. However, the implications of the development of alloantibodies still need to be assessed over longer time periods, alongside the tolerability and efficacy of single and repeated administration of allogeneic MSC before definite conclusions can be established. As discussed elsewhere, some key aspects to be taken into account include both preclinical (e.g., increased vigilance of cellular immunity in preclinical experiments, development of strategies to reduce alloantigen expression on allo-MSCs, determination of optimal tissue source of MSC, combination of allo-MSC therapies with immunomodulatory drugs, replacement of highly immunogenic cells with alternatives, and optimizing the route of administration and culture conditions) and clinical (e.g., prescreening for antidonor responses, tracking the development of humoral immune responses, performance of functionally analyses of adaptive antidonor responses, and systemic public reporting results) approaches [173].

In addition, those studies also raised important questions regarding the protocols related to MSC source and viability, the number of infusions, the number of infused cells, routes of administration, the ability of MSCs to migrate to the injury site, the potency of the MSCs in the context of disease, model, and outcome measure [57]. A key aspect suggests that efficacy may be curtailed by the sequestration within the lungs and early elimination, as discussed previously in Preclinical Studies: Small and Large Animals.

In a meta-analysis of UCB cell-, UCB-MSC-, BM-MSC-, and HSC-based therapies for both DM1 and DM2 individuals ($n = 22$ studies), it was documented that almost 60% of DM1 individuals ($n = 15$ studies, 300 patients, including 40 controls) became insulin-independent for a mean period of 16 months after HSC-MSC treatment (mean dose of $6.99 \pm 3.28 \times 10^6$ cells/kg $CD34^+$), as opposed to a negative response when these patients were treated with UCB cells (mean dose of 1.49×10^7 nucleated cells; mean number of $CD34^+$ cells was 1.26×10^6) [175]. Likewise, UCB-MSC (range, 1.27×10^6/kg to 1.88×10^7/kg; mean dose $2.6 \pm 1.2 \times 10^7$) therapy was superior to BM-MSC (mean dose of 2.75×10^6/kg) therapy for DM1 individuals, when C-peptide levels were compared, but not in terms of decreasing HbA1c. For DM2 individuals ($n = 7$ studies, 224 patients, including 92 controls), no conclusive recommendation was defined, yet DM-MNCs provided a better outcome when compared to UCB-MSCs in improving C-peptide levels and decreasing HbA1c. The administration of cell therapy early after DM diagnosis was more effective than intervention at later stages (relative risk = 2.0). To note, UCB cells ($n = 3$) and BM-MNCs ($n = 107$) were injected intrapancreatically, whereas UCB-MSCs ($n = 22$) were injected intravenously. In addition, mean doses of BM-MNCs were 17.29×10^8 cells/kg (mean number of $CD34^+$ cells was 3.15×10^6) and mean doses of UCB cells were 5.29×10^9 (mean number of $CD34^+$ was 2.88×10^6), whereas mean doses of UCB-MSCs were 1×10^6/kg (either intravenous or intrapancreatic). Whether the number of injected cells and the route of injection (intrapancreatic versus intravenous) are associated with better outcomes, further studies are warranted to reach a definitive conclusion. Importantly, the patient clinical condition may also play a role in cell therapy, as diabetic ketoacidosis may impair its efficacy.

In another recent meta-analysis including DM2 individuals ($n = 6$ studies, 206 patients), treatment with autologous

Table 2: Autologous MSC-based clinical trials.

MSC source	DM type	Age (years)	Time of DM	Number of patients	Number of injections/route of delivery	Number of cells injected	Follow-up (m)	Results	Adverse events	Ref
BM	1	<8 y	<2 m	2	Single dose, liver	180×10^6/kg	12	↑ C-peptide ↓ FBG, HbA1c Negative values of ICA, GAD and anti-insulin antibody levels	None	[161]
BM	1	18-40 y	<3 w	10 treated with insulin and 10 treated with insulin+MSCs	Single dose, IV	2.1-3.6 × 10^6/kg (median 2.75 × 10^6/kg)	12	↑ C-peptide after mixed-meal tolerance test ↔ HbA1c, insulin dose, fasting C-peptide	None	[162]
AT-ISC-MSC+BM-HSC * conditioning	1	Group 1: 20 ± 7 y Group 2: 20 ± 10 y	Group 1: 8.1 ± 3.4 y Group 2: 9.9 ± 7.1 y	Group 1: autologous AT-ISC-MSC +BM-HSC Group 2: allogeneic AT-ISC-MSC +BM-HSC from healthy nondiabetic donors ($n = 10$ each)	Single dose, portal +intrathymus+SC	Group 1: AT-ISC-MSC-2.65 ± 0.8 × 10^4/kg Group 2: AT-ISC-MSC-2.07 ± 0.67 × 10^4/kg	24	Autologous versus allogeneic: (i) Allogeneic: ↓ insulin requirement up to 6 months and ↓ postprandial blood sugar from months 1-24 (ii) Autologous: ↑ C-peptide from months 15-24 (iii) Allogeneic = autologous: ↓ HbA1c, FBG, GAD antibody	None	[157, 158]
BM	2	30-60 y	≥5 y	BM-MSC versus BM-MNCs versus control group (antidiabetic drugs) ($n = 10$ each)	Single dose, superior pancreatic-duodenal artery	MSCs: 1 × 10^6/kg MNCs: 1 × 10^7	12	↓ Insulin requirement ≥ 50% and HbA1c in 60% of individuals (BM-MSC and BM-MNC) ↔ HOMA indexes ↑ Insulin sensitivity index+insulin receptor substrate-1 gene expression in muscle (BM-MSC) ↑ Second phase C-peptide response during hyperglycaemic clamp (BM-MNCs)	Nausea and vomiting ($n = 2$)	[163]
BM	2	37-67 y (52.9 y)	2-15 y (7.15 y)	NPR ($n = 19$) PR ($n = 15$)	Single dose, IV	3.0 × 10^6/kg	6	↓ FBG, hypersensitivity C-reactive protein ↔ HbA1c, plasma IL-6 ↓ Macular thickness and improvement in best corrected visual acuity (proliferative retinopathy group)	None	[164]

AT-ISC-MSC: adipose-derived insulin-secreting mesenchymal stem cells; BM-HSCs: bone marrow-derived hematopoietic stem cells; BM-MNCs: bone marrow-derived mononuclear cells. Autoantibodies to β-cells: GAD65 (glutamic acid decarboxylase) and IA2 antibodies. FBG: fasting blood glucose; HbA1c: glycated haemoglobin; NPR: nonproliferative retinopathy; PR: proliferative retinopathy; SC: subcutaneous; IU: International Units; IV: intravenous; w: week; m: month; y: years.

Table 3: Allogeneic MSC-based clinical trials.

MSC source	DM type	Age	Time of DM	Number of patients	Number of injections/route of delivery	Number of cells injected	Follow-up (m)	Results	Adverse events	Ref
AT-ISC-MSC +HSC *Conditioning	1	13-43 y (21 y)	1-24 y (8.2 y)	11	Single dose, omental vein	Mean total cell quantum transplanted: 96.3 ml (92-118 ml) HSC: $28 \times 10^3/\mu$l (12.2-62.7 $\times 10^3/\mu$l) MSC: $1.2 \times 10^3/\mu$l (0.5-2.1 $\times 10^3/\mu$l)	23	↓ Insulin requirement, HbA1C ↑C-peptide	None	[156]
Placental	2	45-82 y (66 y)	3-20 y (11 y)	10	Single dose, IV	1.35×10^6/kg (1.22-1.51 $\times 10^6$/kg)	3	↓ Insulin requirement, HbA1c ↑ C-peptide, insulin ↔ FBG, GAD antibody	None	[165]
Wharton's jelly-MSC	1	Group 1 (MSC): 17.6 ± 8.7 y Group 2 (saline): 18.2 ± 7.9 y	Newly onset	29 Group 1: $n = 15$ Group 2: $n = 14$	Two doses (4 weeks apart), IV	$2.6 \pm 1.2 \times 10^7$ (1.5-3.2 $\times 10^7$)	21	↓ Insulin requirement, postprandial glucose, HbA1c ↑ C-peptide, C-peptide/glucose ratio	None	[166]
Wharton's jelly-MSC	2	52.9 ± 10.5 y	8.7 ± 4.3 y	22	One dose IV and one dose intrapancreatic	1×10^6/kg	12	↓ FBG, postprandial glucose, HbA1c, insulin requirement ↑ C-peptide, HOMA-β ↓ Serum $CD3^+$ and $CD4^+$ lymphocytes ↔ Serum $CD8^+$ lymphocytes ↓ Serum IL-6, IL-1β ↔ Serum TNF-α and IL-10	Fever ($n = 3$), hematoma ($n = 1$), nausea, vomiting, headache ($n = 1$)	[167]
BM (rexlemestrocel-L) *eGFR: 20-50 ml/min/1.73 m^2	2	Placebo: 74.8 ± 7.9 y Lower dose MSCs: 70.5 ± 7.4 y Higher dose MSCs: 64.8 ± 10.1 y	Time of DM: N/A	30 ($n = 10$ each)	Single dose, IV	150×10^6/kg (lower dose) or 300×10^6/kg (higher dose)	12	↔ eGFR, albuminuria ↔ Lipid profile ↔ Blood pressure ↔ Serum C-reactive protein, TNF-α ↓ Serum IL-6	None	[155]
UC+autologous BM-MNC	1	Standard care: 13-27 y (20.4 y)	Standard care: 2-13 y (7.0 y)	42 ($n = 21$ each)	Single dose of each cell, dorsal pancreatic artery	UCB: 1.1×10^6/kg BM-MNCs: 106.8 ×	12	Cell therapy versus standard care: ↑ AUC of C-peptide	Upper respiratory tract infection ($n = 7$), bleeding ($n = 1$), abdominal pain ($n = 1$)	[168]

Table 3: Continued.

MSC source	DM type	Age	Time of DM	Number of patients	Number of injections/route of delivery	Number of cells injected	Follow-up (m)	Results	Adverse events	Ref
		Cell therapy: 5-28 y (18.3 y)	Cell therapy: 2-16 y (9.2 y)			10^6/kg		↑ AUC of insulin ↓ FBG, HbA1c ↓ Fasting C-peptide ↓ Insulin requirement ↓ Anxiety score ↓ Depression score ↑ Quality of life		

AT-ISC-MSC: adipose-derived insulin-secreting mesenchymal stem cells; AUC: area under the curve; BM-HSCs: bone marrow-derived hematopoietic stem cells; BM-MNCs: bone marrow-derived mononuclear cells; FBG: fasting blood glucose; eGFR: estimated glomerular filtration rate; HbA1c: glycated haemoglobin; IV: intravenous; IU: International Units; m: month; NPR: nonproliferative retinopathy; PR: proliferative retinopathy; SC: subcutaneous; ZO-1: *zonula occludens-1*; y: years.

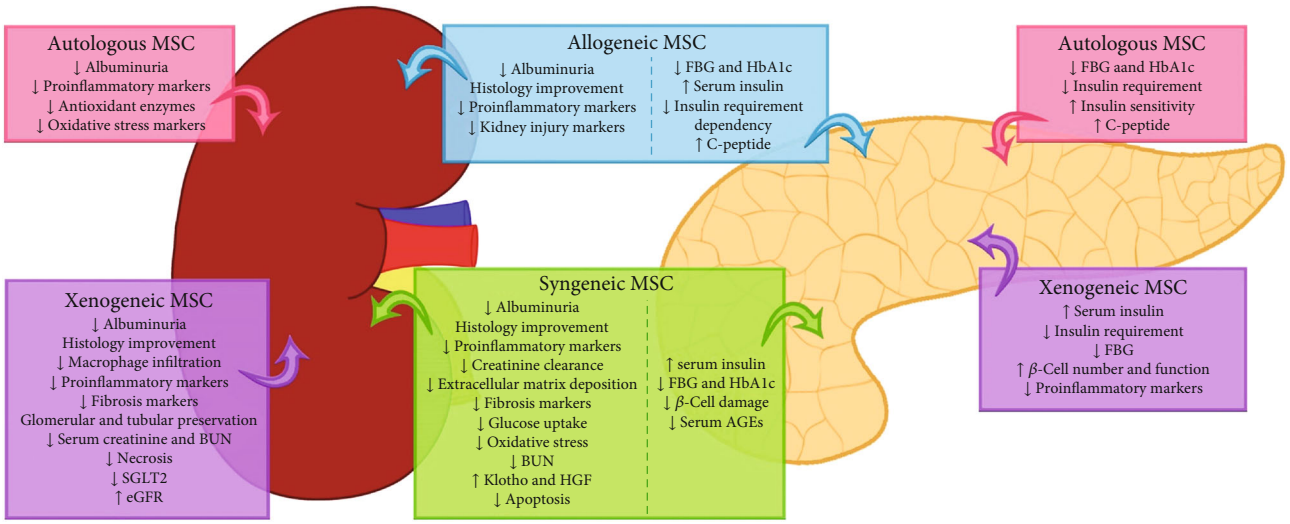

Figure 1: Main findings of preclinical and clinical studies evaluating MSC efficacy.

BM-MNCs (dose ranged from 382.6 ± 10^7 to $2.8 \pm 1.9 \times 10^9$ cells) was effective in reducing HbA1c by 1.18% and insulin requirement during a follow-up of 12 months [176].

3. Future Directions

Strategies such as gene modification, optimization of culture conditions, and pretreatment conditioning may lead to an improvement in MSC functionality and a decrease in heterogeneity. These strategies comprise hypoxia culture, pharmacological agents, trophic factors/cytokines, small molecules, physical factors/materials, and gene modification, which may all contribute to better tissue repair and regeneration mediated by MSCs [177].

MSCs are generally grown in an environment with 21% oxygen tension. However, physiologically, MSCs are found in an environment with a much lower oxygen tension (1% to 7%). Thus, the cultivation or preconditioning of MSCs in a hypoxia environment with 2% or 5% oxygen allows these cells to remain multipotent and have greater proliferative and migratory capacity, in addition to lower senescence rates [178, 179]. Importantly, MSCs preconditioned by hypoxia do not differentiate into fibroblasts associated with tumors *in vitro* and do not induce tumors *in vivo* [178].

In order to reduce the heterogeneity of the MSC profile, which is defined by the different isolation and culture protocols, the preconditioning of these cells with proinflammatory factors has been the focus of investigation. Thus, the preconditioning of MSCs through stimulation with IFN-γ, TNF-α, PGE2, and NO oxide mitigated the heterogeneous behavior of MSCs on T lymphocyte proliferation assays and on late type hypersensitivity response [180].

MSCs can also be tested as carriers of genes or genetic modifications. Due to their ability to migrate to injury sites, MSCs represent a robust platform for delivery of genes associated with regeneration and repair of renal tissue, functioning as a "Trojan Horse" [181]. Thus, several genes associated with trophic factors may be used for these purposes, such as HGF and klotho, since they are renoprotective, as reviewed elsewhere [16].

In addition, genetic modifications of MSCs, which are also very promising in the context of DKD, include the overexpression of erythropoietin, CXCR4, CTLA4Ig, and IL-10/selectin, as well as the transfection of minicircles containing biological drugs, such as etanercept, which is a TNF-α blocker [182], and transfection of nanoparticles containing iron oxide, polymers, and plasmids [183].

Despite MSC-based preconditioning treatment that has not been associated with harmful effects, further studies are required to verify its effectiveness in maintaining MSC properties.

To advance MSC-based therapy, production of a large amount of these cells is challenging. Automated hollow-fiber bioreactors were validated to the development of large-scale manufacturing MSCs, providing cells with preserved characteristics and functionality when compared to the manual multilayer flask method [184, 185]. That approach may be cost- and time-saving at the end of the day.

MSC-derived secretome is a cell-free alternative for treating DM1 and DM2 individuals, which can bypass some issues related to autologous and allogeneic MSCs [186]. Some advantages include the absence of antigenic factors, time-saving obtainment, and the adaptation of MSC to produce preestablished secretome components, designed to target specific pathologies, even separating vesicles from soluble proteins and adapting cell product to each disease scenario.

4. Conclusions

Laboratory research set the basis for establishing further translation research including preclinical development and proof of concept in model systems. Thus, animal models indicate that syngeneic, autologous, allogeneic, and xenogeneic MSCs are effective for treating metabolic dysfunction in the DM setting and for halting the progression of DKD. MSCs demonstrated efficacy in controlling several biological processes, such as apoptosis, autophagy, fibrosis,

inflammation, and oxidative stress, as well as in ameliorating renal functional and structural parameters.

BM-MSCs have been applied as the most valuable source of autologous cell transplants for diabetic complications in animals and humans. Despite differential gene expression, expanded MSCs from DM1 donors are phenotypically and functionally similar to healthy control MSCs with regard to their immunomodulatory, migratory potential, multilineage differentiation, and secretion of growth factors. MSCs from DM2 donors exhibit dysfunctional properties, such as senescence, angiogenesis impairment, higher rates of apoptosis, and lower clonogenic potential. Therefore, hyperglycemia may cause abnormalities in intrinsic BM-MSC, which might lose sufficient therapeutic effects in DM2 individuals. Of importance, DM2 donors are usually older and exhibit hyperglycemia for a longer duration, so that aging may play an additional role in MSC dysfunction. Therefore, preconditioning strategies can be used to recover the characteristics and functions of MSCs of diabetic patients before infusion and, thus, improve their performance in autologous therapies in terms of tissue repair and regeneration.

Together, the bench to bedside pathway has been constructed in the last decade. Laboratory research set the basis for establishing further translation research including preclinical development and proof of concept in model systems [187]. In Figure 1, we summarized the main findings of preclinical and clinical studies. Accordingly, phase I clinical trials (safety studies in humans) have substantiated the safe profile of MSC-based therapy, and phase II clinical trials (proof of concept in trial participants) still need to answer important questions. Therefore, well-designed large-scale randomized studies considering the stem cell type, cell number, and infusion method in DM patients are further needed in order to move to phase III clinical trials (large-scale trials to show significant efficacy). In addition, that pathway should include interactions with regulatory agencies and the protocols involved in the Investigational New Drug (IND) application development.

Clinical trials using MSC-based therapy indicate that infusion of autologous or allogeneic MSCs is generally well tolerated. Phase II clinical trials including a longer period of observation will support the efficacy of MSCs. However, the use of these cells to treat diabetic individuals with DKD awaits clinical validation. In conclusion, MSC-based preclinical and phase I/II clinical data encourage the design of future large-scale controlled clinical trials that evaluate DKD response to MSC therapy, while rigorous reporting of safety and efficacy is still needed.

Abbreviations

AGEs: Advanced glycation end products
AMDCC: Animal Models of Diabetic Complications Consortium
AT-MSC: Adipose tissue-derived MSCs
AT-IS-MSC: AT-insulin-secreting-derived MSCs
BM-MSC: Bone marrow-derived MSCs
BTBR$^{ob/ob}$ mice: Black and tan, obese, and tufted *ob*/*ob* (leptin deficient) mice
CFU-f: Colony Forming Unit-fibroblast
CKD: Chronic kidney disease
CV: Cardiovascular
CXCL: Chemokine (C-X-C motif) ligand
CXCR: C-X-C chemokine receptor
DKD: Diabetic kidney disease
DM: Diabetes mellitus
DPP-4 inhibitors: Dipeptidyl peptidase-4 inhibitors
DRD: Diabetic renal disease
ESKD: End-stage kidney renal disease
EXOs: Exosomes
FBG: Fasting plasma glucose
GBM: Glomerular basement membrane
eGFR: Estimated glomerular filtration rate
GLP1-RA: Glucagon-like peptide 1 receptor agonists
GMCs: Glomerular mesangial cells
GMP: Good Manufacturing Practice
HbA1c: Glycated haemoglobin
HGF: Hepatocyte growth factor
HSCs: Hematopoietic stem cells
IDO: Indoleamine 2,3-dioxygenase
IFN-γ: Interferon-γ
IGF-1: Insulin growth factor
IL: Interleukin
IND: Investigational New Drug
iNOS: Inducible nitric oxide synthase
ISC: Insulin-secreting cells
ISCT: International Society for Cell Therapy
LPS: Lipopolysaccharide
MCP-1: Monocyte chemoattractant protein-1
MHC: Major complex of histocompatibility
MIP: Macrophage inflammatory protein
MSCs: Mesenchymal stem/stromal cells
NO: Nitric oxide
NOD: Nonobese diabetic mouse
PDGF: Platelet-derived growth factor
PGE2: Prostaglandin E2
PKC: Protein kinase C
RAAS: Renin-angiotensin-aldosterone system
Sca-1: Stem cell antigen-1
SDF-1: Stromal-derived factor-1
SGLT(i): Sodium-glucose cotransporter (inhibitors)
STZ: Streptozotocin
TGF-β1: Transforming growth factor-β1
TLR: Toll-like receptors
TNF-α: Tumor necrosis factor-α
TEC: Tubular epithelial cells
UAE: Urinary albumin excretion
UCB-MSCs: Umbilical cord blood-derived MSCs
UTMD: Ultrasound-targeted microbubble destruction
VEGF-A: Vascular endothelial growth factor A.

Data Availability

Our manuscript is based on a review of articles that have been already published on PubMed.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Research Article

Comparison of the Effects of Mesenchymal Stem Cells with Their Extracellular Vesicles on the Treatment of Kidney Damage Induced by Chronic Renal Artery Stenosis

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Background. Chronic renal artery stenosis is considered one of the most common causes of renovascular hypertension (RH). Chronic hypoxia can lead to irreversible damage to renal tissue and to a progressive deterioration of renal function. We have previously shown that bone marrow-derived mesenchymal stem cells (BMSCs) improved renal parenchyma and function in a model of RH (2 kidneys, 1 clip model (2K-1C) in rats. Microvesicles (MVs) and exosomes (EXs) released by MSCs have been shown to induce effects similar to those induced by whole cells but with fewer side effects. In this study, we compared the effects of adipose-derived MSCs (ASCs) with those of the MVs and EXs released by ASCs on tissue inflammation and renal function in 2 K-1C rats. *Results*. Flow cytometry analysis showed that even after 15 days, ASCs were still detected in both kidneys. The expression of a stem cell homing marker (SDF1-α) was increased in ASC-treated animals in both the stenotic and contralateral kidneys. Interestingly, SDF1-α expression was also increased in MV- and EX-treated animals. A hypoxia marker (HIF1-α) was upregulated in the stenotic kidney, and treatments with ASCs, MVs, and EXs were effective in reducing the expression of this marker. Stenotic animals showed a progressive increase in systolic blood pressure (SBP), while animals treated with ASCs, MVs, and EXs showed a stabilization of SBP, and this stabilization was similar among the different treatments. Stenotic animals developed significant proteinuria, which was reduced by ASCs and MVs but not by EXs. The increased expression of Col I and TGFβ in both kidneys was reduced by all the treatments, and these treatments also effectively increased the expression of the anti-inflammatory cytokine IL-10 in both kidneys; however, only ASCs were able to reduce the overexpression of the proinflammatory cytokine IL-1β in both kidneys of 2K-1C animals. *Conclusion*. The results of this study demonstrated that the EVs released by ASCs produced beneficial results but with lower efficacy than whole cells. ASCs produced stronger effects in this model of renal chronic hypoxia, and the use of EVs instead of whole cells should be evaluated depending on the parameter to be corrected.

1. Introduction

Chronic renal artery stenosis is considered to be one of the most common causes of renovascular hypertension (RH) [1]. In addition to hypertension, renal artery stenosis causes chronic hypoxia in the kidney and can lead to irreversible renal tissue injury and progressive deterioration of renal function. The prevalence of RH is estimated to be 2% of the general population of hypertensive patients, and RH has become an important cause of end-stage renal disease, especially in elderly patients [1–4]. Chronic hypoxia is characterized by microvascular rarefaction leading to irreversible damage to the renal parenchyma. In fact, clinical studies have reported that even after resolution of stenosis through angioplasty, the recovery of renal function is limited, and patients often progress to end-stage chronic renal disease

[5–8]. In addition, during unilateral renal stenosis, the contralateral kidney is constantly exposed to high blood pressure and exhibits structural and functional features of a hypertensive kidney.

Due to the limited treatment options and the reduced angiogenic and regenerative abilities of the kidney, cellular therapy appears to be a potential therapeutic strategy. Recent studies from our group have shown that mesenchymal stem cells (MSCs) obtained from bone marrow (BMSCs) produced significant beneficial effects in the experimental model of RH proposed by Goldblatt et al. (2K-1C) [9]. Through paracrine effects, BMSCs stimulated angiogenesis, improving microvascular rarefaction, increasing immunomodulation, and resulting in reduced fibrogenesis and proteinuria. All these effects contributed to the prevention of the progressive increase in blood pressure [10]. BMSC treatment also improved the morphology and attenuated the expression of proinflammatory cytokines in the contralateral kidney [11]. Although MSCs have produced promising results in the improvement of kidney architecture and function, the side effects and potential complications observed after cell transplantation, including tumor formation, thrombosis, and capillary obstruction, must be considered [12].

Extracellular vesicles (EVs) produced and released by different cell types, including MSCs, may be an alternative to whole cells in many strategies of cellular therapy. EVs contain many active molecules, such as DNA fragments, mRNAs, microRNAs, and proteins, that play important roles in cellular communication [13, 14]. EVs may influence target cell behavior by transferring their intravesicular content. Microvesicles (MVs) and exosomes (EXs) consist of heterogeneous populations of EVs that differ according to their biogenesis, size, and sedimentation rate [15]. Recent evidence indicates that MSC-conditioned medium containing EVs exerts therapeutic effects similar to those of whole MSCs [16–18]. EVs have been demonstrated to induce renoprotective effects during ischemic acute kidney injury by restoring morphology, promoting angiogenesis and cell proliferation, reducing fibrosis, and restoring renal function [14, 19, 20]. MVs and EXs may have distinct effects, since they can carry different contents in terms of species and/or amounts, and as recently demonstrated by Bruno et al. [21] in an acute kidney injury model, distinct MSC-derived EV populations have different regenerative effects.

On the other hand, the role of distinct populations of EVs in chronic renal ischemia models has been less explored. Therefore, the aim of this study was to investigate and compare the effects of MSCs obtained from adipose tissue with those of their MVs and EXs in a model of chronic renal ischemia induced by partial clamping of the left renal artery (2K-1C model).

2. Materials and Methods

Male Wistar rats (150–180 g) were purchased from the animal facility (CEDEME) of the Federal University of São Paulo. Animals were housed in cages in groups, were acclimated to a room temperature of 23°C with a 12-h light/dark cycle, and were given free access to rat chow and tap water. All the experimental procedures were approved by the Ethics in Research Committee of the Federal University of São Paulo (CEUA-6972080514).

2.1. Induction of Renal Artery Stenosis (2K-1C Model). Animals were anesthetized with intraperitoneal (IP) injections of 40 and 20 mg/kg ketamine and xylazine, respectively (Vetbrands, SP, Brazil). After a peritoneal incision, the left renal artery was partially obstructed with a 0.2 mm silver clip (Sigma-Aldrich, MO, USA) as previously described [9, 10]. The control animals (sham) were subjected to the same surgical procedure but without renal artery occlusion. Animals were observed for six weeks.

2.2. Systolic Blood Pressure. Systolic blood pressure (SBP) was measured weekly by plethysmography (PowerLab-ADInstruments, CH, Australia). Each animal was maintained in a heating box for 10 minutes to promote the vasodilatation of the caudal vein and to facilitate measurement of the SBP with a tail-cuff sensor that was connected to a computerized system (LabChart-ADInstruments, CH, Australia) with specific integration software (PowerLab-ADInstruments).

2.3. Adipocyte Mesenchymal Stem Cell (ASC) Isolation and Characterization. White adipose tissue was removed from the gonadal region of 6-week-old rats under sterile conditions and washed with PBS (phosphate-buffered saline). The tissue was digested enzymatically at 37°C for 40 minutes with constant stirring in 2 ml of low-glucose DMEM containing 0.1% collagenase type 1A (Sigma-Aldrich, MO, USA) and 20 mg of bovine serum albumin (Sigma-Aldrich). The enzymatic activity was quenched with the same volume of fetal bovine serum (FBS, Thermo Fisher Scientific, MA, USA); the digested adipose tissue was centrifuged at 400 g for 15 minutes and resuspended in low-glucose DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (PS). Subsequently, the resuspended tissue was placed in 10 mm diameter plastic dishes and incubated in an incubator at 37° C with 5% CO_2 for 2 hours. After this period, all the medium containing nonadherent cells was discarded. Fresh culture medium with the same concentrations of FBS and antibiotics was then added. During the three days after extraction, the culture medium was changed every 24 hours to gradually remove the red blood cells and cell debris from the extracted cells.

Immunophenotype assays were performed with antibodies against CD73, CD90, CD29, CD105, CD31, CD34, and CD45 (Becton Dickinson, NJ, USA), and the samples were analyzed by flow cytometry (FACS Canto, Becton Dickson, NJ, USA). ASC multipotentiality was evaluated by adipogenic and osteogenic differentiation. To induce differentiation, 1×10^4 cells/cm^2 were placed in each well of a 6-well culture dish in triplicate. The conventional culture medium (low-glucose DMEM+10% FBS) was replaced with adipogenic or osteogenic medium (StemPro Adipogenesis Differentiation Kit and StemPro® Osteogenesis Differentiation Kit, respectively; Invitrogen, MA, USA) for 14-21 days, and the medium was changed every 3 days. Differentiation was confirmed with specific oil red staining for adipocytes and

Alizarin S staining for osteoblasts. Osteogenic differentiation was demonstrated by the accumulation of calcium in the extracellular matrix, as shown by Alizarin Red staining, on days 14 and 21 of differentiation. Adipogenic differentiation was confirmed by fixing the cells for 1 hour with 4% paraformaldehyde and staining with 0.9% Oil red. After a 5-minute incubation period, the cells were washed with deionized water, and the lipid vacuoles were visualized by orange staining under an inverted microscope (Nikon, TKY, Japan).

2.4. Extracellular Vesicle (EV) Isolation and Characterization. EVs were purified from the ASC culture medium and separated according to size into fractions enriched with microvesicles (MVs) or exosomes (EXs) by ultracentrifugation. The ASCs were cultured in 75 cm^2 flasks containing low-glucose DMEM supplemented with 10% FBS and 1% PS until they reached 90% confluency. After this period, the culture medium was changed to low-glucose DMEM with 1% PS but without FBS. The cell supernatant was collected after 72 hours and centrifuged at 3,000 g for 20 minutes to remove cellular debris and apoptotic bodies. This step was followed by ultracentrifugation (Hitachi, TKY, Japan) at 10,000 g for 30 minutes to obtain microvesicles and then at 100,000 g for 2 hours to obtain exosomes [22]. The vesicles were identified by Nanosight following the manufacturer's protocol, and the expression of MMP2 (MV) and CD63 (EX) (Abcam, MA, USA) was analyzed by Western blotting.

2.5. Experimental Protocol. The rats were divided into five groups: sham ($n = 7$), stenotic ($n = 7$), stenotic+ASC (ASC, $n = 7$), stenotic+MV (MV, $n = 7$), and stenotic+EX (EX, $n = 7$). The ASCs, MVs, and EXs were infused through the tail vein at the 3rd and 5th weeks after clamping. The ASCs were injected at a density of 2×10^5 cells [23] diluted in 200 μl of PBS [10]. The EV-treated groups received 100 μg of MVs or EXs [14, 20] diluted in 200 μl of PBS. Animals were euthanized 6 weeks after clipping by the intraperitoneal injection of an anesthetic overdose of ketamine (160 mg/kg) and xylazine (80 mg/kg).

2.6. Cell Labeling for Tracking Assay. To assess the migration and retention time of the ASCs in the tissues, the cells were incubated with Qtracker 585 nm (Invitrogen, CA, USA) following the protocol recommended by the manufacturer. The cells were administered to an additional group of stenotic rats six weeks after renal artery clamping, and then, four groups were established according to the period after ASC administration: 24 hours ($n = 3$), 48 hours ($n = 3$), 72 hours ($n = 3$), and 15 days ($n = 3$). Animals that did not receive treatment were used as controls. Fragments of the left (stenotic) and right (contralateral) kidneys, heart, and lungs were homogenized through a 70 μM cell filter (Becton Dickinson, Franklin Lakes, NJ, USA), and the cells were washed twice in ice-cold PBS and analyzed by flow cytometry (FACSCanto, Becton Dickinson, NJ, USA).

2.7. Assessment of Renal Function. At the end of six weeks, animals from all the experimental groups were housed in metabolic cages for 24 h urine collection. Then, the animals were anesthetized with ketamine and xylazine, and aortic blood samples were collected. The plasma and urinary concentrations of sodium, potassium (9180 Electrolyte Analyzer, Roche Diagnostics, Indianapolis, USA), and creatinine (Labtest Diagnostics, Lagoa Santa, Brazil) and the urinary excretion of protein (Labtest Diagnostics, Lagoa Santa, Brazil) were determined. Both kidneys were harvested, the cortex and medulla were quickly separated on ice, and the fragments were destined for gene expression analysis.

2.8. Gene Expression Analysis (qPCR). Total RNA was obtained from the cortex and medulla of both kidneys by the phenol and guanidine isothiocyanate-cesium chloride method using the TRIzol kit (Ambion, CA, USA), according to the manufacturer's instructions. Two micrograms of total RNA was treated with DNase (Promega, WI, USA) to avoid genomic DNA contamination and reverse-transcribed into cDNA by the addition of a mixture containing 0.5 mg/ml oligo(dT) (Life Technologies, CA, USA), 10 mM DL-dithiothreitol (Life Technologies, CA, USA), 0.5 mM deoxynucleoside triphosphates (Life Technologies), and 200 units of reverse transcriptase enzyme (SuperScript RT II; Life Technologies). The mRNA expression levels were estimated using qPCR (QuantStudio 7; Applied Biosystems, CA, USA) by the TaqMan or SYBR Green qPCR methods. The specific TaqMan Assay primer sets were as follows: IL-10 (Rn00563409_m1), IL-1β (Rn00580432_m1), β-actin (Rn00667869_m1), and HIF-1α (Rn1472831_m1) (Life Technologies, NY, USA). The following forward and reverse primers used for the SYBR green assays were as follows (forward and reverse, respectively): β-actin (5′ cctctatgccaacacagtgc 3′ and 5′ acatct-gctggaaggtggac 3′), TGFβ (5′ tgacgtcactggagttgtacgg 3′ and 5′ aactattgcttcagctccacagaga 3′), and SDF-1α (5′ gagccatgtcgccagagccaac 3′ and 5′ cacacctctcacatcttgagcctct 3′). The comparative CT method ($\Delta\Delta$CT) was employed to estimate the gene expression, and the relative mRNA levels were calculated as $2^{-\Delta\Delta CT}$. The mRNA expression levels were normalized to β-actin expression, which was used as an endogenous control.

2.9. Western Blot Analysis. Total protein was extracted from microvesicles and exosomes in ice-cold buffer [50 mM TRIS (Sigma-Aldrich, MO, USA), 150 mM NaCl (Labsynth, SP, Brazil), 1.0% nonidet-P-40 (Bio-Rad Laboratories, CA, USA), 0.5% sodium deoxycholate (Sigma-Aldrich), and 0.1% SDS (pH 8.0; Sigma-Aldrich) containing protease inhibitors (AEBSF, aprotinin, bestatin, E-64, leupeptin, pepstatin A; Protease Inhibitor Cocktail; Sigma-Aldrich)] and quantified using a modified Lowry method (Bio-Rad, HH, UK). The protein samples (50 μg) were separated according to size by 12% SDS-PAGE and electroblotted onto nitrocellulose membranes (GE Life Sciences, LC, UK). The membrane blots were probed with primary antibodies overnight at 4°C and with HRP-conjugated secondary antibodies for 1 h at 4°C. The primary antibodies used were as follows: anti-MMP2 diluted at 1 : 100 (Abcam, MA, USA) for the MVs and anti-CD63 diluted at 1 : 100 (Abcam, CBG, UK) for the EXs. Next, the membranes were incubated with HRP-conjugated secondary antibodies (GE Life Sciences). The

protein bands were visualized using the Immobilon Western HRP substrate (Millipore, MO, USA). The obtained bands were quantified using Uvitec analysis software (Uvitec Limited, CBG, UK).

2.10. Statistical Analysis. The results are represented as the mean ± standard deviation. The data were analyzed using one-way ANOVA followed by the Tukey or Newman Keuls posttests when appropriate. The blood pressure data were analyzed using two-way ANOVA followed by the Bonferroni posttest. Statistical significance was defined as $p < 0.05$. The data were analyzed statistically using GraphPad Prism 5 software (GraphPad Software, CA, USA).

3. Results

3.1. Characterization of ASCs and MVs. ASCs were characterized according to their capacity for osteogenic and adipogenic differentiation. For the control, the ASCs were maintained in standard media, and no differentiation was observed (Supplementary Figure 1A). To evaluate osteogenic differentiation, the ASCs were incubated with osteogenic differentiation media for 21 days and then subjected to the Alizarin Red staining protocol, which stained the calcium deposits (Supplementary Figure 1B). To evaluate adipogenic differentiation, the ASCs were incubated with differentiation media and exhibited a phenotypic change similar to adipocytes after 14 days of culture. After staining the cells with Oil Red to visualize the cytoplasmic accumulation of lipids, red staining was observed, as shown in Supplementary Figure 1C. After the third passage, the ASCs exhibited a negative staining pattern for hematopoietic markers (CD34 and CD45) and the endothelial marker (CD31). The ASCs exhibited a positive staining pattern for the adhesion marker (CD29) and mesenchymal markers (CD105, CD90, CD73, and CD44) (Supplementary Figure 1D).

The ASCs were cultured without the addition of FBS to obtain conditioned medium. Three time points (24 h, 48 h, and 72 h) were established for cell survival and viability analysis. As shown in Supplementary Figure 2A-B, there was no significant difference in the survival rate and cell viability at 24 h, 48 h, and 72 h. These data demonstrate that ASC culture without FBS does not affect the cell viability and apoptosis of these cells within 72 h. Due to the higher percentage of EVs/ml in the culture media at the 72 h time point (data not shown), we chose this time point for the isolation of extracellular vesicles.

The characterization of the extracellular vesicles was first performed through the analysis of the concentration and size of the nanoparticles by Nanosight. The microvesicles exhibited variable and larger sizes (115 ± 1 vs. 57 ± 36; $p < 0.05$) compared to the exosomes (Supplementary Figure 2C), demonstrating that ASCs release vesicles that are compatible with both microvesicles and exosomes in terms of size. Fifty micrograms of protein from the MVs and EXs was subjected to Western blotting analysis using antibodies against MMP2 as a marker of microvesicles and CD63 as a marker of exosomes; the results are shown in Supplementary Figure 2D. These data indicate that these proteins can be used to confirm the presence of each type of EV derived from ASCs.

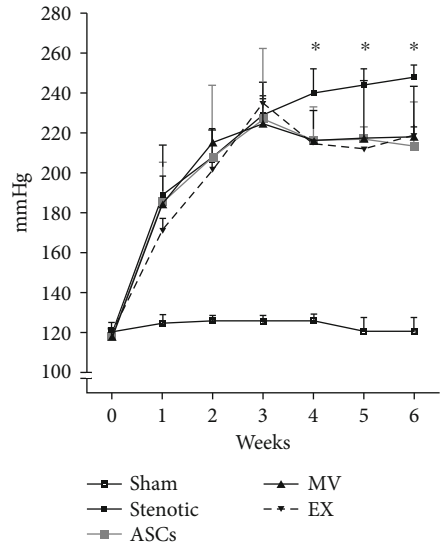

Figure 1: Systolic blood pressure. Values were recorded weekly by plethysmography for six weeks. Groups: sham ($n = 10$), stenotic ($n = 7$), stenotic+ASC ($n = 7$), stenotic+MV ($n = 7$), and stenotic +EX ($n = 7$). SBP is presented as the mean ± SD. $^*p < 0.05$ (two-way ANOVA followed by Bonferroni posttest).

3.2. ASC Tracking and Distribution. To analyze the retention time of the ASCs in the different tissues, Qtracker®-labeled ASCs were injected 6 weeks after the induction of renal artery stenosis, and 4 groups were established according to the time point after the administration of the ASCs (24 hours, 48 hours, 72 hours, and 15 days). These groups were analyzed by flow cytometry (Supplementary Figure 3). The highest percentage of cells in the tissues was observed at 48 hours, and these cells were mainly located the left kidney (28%) and right kidney (28%). The cells were also found in the heart (0.5%) and lung (4%), and after a few days, the presence of ASCs in the lungs (0.05%) decreased considerably, while the percentage of cells in the left kidney (15%) and right kidney (19%) remained elevated, even 15 days after administration. Other animal groups received 2 injections of ASCs at the 3rd and 5th weeks after clamping. These groups also showed retention of these cells in the right kidney (16%) and the left kidney (22%).

3.3. Effect of Treatments on SBP. The basal SBP values were similar among the groups (Figure 1), demonstrating that the conditions of the animals used in the experiments were similar prior to the procedure. After renal artery clamping, all stenotic animals exhibited a progressive increase in SBP until the 3rd week. At that time point, the treated groups (ASCs, MVs, and EXs) showed a slight reduction in SBP, which was followed by a stabilization of SBP in the 4th week and no additional increases until the 6th week. These results were different from those observed in stenotic nontreated animals.

3.4. Effect of Chronic Renal Artery Stenosis on Body, Kidney, and Heart Weights. Hypertensive animals showed lower

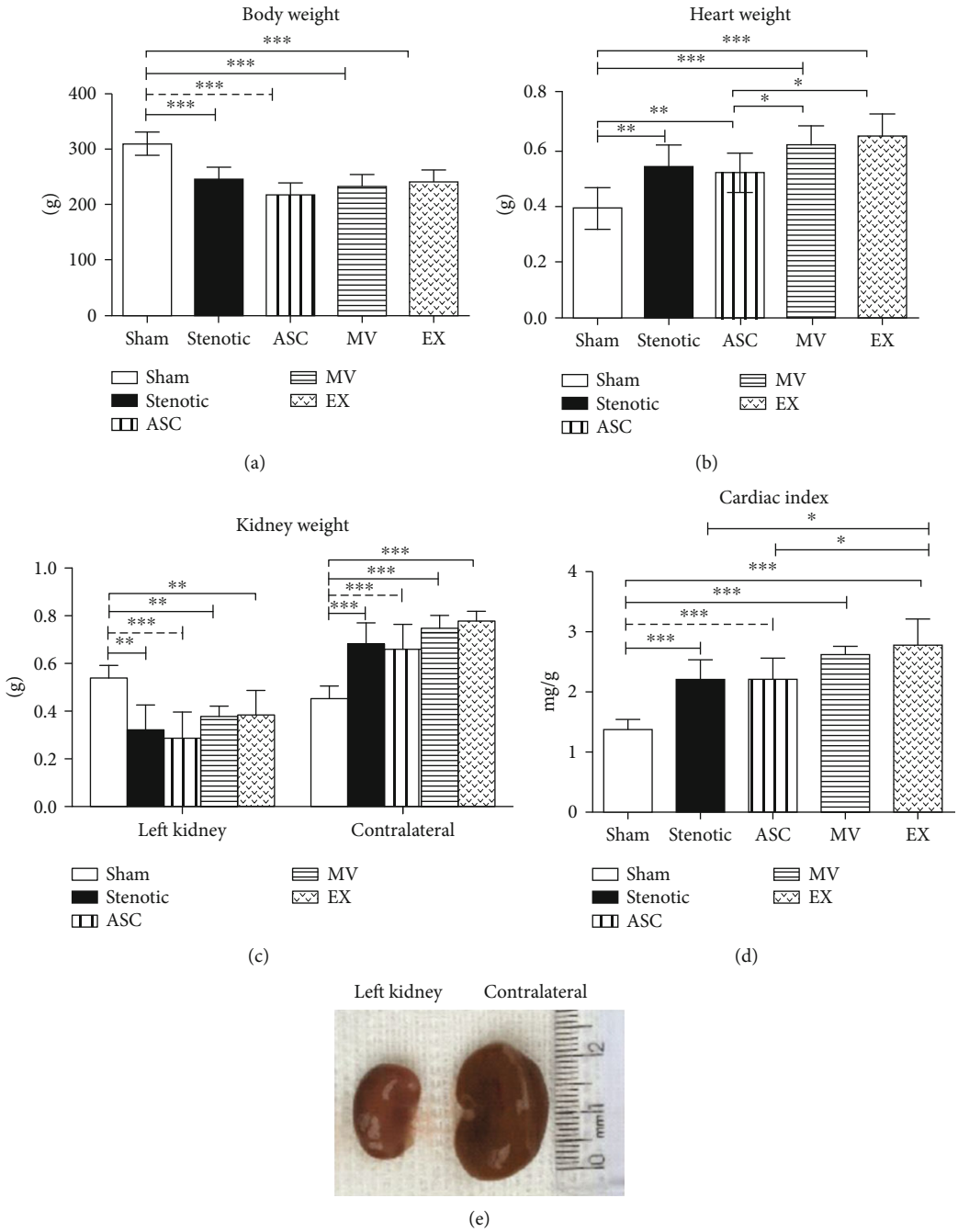

Figure 2: Body weight (a), heart weight (b), kidney weight (c), and cardiac index (d). Groups: sham ($n = 6$), stenotic ($n = 6$), stenotic+ASC ($n = 6$), stenotic+MV ($n = 6$), and stenotic+EX ($n = 6$). Representative images from the stenotic (left) kidney and contralateral kidney after occlusion (e). Data are presented as the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA followed by the Newman-Keuls posttest).

body weight (BW) than sham animals, and the change in BW was not affected by any of the treatments (Figure 2(a)). In contrast to BW, stenotic animals showed a significant increase in heart weight (Figure 2(b)) compared to sham animals, resulting in an increase in the cardiac index (Figure 2(d)). Cardiac hypertrophy was not reversed by any of the treatments (Figure 2(b)). There was a significant reduction in the kidney weight of stenotic animals, whereas the contralateral kidney weight of stenotic animals was significantly higher than that of sham animals. Similar to the heart weight, none of the treatments modified the kidney weight (Figures 2(c) and 2(e)).

3.5. Renal Function Parameters. There was no significant change in the serum creatinine levels (Figure 3(a)) or in the estimated GFR (creatinine clearance, Figure 3(b)) among the groups. In contrast, significant increases in the urinary volume were observed in stenotic animals (Figure 3(c)).

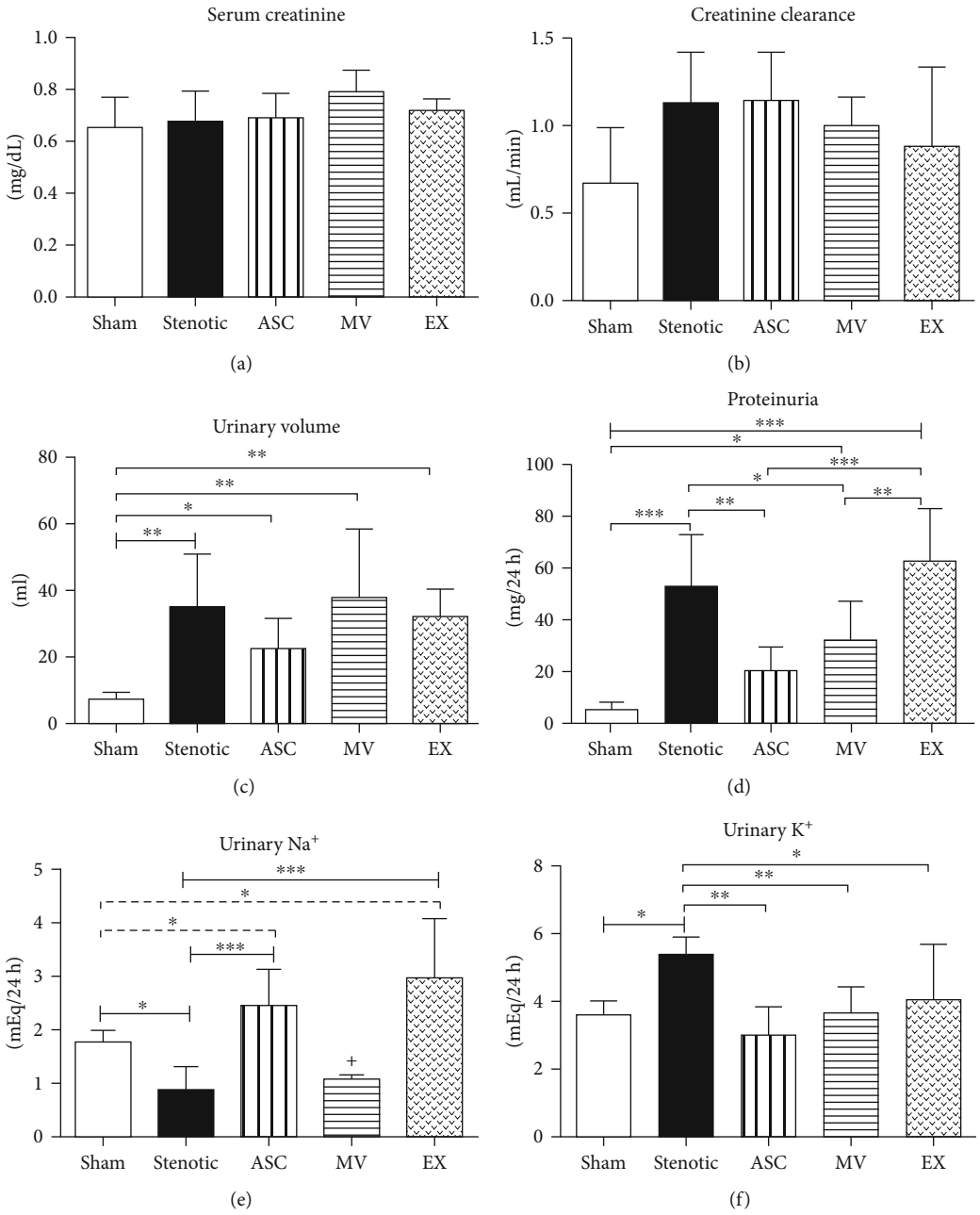

Figure 3: Renal function parameters. Serum creatinine (a), creatinine clearance (b), urinary volume (c), proteinuria (d), urinary sodium excretion (e), and urinary potassium excretion (f). Groups: sham ($n = 6$), stenotic ($n = 6$), stenotic+ASC ($n = 6$), stenotic+MV ($n = 6$), and stenotic+EX ($n = 6$). Data are presented as the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{+}p < 0.001$ (one-way ANOVA followed by the Newman-Keuls posttest).

Polyuria is a typical manifestation of this model, and none of the treatments modified the urinary volume. Despite the absence of detectable changes in serum creatinine, signs of renal dysfunction could be observed in stenotic animals, such as the increase in proteinuria (Figure 3(d)). Interestingly, proteinuria was reduced by the ASC and MV treatments but not by the EX treatment (Figure 3(d)). Stenotic animals presented a reduction in the levels of urinary Na^+ excretion compared to sham animals (Figure 3(e)). The ASCs and EXs were able to elevate the urinary Na^+ levels, but this natriuretic effect was not observed with the MVs. Stenotic animals showed significant urinary K^+ loss, which was corrected by all the treatments (Figure 3(f)).

3.6. ASC and EV Treatments Resulted in Different Gene Expression Responses. The expression of collagen type I (Col I) was increased in both kidneys of hypertensive animals (Figure 4). The overexpression of collagen I in both kidneys was reduced by all the treatments (Figures 4(a)–4(d)). Similar to collagen expression, TGFβ gene expression was increased in both kidneys of stenotic animals (Figures 4(e)–4(h)). The ASCs, MVs, and EXs effectively reduced the expression of

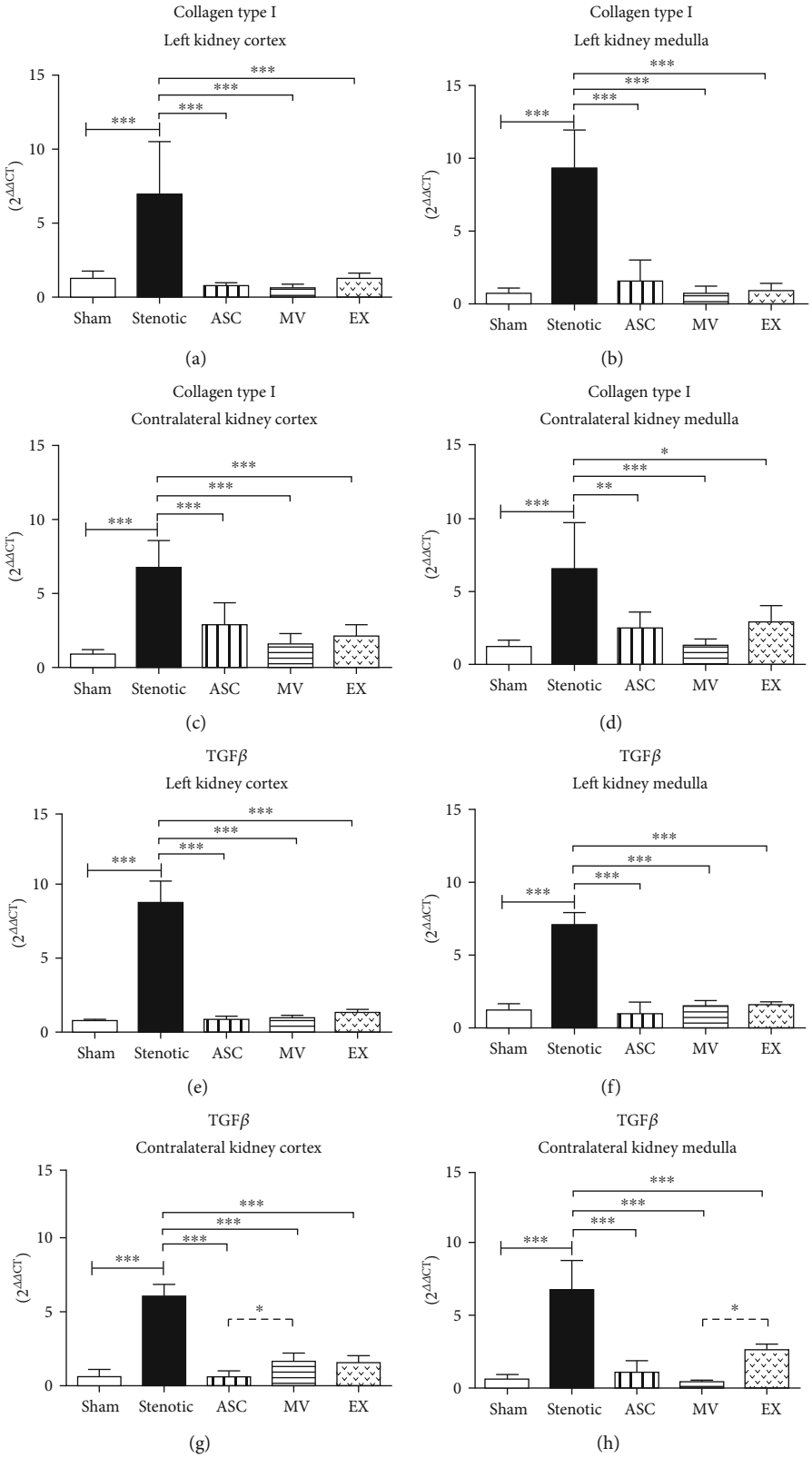

Figure 4: Expression of collagen type I and TGFβ mRNAs determined by quantitative RT-PCR. Collagen mRNA: stenotic kidney cortex (a), stenotic kidney medulla (b), contralateral kidney cortex (c), contralateral kidney medulla (d). TGFβ mRNA: stenotic kidney cortex (e), stenotic kidney medulla (f), contralateral kidney cortex (g), contralateral kidney medulla (h). Groups: sham ($n = 5$), stenotic ($n = 5$), stenotic+ASC ($n = 5$), stenotic+MV ($n = 5$), and stenotic+EX ($n = 5$). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA followed by Tukey's posttest).

TGFβ in the stenotic kidney (Figures 4(e) and 4(f)) and in the contralateral kidney cortex (Figures 4(g) and 4(h)).

There was an increase in the expression of the proinflammatory cytokine IL-1β in both kidneys of hypertensive animals (cortex and medulla) compared to sham animals (Figures 5(a)–5(d)). The expression of IL-1β was effectively reduced in both kidneys, mainly by the ASC treatment (Figures 5(a)–5(d)). In the stenotic kidneys, treatment with ASCs and EXs, but not with MVs, resulted in an improvement in the expression of the proinflammatory cytokine IL-1β in both the cortex and medulla (Figures 5(a) and 6(b)). The effects of the treatments were less consistent in the contralateral kidney. In the cortex, only ASCs was able to decrease IL-1β, whereas MVs and EXs had no effect. In contrast, all the treatments were effective in the medulla (Figures 5(c) and 5(d)). The induction of renal artery stenosis did not significantly modify the expression of the anti-inflammatory cytokine IL-10 in any of the kidneys (Figures 5(e)–5(h)). Despite this finding, the ASC treatment was effective in increasing the IL-10 expression levels in both kidneys. On the other hand, MVs and EXs were less effective in both kidneys. The MV treatment was effective in increasing IL-10 expression in the stenotic kidney cortex and contralateral kidney (Figures 5(e)–5(h)), while the EX treatment was effective in increasing the expression of this marker only in the contralateral kidney cortex (Figure 5(g)).

As expected, the expression of the hypoxia marker HIF1-α was increased in the cortex and medulla of the stenotic kidney (Figures 6(a) and 6(b)) but not in the contralateral kidney (Figures 6(c) and 6(d)). Treatments with ASCs, MVs, and EXs were effective in reducing this marker, and its expression reached values similar to those of the sham group (Figures 6(a) and 6(b)). Animals treated with ASCs showed a significant increase in the expression of the homing stem cell marker SDF1-α in the cortex and medulla in both the stenotic and contralateral kidneys (Figures 6(e)–6(h)). Interestingly, the MV- and EX-treated groups also showed an increase in the expression of SDF1-α in both kidneys, suggesting that this mRNA can be transported via extracellular vesicles.

4. Discussion

In this study, we compared the beneficial effects of whole adipocyte-derived stem cells (ASCs) and 2 distinct populations of secreted EVs (MVs and EXs) in a model of chronic renal hypoxia induced by partial stenosis of the renal artery. In addition to arterial hypertension, renal artery stenosis causes chronic hypoxia, resulting in irreversible renal tissue injury and leading to progressive deterioration of renal function [1], which in turn contributes to worsened hypertension.

We confirmed previous results showing that partial clamping of the left renal artery was effective in producing severe RH, resulting in inflammation and tissue fibrosis in both the clipped and contralateral kidneys [10]. Additionally, hypertensive animals presented lower body weight, cardiac hypertrophy as a consequence of arterial hypertension, reduced stenotic kidney weight, and contralateral kidney hypertrophy as a consequence of hyperflow and hyperfiltration. These results are similar to those observed previously and are characteristics of the model of RH and chronic renal hypoxia [10, 11].

Previous studies [10, 24, 25] demonstrated the beneficial effects of BMSCs on the improvement of renal parenchyma and kidney function in similar animal models of RH [10, 11, 26]. Here, we showed that ASCs induced beneficial effects similar to those induced by BMSCs, with the advantage of being obtained from a more accessible and efficient source than bone marrow.

On the other hand, the benefits of MSCs of different origins have been attributed to their EVs [27], which possess significant potential as a novel alternative to whole-cell therapies [28, 29]. However, it has been demonstrated that different populations of EVs have different contents with distinct biological and regenerative effects [21, 30] and are capable of modulating cellular pathways in recipient cells by different mechanisms, such as direct stimulation, proteins, RNA, miRNA transfer, or surface receptor interaction [31, 32]. The differential ultracentrifugation method used to isolate the EVs in the current study resulted in vesicles that were compatible with EX- and MV-enriched populations; thus, it was possible to compare the regenerative capacity of whole ASCs with that of their distinct EV populations.

ASC treatment resulted in a discrete reduction in SBP; however, compared with that in the untreated hypertensive group, the progressive increase in SBP was blunted in the ASC-treated group, reinforcing the beneficial effects of MSCs of different origins. Interestingly, MVs and EXs produced similar effects on the change in SBP, suggesting that the effects of ASCs on SBP can be mediated by their EVs. Despite the beneficial effects of these treatments in stabilizing SBP, as expected, none of the treatments changed body and organ weight, since both arterial stenosis and hypertension persisted.

It has been shown that EVs derived from MSCs can improve the overall kidney function of CKD patients by increasing the IL-10 levels and repairing the glomerular filtration rate [33]; however, in this study, hypertensive animals showed no alterations in serum creatinine, suggesting a compensatory mechanism of the contralateral kidney and corroborating previously published studies [10, 11, 34]. Despite the lack of change in serum creatinine, signs of functional deterioration, such as significant proteinuria, were observed in the untreated rats. As previously observed with BMSCs [10], the present study showed that ASCs were effective in reducing proteinuria; however, their EVs showed a distinct pattern of protection, since the reduction in proteinuria was also observed with the MV treatment but not with the EX treatment. In contrast, the reduced excretion of sodium observed in hypertensive animals was corrected by ASCs and EXs, but not by MVs. Thus, while ASCs effectively improved all these renal functional parameters, MVs were beneficial in reducing proteinuria and EXs were beneficial in improving Na^+ excretion. These findings can be explained by the diverse bioactive cargo carried by distinct EV populations [35]. It is well known that EX and MV bear distinct biogenesis pathways, since EX are structures formed from multivesicular bodies in the intracellular compartment, whereas MV are released from the plasma membrane. Thus,

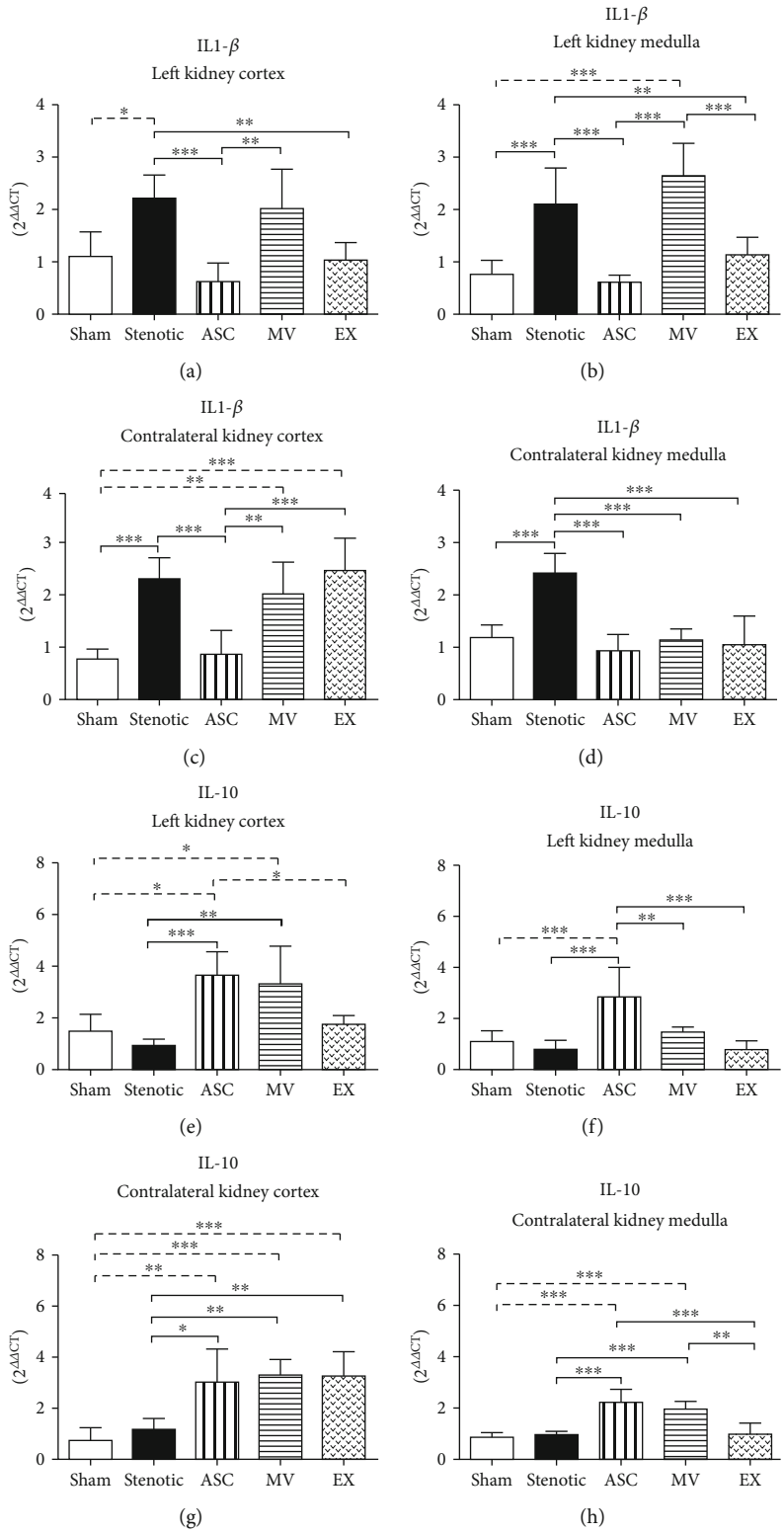

Figure 5: mRNA expression of the proinflammatory cytokine IL-1β and the anti-inflammatory cytokine IL-10 determined by quantitative RT-PCR. IL-1β: stenotic kidney cortex (a), stenotic kidney medulla (b), contralateral kidney cortex (c), contralateral kidney medulla (d). IL-10: stenotic kidney cortex (e), stenotic kidney medulla (f), contralateral kidney cortex (g), contralateral kidney medulla (h). Groups: Sham ($n = 5$), Stenotic ($n = 5$), Stenotic+ASC ($n = 5$), Stenotic+MV ($n = 5$), and Stenotic+EX ($n = 5$). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA followed by Tukey's posttest).

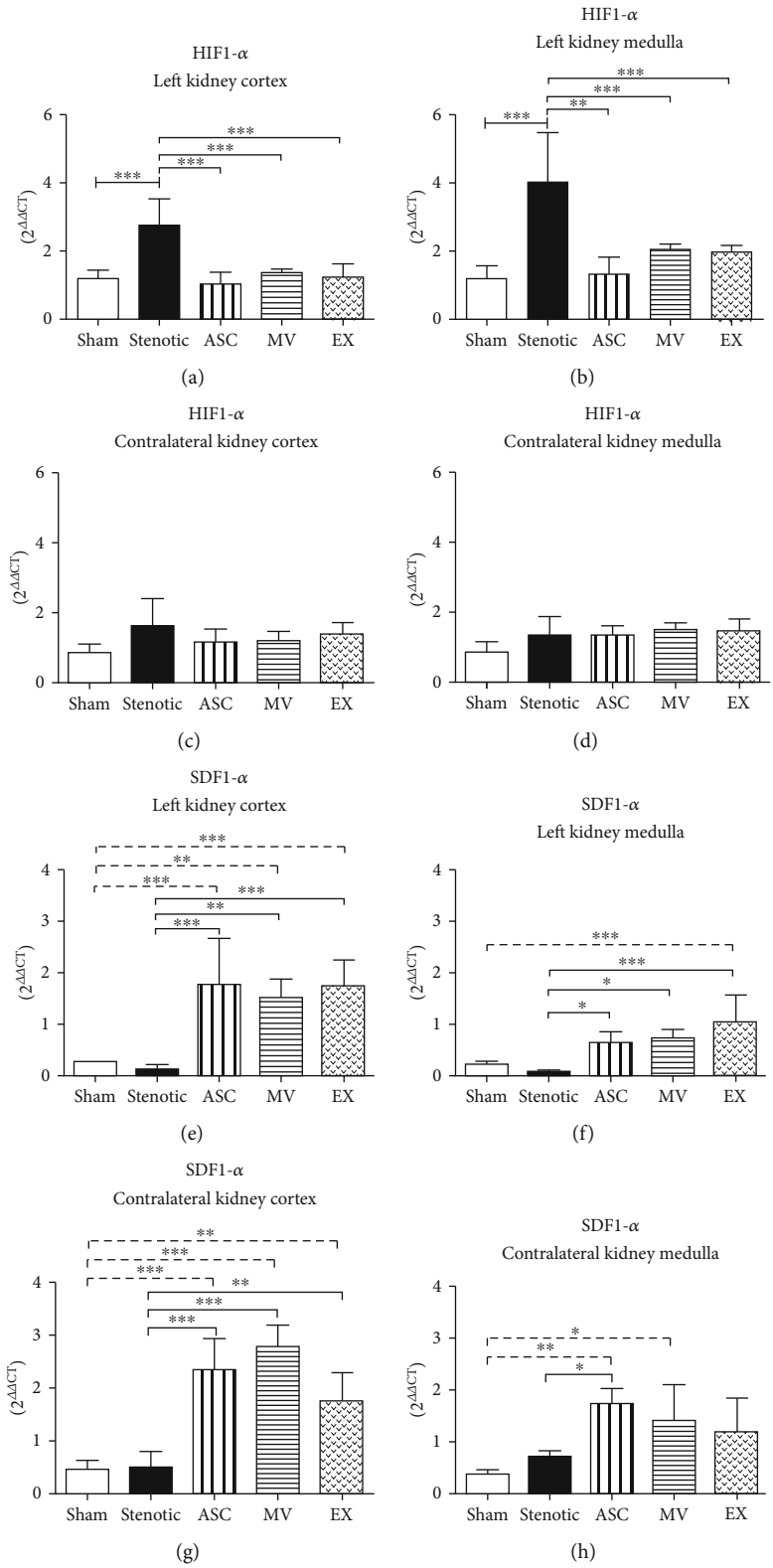

Figure 6: Expression of HIF mRNA determined by quantitative RT-PCR. Stenotic kidney cortex (a), stenotic kidney medulla (b), contralateral kidney cortex (c), contralateral kidney medulla (d). Expression of SDF1-α mRNA determined by quantitative RT-PCR. Stenotic kidney cortex (e), stenotic kidney medulla (f), contralateral kidney cortex (g), contralateral kidney medulla (h). Groups: Sham ($n = 5$), Stenotic ($n = 5$), Stenotic+ASC ($n = 5$), Stenotic+MV ($n = 5$), and Stenotic+EX ($n = 5$). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA followed by Tukey's posttest).

differences in the content of distinct EV populations may result in distinct responses. Taken together, these results suggest that the choice of ASCs and/or their specific EV populations for the treatment of RH would be dependent on the parameter that needs to be corrected.

The beneficial effects of ASCs on the renal function parameters, particularly proteinuria and natriuresis, were also previously observed with BMSCs, and these benefits were attributed to reduced inflammation and improved tissue perfusion by neoangiogenesis [11]. On the other hand, the contralateral kidney is the main kidney responsible for sodium excretion in this model of RH, and we have recently shown that treatment with BMSCs improved the capacity of the contralateral kidney to excrete sodium, contributing to the mitigation of volume-dependent hypertension [36]. Despite the compensatory increase in the renal function of the contralateral kidney and its ability to excrete sodium, the contralateral kidney exhibits characteristics of a hypertensive kidney, including inflammation and fibrosis [11]. The cargo of EVs derived from MSCs is associated with many biological functions, such as the regulation of inflammation, the cell cycle, and cell migration [37, 38]; indeed, we observed that all the treatments were equally effective in reducing the expression of the fibrosis markers collagen 1 and TGFβ. In contrast, the treatments were not equally effective in reducing the inflammatory cytokine IL-1β or in improving the expression of the anti-inflammatory cytokine IL-10. Although all the treatments elevated the expression of the anti-inflammatory IL-10, ASCs were the most effective. We believe that these differences can be attributed to the cargo released by each extracellular vesicle type and/or to the ability of these vesicles to reach the proper site in the damaged tissue, but further studies are required to answer these questions.

As expected, the chronic hypoxia in the stenotic kidneys caused an elevation in the expression of hypoxia induction factor-1α (HIF-1α), and this expression was reduced in the ASC-, MV-, and EX-treated groups. It was previously demonstrated that MSCs were able to induce neoangiogenesis in the stenotic kidneys that could improve renal perfusion and thus reduce HIF1-α synthesis. In the present study, we showed that in addition to ASCs, MVs and EXs were also able to suppress HIF1-α expression. Taken together, these results suggest that ASCs and both populations of EVs were able to induce neoangiogenesis, improve renal perfusion, and thus reduce HIF1α expression.

It has been shown that stem cells have the capacity to migrate to injured tissues in response to chemoattractant factors [39, 40]. The activation of HIF1-α can contribute to the recruitment of MSCs, and this may be a key mechanism for the recruitment and migration of MSCs [41]. One piece of evidence that the ASCs were attracted to the injured tissue was the increase in the synthesis of stromal cell-derived factor 1 (SDF-1), which, in turn, played an important role in stem cell homing.

SDF-1α can be induced under many physiopathological conditions, including hypoxic and angiogenic environments [42], and can lead to a variety of biological effects, including the homing of cells to the kidney after ischemic injury [43]. The presence of ASCs in both kidneys was verified, indicating that the chemoattraction of these cells to the contralateral kidney was not induced by HIF1-α but probably by the presence of high levels of inflammatory molecules in the hypertensive kidney [11, 44].

In the present study, we demonstrated that the highest percentage of ASCs was retained in the stenotic kidney in the first 72 hours, but after 15 days, the cells were still observed in the stenotic kidney and in the contralateral kidney. This fact can be explained by the longer period required for the contralateral kidney to exhibit damage due to severe hypertension. Interestingly, treatment with MVs and EXs also resulted in the upregulation of SDF-1α in the kidneys, suggesting that this molecule can be transported through EVs.

5. Conclusions

ASCs were as effective as stem cells derived from bone marrow in minimizing the renal effects of renovascular hypertension and chronic renal hypoxia. The EVs released by ASCs exerted beneficial effects similar to those produced by ASCs but with less efficacy in correcting some of the parameters analyzed. The results suggest that differences in the contents of EVs may define their therapeutic roles, and considering the advantages of these vesicles compared with ASCs, the results suggest that the choice of therapeutic strategy should be evaluated according to the parameters to be corrected.

Abbreviations

RH: Renovascular hypertension
MSCs: Mesenchymal stem cells
ASCs: Adipose-derived stem cell
BMSCs: Bone marrow stem cells
2K-1C: 2 kidneys, 1 clip model
EVs: Extracellular vesicles
MVs: Microvesicles
EXs: Exosomes
IP: Intraperitoneal
SBP: Systolic blood pressure
PBS: Phosphate-buffered saline
FBS: Fetal bovine serum
BW: Body weight
HIF-1α: Hypoxia induction factor-1α
SDF-1: Stromal cell-derived factor 1
IL-10: Interleukin 10
IL-1β: Interleukin 1 beta.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

All the experimental procedures were approved by the Ethics in Research Committee of the Federal University of São Paulo (CEUA-6972080514).

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

CI contributed to the study design, data acquisition, analysis, interpretation, discussion, and preparation of the manuscript. MO, EM, RR, and AN contributed to conducting the experiments. MB contributed to the study design, data analysis and interpretation, financial support, discussion, and the review and editing of the manuscript.

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Supplementary Materials

Supplementary Figure 1: mesenchymal stem cell characterization. Representative image of a control culture of Wistar rat adipose tissue (ASCs) (5x magnification) (A). Representative image of calcium deposition in the extracellular matrix stained with Alizarin Red S after 21 days of osteogenic differentiation (5x magnification) (B). Representative image of lipid droplets stained with oil red after 14 days of adipogenic differentiation (5x magnification) (C). Immunophenotyping characterization of ASCs in the third passage (D). Supplementary Figure 2: characterization of microvesicles and exosomes derived from ASCs. Analysis of cell survival at different times ($n = 4$) (A), cell viability ($n = 4$) (B), nanoparticle quantification by Nanosight ($n = 3$) (C), and characterization of microvesicles and exosomes by Western blotting ($n = 3$) (D). Data are presented as the mean ± SD. $^{**}p < 0.01$ vs. MV (one-way ANOVA followed by Tukey's posttest, Student's t-test). Supplementary Figure 3: administration and tracking of ASCs labeled with QTracker® and injected intravenously at different times. Representative images of ASCs stained with fluorescent markers and analyzed by flow cytometry from the following tissues from stenotic animals: cortex and medulla of the stenotic kidney (A) and cortex and medulla of the unclipped kidney (B), lung, and heart (C). Percentage of cells stained from the cortex and medulla of the stenotic and unclipped kidney, lung, and heart as determined by flow cytometry ($n = 2$) (D). *(Supplementary Materials)*

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Review Article

Differentiating Induced Pluripotent Stem Cells into Renal Cells: A New Approach to Treat Kidney Diseases

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Renal disease is a major issue for global public health. Despite some progress in supportive care, the mortality rates among patients with this condition remain alarmingly high. Studies in pursuit of innovative strategies to treat renal diseases, especially stimulating kidney regeneration, have been developed. In this field, stem cell-based therapy has been a promising area. Induced pluripotent stem cell-derived renal cells (iPSC-RCs) represent an interesting source of cells for treating kidney diseases. Advances in regenerative medicine using iPSC-RCs and their application to the kidney are discussed in this review. Furthermore, the way differentiation protocols of induced pluripotent stem cells into renal cells may also be applied for the generation of kidney organoids is also described, contributing to studies in renal development, kidney diseases, and drug toxicity tests. The translation of the differentiation methodologies into animal model studies and the safety and feasibility of renal differentiated cells as a treatment for kidney injury are also highlighted. Although only few studies were published in this field, the results seem promising and support the use of iPSC-RCs as a potential therapy in the future.

1. Introduction

Kidney disease is a condition characterized by impaired renal function and/or structure [1, 2]. Its incidence has increased over the years and represents a considerable concern worldwide [3, 4]. Kidney diseases can be distinguished into acute kidney injury (AKI) and chronic kidney disease (CKD), although intercommunication between these two pathologies has been observed [5].

AKI is characterized by a rapid decline in renal function and excessive renal inflammation, as well as programmed death of resident cells [6–8]. In addition, AKI shows high morbidity and mortality and may progress to CKD [6]. Conversely, CKD is defined as the irreversible impairment of renal function and/or structure for 3 months or more [9] and its major causes are systemic arterial hypertension and diabetes [10]. Both AKI and CKD may progress to end-stage renal disease (ESRD), a condition with very few effective and specific available therapies, except for supportive care [11]. ESRD reduces quality of life in patients, significantly diminishes life expectancy, and increases health care costs [12].

The high incidence of renal diseases has caused a relentless pursuit of effective therapeutic methods, aiming to slow down or even stop the progress of the disease. Several strategies have been developed over the time, including the first attempt to create an artificial kidney in the 1940s [13], the long-term successful human kidney transplantation from a living donor [14], the introduction of outpatient dialysis in

the 1960s [15, 16], and the discovery of drugs that delay the progression of kidney disease, such as the renin-angiotensin-aldosterone system blockers [17]. Nevertheless, further strategies that effectively and ideally remove patients from the transplant queue are still needed. Therefore, the development of new therapeutic strategies is crucial and cellular therapy has emerged as a promising field to achieve this goal.

Adult renal tissue has a limited regeneration capacity after an injury [18]. In this context, there is growing interest in the study of regenerative cell therapy in kidney diseases, especially those involving the use of renal cells derived from induced pluripotent stem cells (iPSC). Since iPSC are immature cells [19] and can originate almost any cell type in the body, differentiation protocols commonly attempt to mimic the embryonic development of the kidneys [20]. Unlike pluripotent stem cells, renal cells have a limited number of divisions and are at a more mature stage of differentiation, representing a safer option for cell therapy [21].

Potential applications of iPSC-RCs are described in the present review, as well as discussions on the advances in regenerative medicine and the safety and feasibility of renal differentiated cells as a treatment for kidney injury.

2. Embryonic Development of the Mammalian Kidney

Understanding kidney organogenesis is important to establish a wide range of cell differentiation methodologies. The mammalian kidney originates from the intermediate mesoderm (IM) by the sequential induction of three distinct kidneys: pronephros, mesonephros, and metanephros [22, 23]. During the development process, these structures receive various inductive signals and interactions from the environment in order to become kidneys [22]. The expression of transcription factors PAX2, PAX8, and LHX1 is common to all of them [24, 25]. The first structure to arise is the pronephros, followed by the mesonephros, both degenerating before birth [23]. However, the metanephros is the last to arise and the only one to persist and form the permanent organ with all its individual functional units—the nephrons [22, 23, 26].

In the adult kidney, nephrons are originated through reciprocal signal induction between two IM structures: ureteric bud (UB) and metanephric mesenchyme (MM) [22]. The UB is an epithelial side branch of the Wolffian duct [27], and after induction by glial cell line-derived neurotrophic factor (GDNF), produced by the MM, it evolves towards the MM initiating a series of dichotomous branching and leading to the ureteric epithelial tree development, which in turn will originate the collecting ducts in the metanephros [26–28]. At this stage, the GDNF is continued produced by a specific mesenchymal cell population, named cap mesenchyme, which represents nephron progenitor cells (NPCs) and expresses SIX2 transcription factor [29]. The expression of SIX2 is essential for maintaining the NPC in an immature stage, and its cessation is related to the initiation of nephron commitment [30]. Increased levels of the canonical Wnt9b signaling [31], as well as the Notch signaling [30], have been suggested as inductive of a mesenchyme-to-epithelial transition (MET), initiating the differentiation into nephron cells [27, 30].

Subsequently, a pretubular aggregate of mesenchyme gives rise to a renal vesicle, which develops a lumen and grows towards the distal end of the ureteric tip to form a contiguous lumen with the ureteric epithelium, enabling the appropriate drainage from the nephron through the collecting ducts [32]. The renal vesicle then elongates into a comma-shaped body that undergoes further morphological alteration into S-shaped body [30]. Following that, a glomerulus formation is initiated with a capillary loop invasion into a region denominated glomerular cleft, located between the primitive podocytes and the proximal tubule, in the S-shaped body [30, 33, 34]. During the glomerular maturation, the capillary is divided in several loops, endothelial cells became fenestrated, all the capillary structure is enveloped by the glomerular basement membrane, and podocytes extend their foot processes around the endothelial cells [34]. Some of the transcription factors expressed in early stages of podocytes maturation are LMX1B, FOXC2, POD1, FOXD2, and MAFB [35–39].

Regarding other renal development markers, PAX2 and WT1 are expressed at the beginning of the kidney rising and then downregulated [40]. However, they become active again at the final stages of nephron formation. Furthermore, OSR1 transcription factor is expressed in the intermediate mesoderm, while HOX11 is expressed in the metanephric mesenchyme and the coexpression of SIX2, SALL1, WT1, and PAX2 characterizes a NPC [22] (Figure 1).

3. Pluripotent Stem Cell for Cell-Based Therapy

Over the past two decades, we have experienced growing interest in the use of stem cells as a therapeutic alternative for regenerating damaged tissues and organs. Stem cells are characterized by a large proliferative ability and potential to differentiate into distinct specialized cells. It is also noteworthy that not all stem cell types possess the same differentiation and therapeutic potentials, since pluripotent stem cells exhibit higher potential than multipotent ones [41].

Pluripotent stem cells are self-renewing, clonogenic, and able to undergo lineage commitment into the three different embryonic germ lines: ectoderm, mesoderm, and endoderm [42]. The most famous source of these cells is human embryos at blastocyst phase, namely, embryonic stem cells (ESCs). However, the use of ESC for cellular therapy is quite complex, considering ethical conflicts concerning manipulation of human embryos and safety concerns related to their immunogenicity, as well as the risk of uncontrolled growth and teratoma formation when administrated in vivo [43].

In an attempt to overcome these issues, a new reprogramming technology has led to the generation of iPSC from somatic cells through the introduction of four factors: Oct4, Sox2, c-Myc, and Klf4 [19]. iPSC share with ESCs many features including pluripotency and high differentiation capacity, representing a promising alternative as a source of pluripotent stem cells without ethical concerns and immunorejection, since they can be generated from patient-derived adult cells [41].

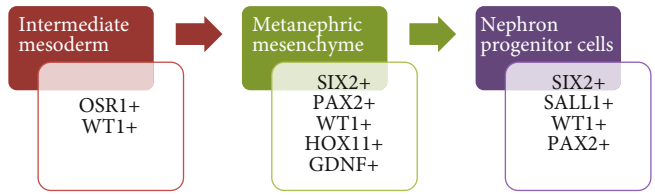

Figure 1: Kidney development stages and expression of transcription factors. OSR1 and WT1 are expressed in the intermediate mesoderm; SIX2, PAX2, WT1, HOX11, and GDNF are expressed in the metanephric mesenchyme; coexpression of SIX2, SALL1, WT1, and PAX2 characterizes a NPC.

Although the application of iPSC in regenerative medicine seems to be promising, their use *per se* in cellular therapy is challenging. Some limitations still persist and include the efficiency of their derivation, the risk of tumor development following transplantation due to their high proliferative potential [21, 44], and the use of viral vectors for reprogramming [45], restricting the iPSC application in an immature stage. Therefore, an alternative approach is to differentiate iPSC into a specific cell type before cellular transplantation. Such differentiation protocols enable the management of crucial variables for cell therapy, some of which are cell fate and expansion in culture.

4. iPSC-Derived Renal Cells

Until recently, renal studies were made only with immortalized kidney cell lines or animal model systems [46]. However, immortalized kidney cells obtained from primary cultures have some limitations, including complications for successful isolation, short-time life periods in culture, and restricted functional and/or morphological characteristics when compared to their native counterparts [47, 48]. Since the iPSC advent [19], great interest has arisen in studying these cells for several diseases and drug development models. A major advantage in the use of iPSC is that they can be generated from somatic cells, enabling immunocompatible transplantation and development of patient-specific models of disease [44].

In vitro differentiation of iPSC into kidney cells can be achieved by the induction of specific nephrogenic factors. In general, a common step in the differentiation protocols is the use, among other substances, of at least two of these three nephrogenic factors: activin A, retinoic acid (RA), and bone morphogenetic proteins (BMPs). These factors have an important role in the generation of kidney structures and specification of renal progenitor cells during renal development. The use of activin A and RA has been described as capable of generating structures related to kidney development [49, 50]. The ureteric bud produces activin A during the kidney growth phase, and it is an important nephrogenic factor, inducing the differentiation into metanephric mesenchyme [51]. Similarly, RA is a crucial factor during kidney development and the specification of renal progenitor cells [52]. The blockage of RA action in this phase causes serious abnormalities to the urinary system [53]. BMP7 also plays an important role in the kidney formation, and its genetic ablation results in highly disorganized and undeveloped kidneys, with an expanded interstitium [54]. In this context, diverse differentiation methodologies have been described in the past few years, aiming to transform iPSC into renal cells with similar properties to those observed in vivo (Table 1).

4.1. Differentiation Protocols. Kim and Dressler were the first to use a combination of activin A, BMP7, and RA to differentiate pluripotent stem cells into renal cells [55]. They induced embryoid body (EB) formation and then differentiated mouse embryonic stem cells (ESC) into cells expressing markers for intermediate mesoderm and early derivatives of the metanephric mesenchyme, such as PAX2, WT1, LIM1, GDNF, Cadherin-6, and EYA1. In addition, they injected the resulting cells into a developing kidney and observed their integration into tubules, along with the expression of proximal tubule markers.

Following this work, Morizane et al. [56] have used iPSC for the generation of kidney cells, which expressed SIX2, WT1, PAX2, Nephrin, and KSP (the last one being a tubular specific marker). The authors generated iPSC from mouse fibroblasts and then initiated the differentiation by the induction of embryoid body (EB) formation, followed by cell plating in gelatin-coated dishes. During the entire process, activin, GDNF, and BMP7 or only activin was added to the differentiation media. When the three nephrogenic factors were used, the authors found that the iPSC could differentiate into metanephric mesenchyme cells, while the sole use of activin enables the generation of tubular cells.

In 2012, Song and collaborators described the direct differentiation into renal cells using human iPSC [57]. The iPSC were generated from normal human kidney mesangial cells and induced to differentiate into renal progenitor cells (RPCs). Activin A, BMP7, and RA were used as nephrogenic factors. The protocol was initiated with the EB formation, followed by adherent culture, for 10 days. At the end of the protocol, cells were characterized and they showed the expression of Nephrin, Synaptopodin, PAX2, and WT1, as well as functional properties similar to those observed in podocytes from primary culture. Furthermore, the cells were able to proliferate in vitro and could be maintained up to 3 months. Later, several new studies were published reporting the generation of different types of renal cells and improving the differentiation protocols [58–84] available.

4.2. Kidney Organoids. The evolution on the knowledge related to kidney organogenesis enabled the creation of enhanced methodologies, in special the ones involving 3D mini-organs, the organoids, which host several kinds of renal

Table 1: Protocols for iPSC differentiation into renal cells.

Authors	Differentiation factors	Differentiation period	Starting iPSC type	Induced cell type
Morizane et al. 2009 [56]	Activin A, GDNF, BMP7	14 days or 18 days	Murine iPSC	Tubular cells, metanephric mesenchyme cells
Song et al. 2012 [57]	Activin A, BMP7, and RA	10 days	Human iPSC	Podocyte-like cells
Mae et al. 2013 [58]	CHIR99021, Activin A, and BMP7	~10 to 20 days	OSR1-GFP human iPSC	Intermediate mesoderm cells
Xia et al. 2013 [59]	BMP4, FGF2, RA, activin A, and BMP2	4 days	Human iPSC	Ureteric bud kidney progenitor-like cells
Taguchi et al. 2014 [60]	BMP4, activin A, basic FGF, CHIR RA, and FGF9	14 days	Human iPSC	Kidney organoid—Metanephric nephron progenitors
Araoka et al. 2014 [61]	CHIR99021 and AM580 or TTNPB	5 days	Human iPSC and OSR1-GFP human iPSC	Intermediate mesoderm cells
Lam et al. 2014 [62]	CHIR99021, FGF2, RA, FGF9, and activin A	9 days	Human iPSC	Intermediate mesoderm cells
Kang & Han 2014 [63]	Activin A, Wnt3a, BMP4, FGF2, RA, BMP7	26 days	Human iPSC	Nephron progenitor cells
Imberti et al. 2015 [64]	RA, RhoA inhibitor and PI3K inhibitor, activin A, FGF2, BMP7, and GDNF	19 days	Human iPSC	Renal progenitor cells
Toyohara et al. 2015 [65]	Activin A, CHIR, BMP7, TTNPB, TGF-β1, and DMH1	28 days	Human iPSC	Renal progenitor cells
Li et al. 2015 [66]	RA, BMP7, activin A, renal epithelial cell growth medium alone	10 days	Mouse iPSC	Renal progenitor cells
Kandasamy et al. 2015 [67]	Renal epithelial growth medium, Rho kinase, BMP2, and BMP7	8 days	Human iPSC	Proximal tubular-like cells
Takasato et al. 2015 [68, 69]	CHIR, FGF9, heparin	25 days	Human iPSC	Kidney organoid–nephron segment cells
Morizane et al. 2015 [70]	FGF2, CHIR, Noggin, activin A, and FGF9	9 days (NPCs) 21-35 days (organoids)	Human iPSC	Kidney organoid–nephron progenitor cells and nephron epithelia
Freedman et al. 2015 [71]	CHIR and B27	16–23 days	Human iPSC	Kidney organoid–nephron segment cells
Ciampi et al. 2016 [72]	N2 and B27 supplements, CP21R7 (Roche), BMP4, retinoic acid, BMP7, FGF9, vitamin D3	13 days	Human iPSC	Podocyte-like cells
Musah et al. 2017 [73]	Activin A, CHIR, BMP7, VEGF, and retinoic acid	26 days	Human iPSC	Podocyte-like cells
Taguchi & Nishinakamura 2017 [74]	Activin, Bmp4, CHIR, FGF9, FGF1, GDNF, LDN193189, SB431542, retinoic acid, and B27	12.5 days	Human iPSC	Ureteric bud-like cells
Wu et al. 2018 [75]	CHIR, FGF9, heparin, Noggin, activin, and NTRK2 inhibitor K252a	25–26 days	Human iPSC	Kidney organoid–nephron progenitor cells
Przepiorski et al. 2018 [76]	CHIR and KnockOut Serum Replacement (KOSR)	14–26 days	Human iPSC	Kidney organoid–nephron progenitor cells
Rauch et al. 2018 [77]	Activin A, BMP7, and retinoic acid	10 days	Human iPSC	Podocyte-like cells
Mae et al. 2018 [78]	Activin A, CHIR, BMP4, LDN193189, A83-01, retinoic acid, PD0325901, FGF8, TTNPB, GDNF, FGF1, thiazovivin	15 days	Human iPSC	Wolffian duct cells
Qian et al. 2019 [79]	CHIR and B27	16 days	Human iPSC	Podocyte-like cells
Hariharan et al. 2019 [80]	Activin A, BMP4, retinoic acid, GDNF, HGF, REGM, FGF2, and BMP7	6 - 14 days	Human iPSC	Renal progenitor cell–multiple nephronal cell

Table 1: Continued.

Authors	Differentiation factors	Differentiation period	Starting iPSC type	Induced cell type
Ahmadi et al. 2019 [81]	CHIR, PD032590, activin A, TTNPB, BMP7, LIF, GDNF, retinoic acid, vitamin D3, dexamethasone	22 days	Mouse iPSC	Podocyte-like cells
Garreta et al. 2019 [82]	CHIR, FGF9, heparin, activin A	21 days	Human iPSC	Kidney organoid–nephron segment cells

cells [60]. Takasato et al. [68, 69] have developed a 3D differentiation protocol by which kidney organoids were generated. Using CHIR, FGF9, and heparin in a series of methodological steps for 25 days, the authors described the formation of a 3D structure, which consisted of multiple nephron segment cells, expressing markers for glomerulus ($WT1^+$ cells), early distal tubule ($GATA3^-$ LTL^- $ECAD^+$ cells), early proximal tubule (LTL^+ $ECAD^-$ cells), and collecting duct ($GATA3^+$ $ECAD1^+$ cells). Renal structures observed during the differentiation protocol resembled in vivo kidney tissue organization, and each organoid comprised a substantial size with more than 500 nephrons.

Morizane and collaborators [70] also described a kidney organoid generation, mainly through CHIR and FGF9 induction in a 3D culture. The authors first differentiated human iPSC into primitive streak cells, following induction into posterior intermediate mesoderm and nephron progenitor cells. These cells were transferred to a 3D culture and treated with CHIR and FGF9 and by day 21; the renal organoids were spontaneously organized in elongated epithelial nephron structures expressing several nephron markers. These structures expressed nephron markers in a contiguous arrangement, including loops of Henle (E-cadherin $(CDH1)^+$ uromodulin $(UMOD)^+$ $BRN1^+$ $AQP1^+$), distal convoluted tubules ($CDH1^+UMOD^-$), glomerular podocytes ($NPHS1^+$ $PODXL^+WT1^+$), and proximal tubules (LTL^+AQP1^+).

Over the past few years, several other protocols involving kidney organoid generation [71, 75, 76, 82] were also described, enabling the use of such differentiated cells for experimental models in kidney disease.

5. iPSC-Derived Renal Cells as Cell Therapy for Kidney Diseases

The ability to self-renew and differentiate makes stem cells a promising strategy for regenerating damaged kidneys. Our group has recently published a work [44] studying the therapeutic potential of iPSC in a CKD model in rats (the 5/6 model). Although iPSC ameliorated CKD rats, they also generated Wilms' tumors, justifying the essential step of differentiating iPSC into renal cells prior to their transplantation into kidney disease models [21, 44].

Few reports have addressed the regenerative potential of iPSC-derived renal cells in kidney diseases (Table 2). Imberti et al. [64] described the generation of renal progenitor cells from human iPSC and studied their therapeutic potential in a mouse model of AKI. Intravenously infused RPCs integrated into mouse renal tissue as early as 24 h after transplantation, especially into tubuli. Results showed a reduction of blood urea nitrogen (BUN) levels and improved renal histology in mice when compared to the control group.

Toyohara et al. [65] have injected $OSR1^+SIX2^+$ RPCs into the renal subcapsule of induced AKI mice and observed that although the cells did not differentiate into tubular structures, kidney function was improved in the treated animals. In addition, histological analysis demonstrated a significant reduction in renal parenchyma damage. Similarly, Li et al. [66] have transplanted RPCs into an ischemia/reperfusion-induced AKI model in rats and observed improved renal function and histological aspects in the treated group.

More recently, Hoshina et al. [83] studied hiPSC-derived RPCs ($CD9^-CD140a^+CD140b^+CD271^+$ cells) as a therapy for AKI. Cells were injected into renal subcapsules after the induction of AKI in a mouse model. The authors described improved renal function and reduced tissue damage, indicated by decreased fibrosis, tubular dilatation, and loss of tubular borders. Ahmadi et al. [81] also studied the potential of renal cells in kidney disease, specifically using iPSC-derived podocytes in a mouse model of membranous nephropathy. As early as 10 days after the cell transplantation, proteinuria was significantly decreased in the treated animals and there was also reduction in the urine albumin/-creatinine ratio, indicating the benefits of using mature renal cells (iPSC-podocyte) as cell therapy.

6. Future Perspectives

A nephron is a complex structure, composed of multiple varieties of cells [84]. Therefore, addressing which one should be transplanted for treating specific kidney injuries remains a challenge. However, it is expected that the transplantation of kidney progenitors enables the final cell differentiation into the tissue and provides a source of several types of cells, which can be used for renal regeneration and improvement of kidney function [85] (Figure 2).

iPSC represent a valuable choice for cell therapy, considering their ability to generate renal cells at their more primitive lineage stage. Such cells may then be employed for therapeutic proposes, differentiated into a mature cell, or even be used for repopulating decellularized native kidney [86, 87]. The advance in the understanding of the kidney development has provided the refinement of differentiation methodologies leading to improved cost-effective protocols and generation of more types of cells and even more complex and organized structures [88, 89]. 3D

Table 2: Published studies involving iPSC-derived renal cells in kidney diseases.

Authors	iPSC-derived cell type	Kidney disease type
Imberti et al. 2015 [64]	Renal progenitor cell	Cisplatin-induced AKI
Toyohara et al. 2015 [65]	Renal progenitor cell	Ischemia/reperfusion-induced AKI
Li et al. 2015 [66]	Renal progenitor cell	Ischemia/reperfusion-induced AKI
Hoshina et al. 2018 [83]	Renal progenitor cell	Ischemia/reperfusion-induced AKI
Ahmadi et al. 2019 [81]	Podocytes	Membranous nephropathy

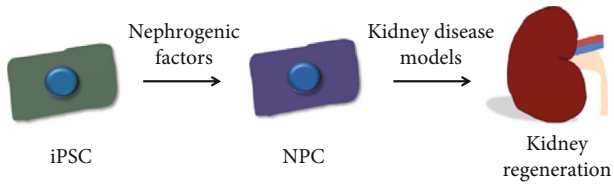

Figure 2: iPSC-derived renal cells in kidney diseases. iPSC: induced pluripotent stem cells; NPC: nephron progenitor cell.

conformation culture, associated with specific growth factors, is aimed at mimicking the developmental stages and provides generation of organoids, with a wide range of cell types that are also self-organized in organ-specific structures, resembling their native counterparts [90].

The development of kidney organoids allows their use for regenerative medicine as a source of several types of renal cells (from RPCs to mature podocytes or tubular cells), which could be applied for cell therapy [91]. In addition, such organoids may be used for studying renal embryonic development and diseases, as well as for testing drug toxicity and, therefore, providing a valuable tool for improving in vitro scale, structure, and functional maturation of the kidney in the future [92–94].

Although the studies underlying the use of iPSC-derived renal cells in kidney diseases have promising results, only a few were published and further investigation on whether these cells could effectively be applicable as a treatment or not is needed. Studies in this direction may provide a better understanding of the action mechanisms of renal cells in kidney diseases and their efficacy and safety, as well as the possibility to translate these discoveries from bench to bedside. Further studies are necessary to address the use of iPSC-derived renal cells in CKD. Such cells may represent a promising strategy to slow down the progression of disease and regenerate the damaged tissue.

7. Conclusions

The development of innovative iPSC differentiation protocols into renal cells and the advanced knowledge in kidney development enable the emergence of new studies focused on the treatment of kidney diseases. Such studies demonstrate the therapeutic potential of differentiated renal cells, supporting their promising use as cell therapy. Long-term studies are necessary to address the beneficial effects and safety of iPSC-derived renal cells.

Data Availability

The data supporting this review are from previously reported studies and datasets, which have been cited in the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

The Efficacy of Mesenchymal Stem Cells in Therapy of Acute Kidney Injury Induced by Ischemia-Reperfusion in Animal Models

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Mesenchymal stem cells (MSCs), discovered and isolated from the bone marrow in the 1960s and with self-renewal capacity and multilineage differentiation potential, have valuable immunomodulatory abilities. Acute kidney injury (AKI) refers to rapid renal failure, which exhibits as quickly progressive decreasing excretion in few hours or days. This study was performed to assess the efficacy of MSCs in the treatment of AKI induced by ischemia-reperfusion using a meta-analysis method. A literature search using corresponding terms was performed in the following databases: Embase, Cochrane Library, PubMed, and ISI Web of Science databases up to Dec 31, 2019. Data for outcomes were identified, and the efficacy of MSCs for AKI was assessed using Cochrane Review Manager Version 5.3. Nineteen studies were eligible and recruited for this meta-analysis. MSC treatment can reduce the Scr levels at 1 day, 2 days, 3 days, 5 days, and >7 days (1 day: WMD = −0.56, 95% CI: -0.78, -0.34, $P < 0.00001$; 2 days: WMD = −0.58, 95% CI: -0.89, -0.28, $P = 0.0002$; 3 days: WMD = −0.65, 95% CI: -0.84, -0.45, $P < 0.00001$; 5 days: WMD = −0.35, 95% CI: -0.54, -0.16, $P = 0.0003$; and >7 days: WMD = −0.22, 95% CI: -0.36, -0.08, $P = 0.002$) and can reduce the levels of BUN at 1 day, 2 days, 3 days, and 5 days (1 day: WMD = −11.72, 95% CI: -18.80, -4.64, $P = 0.001$; 2 days: WMD = −33.60, 95% CI: -40.15, -27.05, $P < 0.00001$; 3 days: WMD = −21.14, 95% CI: -26.15, -16.14, $P < 0.00001$; and 5 days: WMD = −8.88, 95% CI: -11.06, -6.69, $P < 0.00001$), and it also can reduce the levels of proteinuria at 3 days and >7 days and alleviate the renal damage in animal models of AKI. In conclusion, MSCs might be a promising therapeutic agent for AKI induced by ischemia-reperfusion.

1. Introduction

Mesenchymal stem cells (MSCs), discovered and isolated from bone marrow in the 1960s and with self-renewal capacity and multilineage differentiation potential, have valuable immunomodulatory abilities and exist in almost all human tissue lineages [1–3]. MSCs can secrete a wide range of growth factors, such as cytokines, chemokines, and extracellular vesicles—collectively termed the secretome [4, 5]. MSCs support revascularization, inhibition of inflammation, regulation of apoptosis, and promotion of the release of beneficial factors [6, 7]. MSC transplantation is a fast-developing therapy in cell-based therapies and regenerative medicine [8–10]. Thus, they are regarded as a promising candidate for the repair and regeneration of some diseases [6, 11–13].

Acute kidney injury (AKI) refers to rapid renal failure, which exhibits as quickly progressive decreasing excretion in few hours or days [14]. It is mainly characterized by oliguria or accumulation of serum creatinine, which is elevated by 0.3 mg/dl within 48 hours or more than 50% of the baseline [15, 16]. Ischemia-reperfusion is one of the common pathological conditions in AKI. It indicates that organs regain perfusion after temporary restriction of blood flow. In response to the sudden interruption of blood supply in IRI, oxidative stress and inflammation appear frequently in AKI [17, 18]. A series of cytokines, such as interleukins and tumor necrosis factor-α (TNF-α), are activated in this procedure. By promoting oxidative stress or apoptotic processes, they finally enhance renal inflammation and dysfunction [18–20].

This study was performed to assess the efficacy of MSCs in the treatment of AKI induced by ischemia-reperfusion using a meta-analysis method.

2. Materials and Methods

2.1. Search Strategy. A comprehensive search strategy for literature, which was restricted to English-language literature, was conducted in the Embase, Cochrane Library, PubMed, and ISI Web of Science databases up to Dec 31, 2019, using the following search corresponding terms: (mesenchymal stem cells OR MSC OR MSCs OR multipotent stromal cells OR mesenchymal stromal cells OR mesenchymal progenitor cells OR stem cells OR stromal cells) AND (acute kidney injury OR AKI OR acute renal failure OR ARF OR renal ischemia-reperfusion). The manual reference searches in the recruited articles were also conducted to identify additionally eligible reports.

2.2. Inclusion and Exclusion Criteria

2.2.1. Inclusion Criteria. The inclusion criteria are the following: (1) research object: animal experiment used mice or rat, (2) object of the study: AKI, (3) interventions for study: MSCs for treatment, and (4) outcome: efficacy.

2.2.2. Exclusion Criteria. The exclusion criteria are the following: (1) letters, case reports, reviews, clinical studies, editorials, meta-analysis, and systematic reviews; (2) studies lacking the targeted indicators or number of the case group or the control group and conducted in humans; (3) the AKI disease not induced by ischemia-reperfusion; and (4) the therapeutic regimen for AKI including other agents with undefined effects.

2.3. Outcome Measures. The following outcomes regarding the efficacy of MSC treatment on AKI induced by ischemia-reperfusion were identified from the recruited studies: serum creatinine (Scr), blood urea nitrogen (BUN), proteinuria, malondialdehyde (MDA), L-glutathione (GSH), CAT, superoxide dismutase (SOD), NADPH oxidase-1 (NOX1), NADPH oxidase-2 (NOX2), poly(ADP-ribose) polymerase-1 (PARP1), Caspase 3 (mRNA and protein), tumor necrosis factor-α (TNF-α), Bcl-2 associated X protein (Bax), nuclear factor kappa beta (NFκB), interleukin 1β (IL1β; mRNA and protein), interleukin 4 (IL4), interleukin 6 (IL6) mRNA, interleukin 10 (IL10; mRNA and protein), transforming growth factor-β1 (TGF-β), and renal damage score. When disagreements were addressed, a mutual consensus was conducted to resolve it.

2.4. Quality Assessment. The Cochrane Handbook for Interventions was used to evaluate the methodological quality by two investigators independently (Tianbiao Zhou and Chunling Liao). The principal assessment included the following sections for each investigation: selection bias, attrition bias, performance bias, detection bias, reporting bias, and other bias. Each item was classified as unclear, high risk, or low risk.

2.5. Statistical Analysis. Review Manager Version 5.3 was used to explore whether MSC treatment can get a good efficacy on AKI induced by ischemia-reperfusion, and STATA 12.0 was applied to test the publication bias. Heterogeneity of variation among individual studies was quantified and described with I^2. When the P value was ≥ 0.1, the fixed-effects model was used, based on the heterogeneity test. Otherwise, we will use the random-effects model to pool the results for the meta-analysis. Weighted mean differences (WMDs) for the mean values were used to compute the continuous variables, and 95% confidence intervals (95% CI) were calculated for the included studies using the Mantel-Haenszel (M-H) method. Both Begg's rank correlation test and Egger's linear regression method were applied to detect the publication bias among the studies. A P value < 0.05 was considered as statistical significance.

3. Results

3.1. Search Results. The databases mentioned above were searched for this meta-analysis, and we only recruited these studies in mice or rat for evaluation of therapeutic efficiency of MSC treatment on AKI. Nineteen studies [21–39] were eligible and recruited for this meta-analysis, and the flowchart of inclusion of studies is presented in Figure 1. The included study characteristics are shown in Table 1.

3.2. Quality Assessment of Included Studies. In the recruited studies, the methodological quality was considered as acceptable, for the result that most of the domains of the recruited investigations were ranked as unclear risk of bias or low risk of bias. Unclear risk of bias was mostly detected in performance bias and selection bias. Low risk of bias mostly occurred in detection bias, reporting bias, and attrition bias. Figure 2 shows the summary of the risk of biases of the recruited investigations.

3.3. Scr. 19 studies [21–39] were included to assess the effect of MSCs on Scr, 12 for 1 day, four for 2 days, 14 for 3 days, four for 5 days, seven for 7 days, and five for >7 days, and the results showed that the difference between the MSC treatment group and the control group was notable for 1 day, 2 days, 3 days, 5 days, and >7 days (1 day: WMD = −0.56, 95% CI: -0.78, -0.34, $P < 0.00001$; 2 days: WMD = −0.58, 95% CI: -0.89, -0.28, $P = 0.0002$; 3 days: WMD = −0.65, 95% CI: -0.84, -0.45, $P < 0.00001$; 5 days: WMD = −0.35, 95% CI: -0.54, -0.16, $P = 0.0003$; and >7 days: WMD = −0.22, 95% CI: -0.36, -0.08, $P = 0.002$; Figure 3 and Table 2). However, the difference between the MSC treatment group and the control group was not notable for 7 days (WMD = −0.14, 95% CI: -0.28, -0.00, $P = 0.05$; Figure 3 and Table 2).

3.4. BUN. 12 studies [21, 24–28, 30, 31, 33, 36, 38, 39] were included to assess the effect of MSCs on Scr, 7 for 1 day, 3 for 2 days, 10 for 3 days, 2 for 5 days, 2 for 7 days, and 2 for >7 days, and the results indicated that the difference between the MSC treatment group and the control group was notable for 1 day, 2 days, 3 days, and 5 days (1 day: WMD = −11.72, 95% CI: -18.80, -4.64, $P = 0.001$; 2 days:

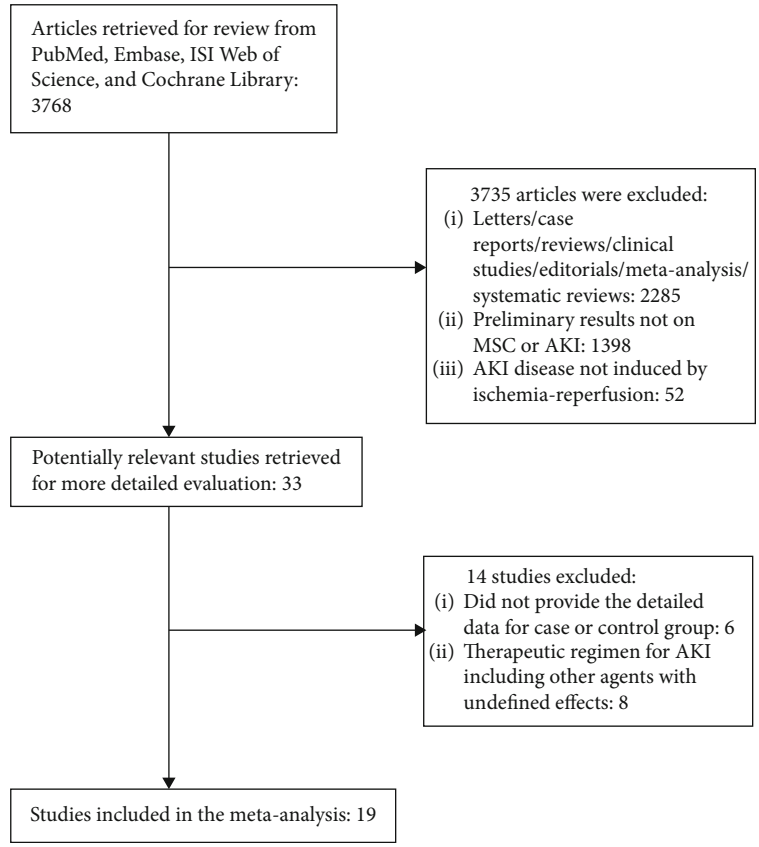

Figure 1: Flow diagram of the selection process.

WMD = −33.60, 95% CI: -40.15, -27.05, $P < 0.00001$; 3 days: WMD = −21.14, 95% CI: -26.15, -16.14, $P < 0.00001$; and 5 days: WMD = −8.88, 95% CI: -11.06, -6.69, $P < 0.00001$; Figure 4 and Table 2). However, the difference between the MSC treatment group and the control group was not notable for 7 days and >7 days (7 days: WMD = −0.72, 95% CI: -13.49, -12.05, $P = 0.91$; >7 days: WMD = −90.84, 95% CI: -257.31, 75.62, $P = 0.28$; Figure 4 and Table 2).

3.5. Proteinuria. Five studies [24, 28, 30, 33, 37] were recruited into the meta-analysis for the assessment of MSCs on proteinuria, three for 3 days and two for >7 days. The results showed that the MSC group had lower proteinuria than the control group for 3 days and for >7 days (3 days: WMD = −0.45, 95% CI: -0.61, -0.30, $P < 0.00001$; >7 days: OR = −108.55, 95% CI: -110.31, -106.78, $P < 0.00001$; Table 2).

3.6. Oxidative Stress and Apoptosis-Related Factors. In this meta-analysis, four studies [21, 24, 32, 39] were included for the assessment of MDA, two [24, 39] for GSH, two [21, 24] for CAT, two [21, 39] for SOD, three [28, 30, 33] for NOX1, four [21, 28, 30, 33] for NOX2, four [21, 28, 30, 33] for PARP1, two [21, 27] for Caspase 3 (mRNA), three [28, 30, 33] for Caspase 3 (protein), and three [28, 30, 33] for Bax. The results indicated that the difference between the MSC treatment group and the control group was notable for MDA, SOD, NOX1, NOX2, PARP1, Caspase 3 mRNA, Caspase 3 protein, and Bax (MDA: WMD = −5.51, 95% CI: -10.57, -0.45, $P = 0.03$; SOD: WMD = 18.95, 95% CI: 16.86, 21.04, $P < 0.00001$; NOX1: WMD = −0.32, 95% CI: -0.54, -0.10, $P = 0.004$; NOX2: WMD = −0.19, 95% CI: -0.28, -0.10, $P < 0.0001$; PARP1: WMD = −0.22, 95% CI: -0.34, -0.09, $P = 0.0006$; Caspase 3 mRNA: WMD = −3.40, 95% CI: -6.13, -0.68, $P = 0.01$; Caspase 3 protein: WMD = −0.15, 95% CI: -0.21, -0.08, $P < 0.00001$; and Bax: WMD = −0.25, 95% CI: -4.42, -0.08, $P = 0.004$; Table 2). However, the difference for GSH and CAT between the MSC treatment and the control group was not significant (GSH: WMD = −31.40, 95% CI: -21.52, 84.31, $P = 0.24$; CAT: WMD = 10.82, 95% CI: -4.30, 25.95, $P = 0.16$; Table 2).

3.7. Assessment of Cytokines. The levels of TNF-α, NFκB, IL1β (mRNA), IL1β (protein), IL4, IL6 (mRNA), IL10 (mRNA), IL10 (protein), and TGF-β were detected, and five studies [25, 28, 30, 33, 37] for TNF-α, three studies [28, 30, 33] for NFκB, two studies [21, 37] for IL1β (mRNA), three studies [25, 30, 33] for IL1β (protein), two studies [28, 33] for IL4, two studies [37, 38] for IL6 (mRNA), two studies [21, 37] for IL10 (mRNA), three studies [25, 28, 33] for IL10 (protein), and two studies [30, 37] for TGF-β were recruited for the evaluation of the treatment effect of MSC treatment on these cytokines. We also found that the difference between the MSC

Table 1: Characteristics of the studies included in this meta-analysis.

Author, year	n	Type of animal	MSC type	Number of MSC	Route of delivery	Endpoints for this meta-analysis
Tögel, 2005	12	Rat	BM-MSCs	0.1×10^6	Artery	Scr
Duffield, 2005	14	Mice	BM-MSCs	0.5×10^6	Intravenous	Scr
Tögel, 2009	36	Rat	BM-MSCs	2×10^6	Artery	Scr
Burst, 2010	28	Rat	BM-MSCs	2×10^6	Intravenous	Scr
La Manna, 2011	12	Rat	FM-MSCs	1×10^6	Intravenous	Scr, renal damage score
Zhuo, 2013	24	Rat	BM-MSCs	1×10^6	Intravenous or artery	Scr, BUN, MDA, GSH, SOD, renal damage score
Sadek, 2013	10	Rat	BM-MSCs	—	Intravenous	Scr, BUN
Zhao, 2014	20	Rat	BM-MSCs	1×10^6	Intravenous	Scr, BUN, IL6 mRNA
Tsuda, 2014	54	Rat	FM-MSCs	0.5×10^6	Intravenous	Scr, BUN, renal damage score
Hattori, 2015	22	Mice	BM-MSCs	1×10^6	Kidney subcapsular injection	Scr, BUN
Lin, 2016	16	Rat	AD-MSCs	1.2×10^6	Intravenous	Scr, BUN, proteinuria, NOX1, NOX2, PARP1, Caspase 3 protein, Bax, TNF-α, NFκB, IL1β protein, TGF-β, renal damage score
Hussein, 2016	36	Rat	AD-MSCs	1×10^6	Intravenous	Scr, BUN, Caspase 3 mRNA
Sheashaa, 2016	42	Rat	AD-MSCs	1×10^6	Intravenous	Scr, MDA
Zhang, 2017	12	Rat	AD-MSCs	2×10^6	Intravenous	Scr, proteinuria, TNF-α, IL1β mRNA, IL6 mRNA, IL10 mRNA, TGF-β
Fahmy, 2017	16	Rat	UC-MSCs	1×10^6	Intravenous	Scr, BUN, proteinuria, MDA, GSH, CAT
Sung, 2017	16	Rat	AD-MSCs	1.2×10^6	Intravenous	Scr, BUN, proteinuria, NOX1, NOX2, PARP1, Caspase 3 protein, Bax, TNF-α, NFκB, IL1β protein, IL4, IL10 protein, renal damage score
Guo, 2018	36	Mice	UC-MSCs	1×10^6	Intravenous	Scr, BUN, TNF-α, IL1β protein, IL10 protein
Ko, 2018	12	Rat	iPSC-MSC	1.2×10^6	Intravenous	Scr, BUN, proteinuria, NOX1, NOX2, PARP1, Caspase 3 protein, Bax, TNF-α, NFκB, IL4, IL10 protein
Alzahrani, 2019	20	Rat	BM-MSCs	1×10^6	Artery	Scr, BUN, MDA, CAT, SOD, NOX2, PARP1, Caspase 3 mRNA, IL1β mRNA, IL10 mRNA, renal damage score

Note: FM-MSCs: fetal membrane-derived mesenchymal stem cells; BM-MSC: bone marrow-derived mesenchymal stem cells; AD-MSCs: adipose tissue-derived MSCs; UC-MSCs: umbilical cord mesenchymal stem cells; iPSC-MSCs: inducible pluripotent stem cell-derived mesenchymal stem cells; Scr: serum creatinine; BUN: blood urea nitrogen; MDA: malondialdehyde; GSH: L-glutathione; SOD: superoxide dismutase; NOX1: NADPH oxidase-1; NOX2: NADPH oxidase-2; PARP1: poly(ADP-ribose) polymerase-1; TNF-α: tumor necrosis factor-α; Bax: Bcl-2 associated X protein; NFκB: nuclear factor kappa beta; IL1β: interleukin 1β; IL4: interleukin 4; IL6: interleukin 6; IL10: interleukin 10; TGF-β: transforming growth factor-β.

treatment group and the control group was significant for NFκB, IL1β mRNA and protein, IL4, and IL10 mRNA and protein (NFκB: WMD = −0.36, 95% CI: -0.66, -0.05, P = 0.02; IL1β mRNA: WMD = −3.26, 95% CI: -4.37, -2.15, $P < 0.00001$; IL1β protein: WMD = −0.37, 95% CI: -0.57, -0.17, $P = 0.0003$; IL4: WMD = 0.13, 95% CI: 0.02, 0.23, $P = 0.02$; IL10 mRNA: WMD = 0.27, 95% CI: 0.24, 0.29, $P < 0.00001$; and IL10 protein: WMD = 0.45, 95% CI: 0.04, 0.86, $P = 0.03$; Table 2). However, the difference for TNF-α, IL6 mRNA, and TGF-β between the MSC treatment and control groups was not significant (TNF-α: WMD = −0.15, 95% CI: -0.31, -0.02, $P = 0.08$; IL6 mRNA: WMD = −2.34, 95% CI: -4.75, 0.07, $P = 0.06$; and TGF-β: WMD = −18.89, 95% CI: -55.79, 18.02, $P = 0.32$; Table 2).

3.8. Assessment of Renal Damage Score. Four studies [29, 35, 36, 39] for 1 day and four studies [21, 30, 33, 36] for 3 days were included in this meta-analysis. The results indicated that the difference of the renal damage score for 1 day and for 3 days between the MSC treatment and control groups was significant (1 day: WMD = −14.50, 95% CI: -19.10, -9.90, $P < 0.00001$; 3 days: WMD = −1.19, 95% CI: -1.72, -0.66, $P < 0.0001$; Table 2).

3.9. Publication Bias. The publication bias was tested in this meta-analysis, and a funnel plot was generated used STATA 12.0 for the primary outcome, and Begg's test and Egger's test suggested that publication bias was found (Egger's: $P = 0.000$, Begg's: $P = 0.000$; Figure 5).

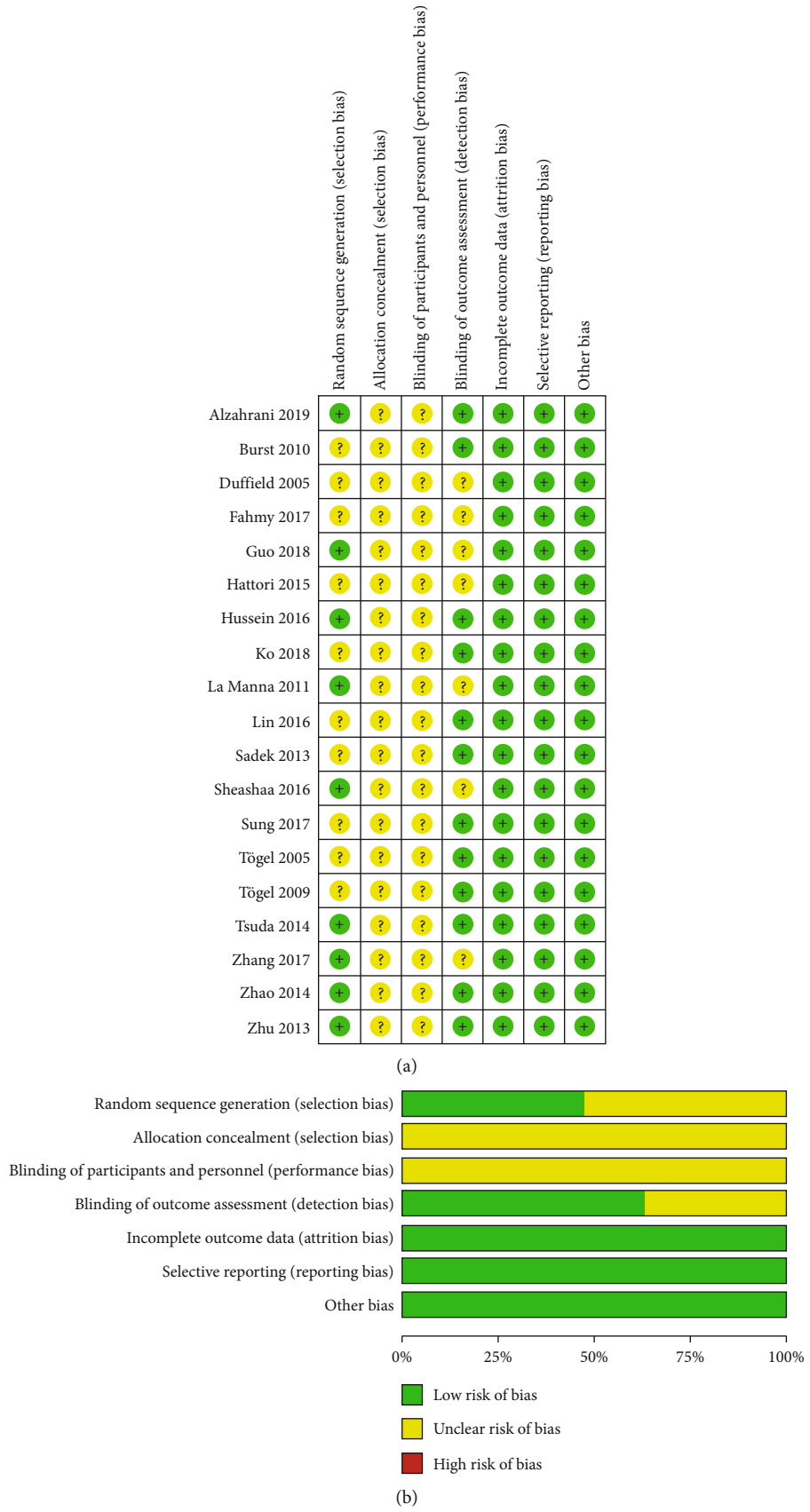

Figure 2: (a) Aggregate risk of bias graph for each experimental animal studies; (b) risk of bias summary.

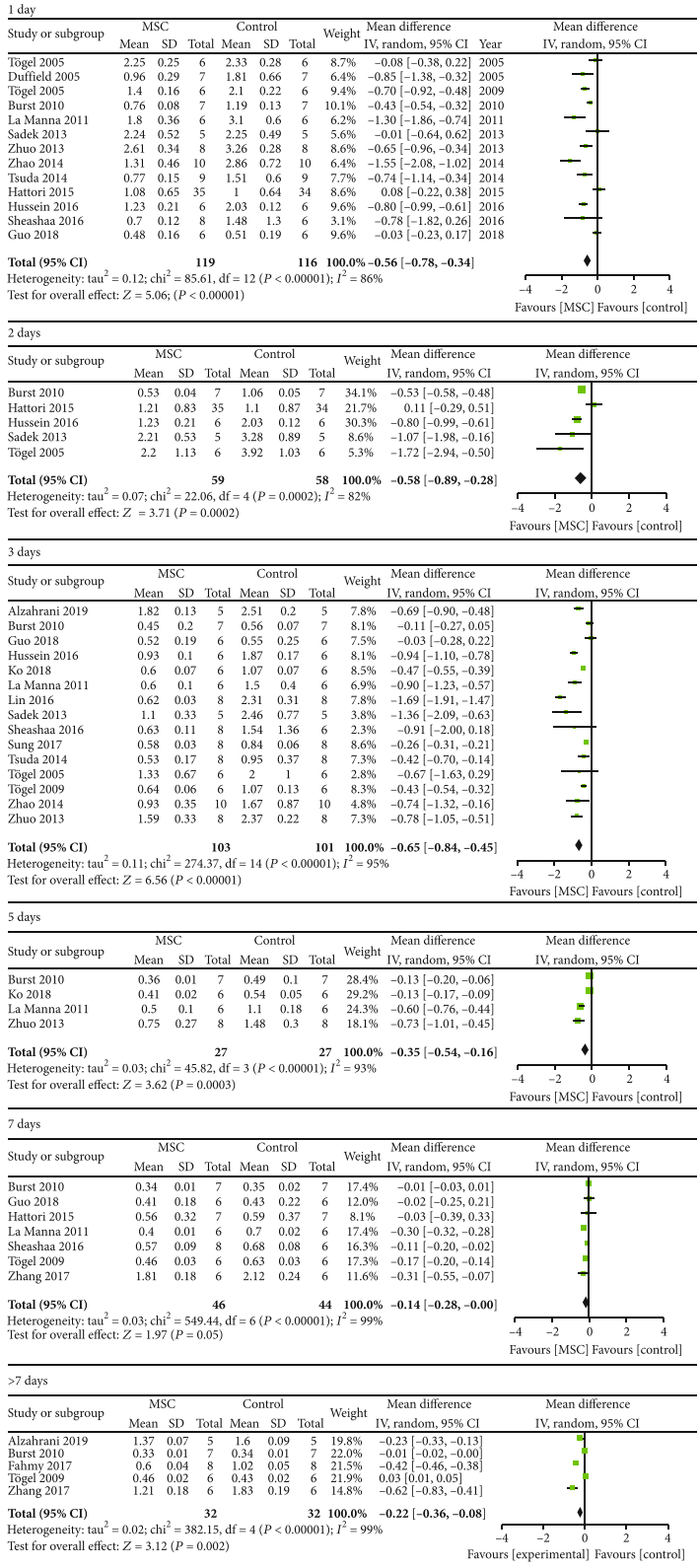

Figure 3: Effect of MSC on Scr.

4. Discussion

In this study, we found that MSC treatment can reduce the Scr levels at 1 day, 2 days, 3 days, 5 days, and >7 days in animal models of AKI. Furthermore, MSC treatment also can reduce the levels of BUN at 1 day, 2 days, 3 days, and 5 days, and it also can reduce the levels of proteinuria at 3 days and >7 days. The renal damage score was also detected, and we

Table 2: Meta-analysis of the efficacy of MSC in therapy of acute kidney injury induced by ischemia-reperfusion.

Indicators	Timepoint	Study number	Q test P value	Model selected	WMD (95% CI)	P
Scr	1 day	13	<0.00001	Random	-0.56 (-0.78, -0.34)	<0.00001
	2 days	5	0.0002	Random	-0.58 (-0.89, -0.28)	0.0002
	3 days	15	<0.00001	Random	-0.65 (-0.84, -0.45)	<0.00001
	5 days	4	<0.00001	Random	-0.35 (-0.54, -0.16)	0.0003
	7 days	7	<0.00001	Random	-0.14 (-0.28, -0.00)	0.05
	>7 days	5	<0.00001	Random	-0.22 (-0.36, -0.08)	0.002
BUN	1 day	7	0.04	Random	-11.72 (-18.80, -4.64)	0.001
	2 days	3	0.38	Fixed	-33.60 (-40.15, -27.05)	<0.00001
	3 days	10	<0.00001	Random	-21.14 (-26.15, -16.14)	<0.00001
	5 days	2	0.20	Fixed	-8.88 (-11.06, -6.69)	<0.00001
	7 days	2	0.79	Fixed	-0.72 (-13.49, -12.05)	0.91
	>7 days	2	<0.00001	Random	-90.84 (-257.31, 75.62)	0.28
Proteinuria	3 days	3	<0.00001	Random	-0.45 (-0.61, -0.30)	<0.00001
	>7 days	2	0.21	Fixed	-108.55 (-110.31, -106.78)	<0.00001
MDA	—	4	0.0001	Random	-5.51 (-10.57, -0.45)	0.03
GSH	—	2	0.0002	Random	-31.40 (-21.52, 84.31)	0.24
CAT	—	2	<0.00001	Random	10.82 (-4.30, 25.95)	0.16
SOD	—	2	0.41	Fixed	18.95 (16.86, 21.04)	<0.00001
NOX1	—	3	<0.00001	Random	-0.32 (-0.54, -0.10)	0.004
NOX2	—	4	<0.00001	Random	-0.19 (-0.28, -0.10)	<0.0001
PARP1	—	4	<0.00001	Random	-0.22 (-0.34, -0.09)	0.0006
Caspase 3 (mRNA)	—	2	<0.00001	Random	-3.40 (-6.13, -0.68)	0.01
Caspase 3 (protein)	—	3	<0.00001	Random	-0.15 (-0.21, -0.08)	<0.00001
Bax	—	3	<0.00001	Random	-0.25 (-4.42, -0.08)	0.004
TNF-α	—	5	<0.00001	Random	-0.15 (-0.31, -0.02)	0.08
NFκB	—	3	<0.00001	Random	-0.36 (-0.66, -0.05)	0.02
IL1β (mRNA)	—	2	0.007	Random	-3.26 (-4.37, -2.15)	<0.00001
IL1β (protein)	—	3	<0.00001	Random	-0.37 (-0.57, -0.17)	0.0003
IL4	—	2	<0.00001	Random	0.13 (0.02, 0.23)	0.02
IL6 (mRNA)	—	2	<0.00001	Random	-2.34 (-4.75, 0.07)	0.06
IL10 (mRNA)	—	2	0.13	Fixed	0.27 (0.24, 0.29)	<0.00001
IL10 (protein)	—	3	<0.00001	Random	0.45 (0.04, 0.86)	0.03
TGF-β	—	2	<0.00001	Random	-18.89 (-55.79, 18.02)	0.32
Renal damage score	1 day	4	<0.00001	Random	-14.50 (-19.10, -9.90)	<0.00001
	3 days	4	<0.00001	Random	-1.19 (-1.72, -0.66)	<0.0001

Note: Scr: serum creatinine; BUN: blood urea nitrogen; MDA: malondialdehyde; GSH: L-glutathione; SOD: superoxide dismutase; NOX1: NADPH oxidase-1; NOX2: NADPH oxidase-2; PARP1: poly(ADP-ribose) polymerase-1; TNF-α: tumor necrosis factor-α; Bax: Bcl-2 associated X protein; NFκB: nuclear factor kappa beta; IL1β: interleukin 1β; IL4: interleukin 4; IL6: interleukin 6; IL10: interleukin 10; TGF-β: transforming growth factor-β.

found that MSC treatment can significantly reduce the renal damage score in animal models of AKI. The results indicated that MSCs can get a protective role against AKI.

The dysfunction of oxidative stress is associated with AKI induced by ischemia-reperfusion, and cell injury or cell apoptosis takes part in the pathogenesis of AKI. As those mentioned above, the result indicated that the MSCs can improve the injury of AKI in animal models. We further collected the data about oxidative stress and apoptosis-related factors. In this study, the results indicated that MSC treatment can reduce MDA, NOX1, NOX2, PARP1, Caspase 3, and Bax and increase SOD. Previously, there were some studies indicating that MSC treatment can suppress oxidative stress and take the protective role. Song et al. [40] conducted a study in adriamycin-induced nephropathy rats and reported that MSCs can attenuate the nephropathy by diminishing oxidative stress and inhibiting the inflammation via downregulation of NFκB. de Godoy et al. [41] evaluate the neuroprotective potential of MSCs against the deleterious impact of amyloid-β peptide on hippocampal neurons and reported that MSCs protect hippocampal neurons against oxidative stress and synapse damage. Chang et al. [42]

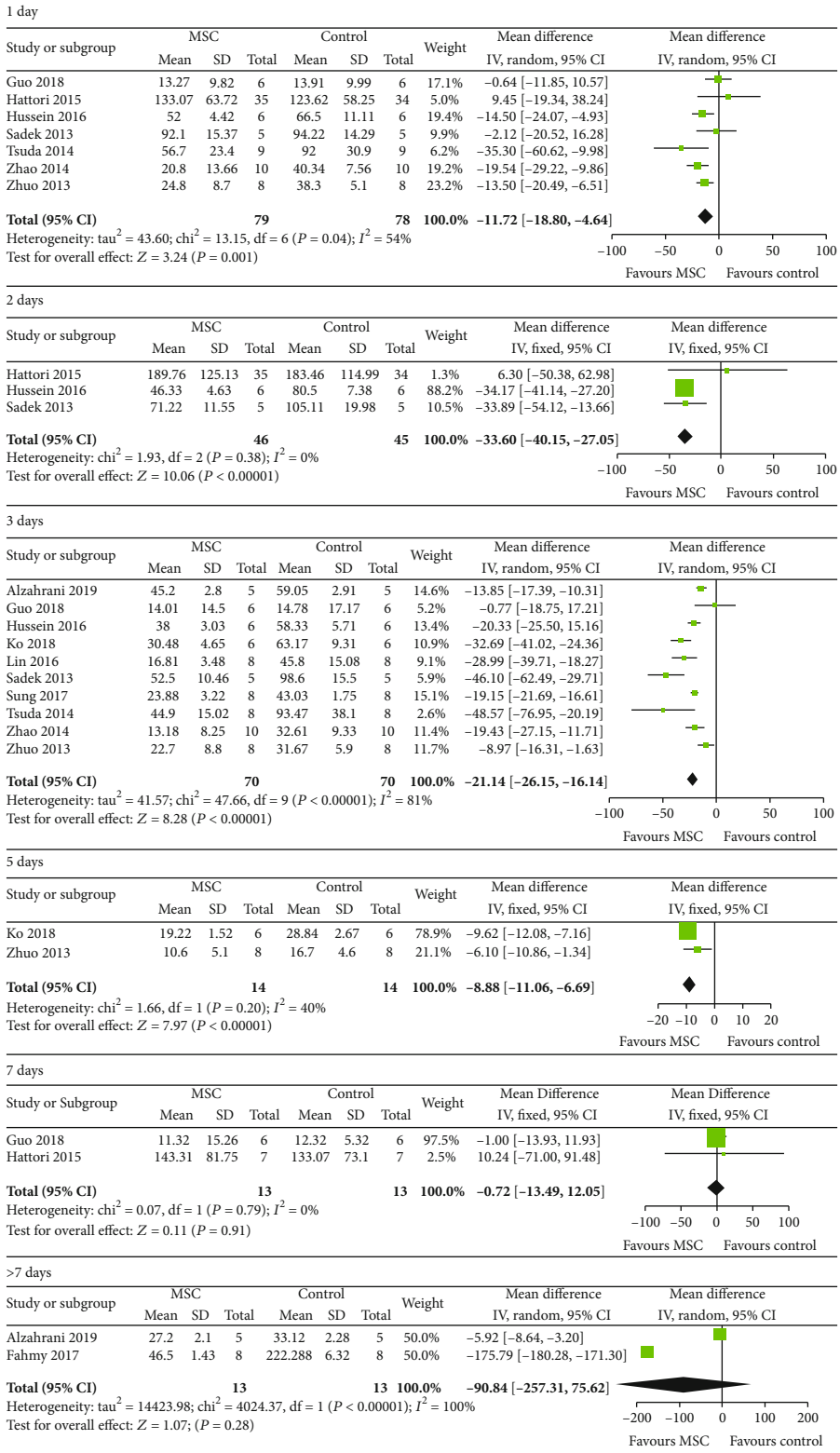

Figure 4: Effect of MSC on BUN.

reported that MSC transplantation successfully alleviates glomerulonephritis through antioxidation and antiapoptosis in nephritic rats.

Activation of some cytokines takes part in the pathogenesis of AKI induced by ischemia-reperfusion. In our study, we found that MSC treatment can inhibit NFκB and IL1β

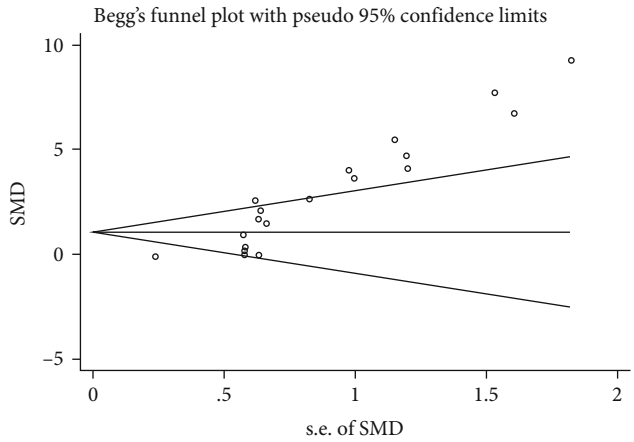

Figure 5: Publication bias.

and increased IL4 and IL10. Song et al. [40] indicated that MSCs can attenuate the nephropathy by inhibiting oxidative stress and alleviating the inflammation via inhibiting NFκB. There were also some studies reporting the association of MSCs with ILs.

However, there were some limitations in our meta-analysis. First, the sample size for the recruited investigation was small, and the longer-term endpoints were missed. Furthermore, the animal type was different (mouse and rat), and the normal values of the parameters, such as BUN and Scr, for rats or mice were different. The type of MSCs and the dose of MSCs administered were not exactly the same. These factors mentioned above may cause our results to be less robust.

5. Conclusions

MSC treatment can reduce the Scr levels at 1 day, 2 days, 3 days, 5 days, and >7 days and can reduce the levels of BUN at 1 day, 2 days, 3 days, and 5 days, and it also can reduce the levels of proteinuria at 3 days and >7 days and alleviate the renal damage in animal models of AKI. The results indicated that MSCs can get a protective role against AKI. However, more well-designed studies with larger sample sizes and longer-term endpoints should be conducted to identify additional and robust outcomes in the future.

Data Availability

The data supporting this meta-analysis are from previously reported studies and datasets, which have been cited. The processed data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

TBZ contributed to the conception and design of the study. TBZ and CLL were responsible for collection of data and performing the statistical analysis and manuscript preparation. SJL, WSL, HZZ, and SYH were responsible for checking the data. All authors were responsible for drafting the manuscript, read, and approved the final version.

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Research Article

Adipose-Derived Mesenchymal Stem Cells Modulate Fibrosis and Inflammation in the Peritoneal Fibrosis Model Developed in Uremic Rats

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Peritoneal fibrosis (PF) represents a long-term complication of peritoneal dialysis (PD), affecting the peritoneal membrane (PM) function. Adipose tissue-derived mesenchymal stem cells (ASC) display immunomodulatory effects and may represent a strategy to block PF. The aim of this study was to analyze the effect of ASC in an experimental PF model developed in uremic rats. To mimic the clinical situation of patients on long-term PD, a combo model, characterized by the combination of PF and chronic kidney disease (CKD), was developed in Wistar rats. Rats were fed with a 0.75% adenine-containing diet, for 30 days, to induce CKD with uremia. PF was induced with intraperitoneal injections of chlorhexidine gluconate (CG) from day 15 to 30. 1×10^6 ASC were intravenously injected at days 15 and 21. Rats were divided into 5 groups: control, normal rats; CKD, rats receiving adenine diet; PF, rats receiving CG; CKD+PF, CKD rats with PF; CKD+PF+ASC, uremic rats with PF treated with ASC. PF was assessed by Masson trichrome staining. Inflammation- and fibrosis-associated factors were assessed by immunohistochemistry, multiplex analysis, and qPCR. When compared with the control and CKD groups, GC administration induced a striking increase in PM thickness and inflammation in the PF and CKD+PF groups. The development of PF was blocked by ASC treatment. Further, the upregulation of profibrotic factors (TGF-β, fibronectin, and collagen) and the increased myofibroblast expression observed in the CKD+PF group were significantly ameliorated by ASC. Beyond the antifibrotic effect, ASC showed an anti-inflammatory effect avoiding leucocyte infiltration and the overexpression of inflammatory cytokines (IL-1β, TNF-α, and IL-6) in the PM induced by GC. ASC were effective in preventing the development of PF in the experimental model of CKD+PF, probably due to their immunomodulatory properties. These results suggest that ASC may represent a potential strategy for treating long-term PD-associated fibrosis.

1. Introduction

Peritoneal dialysis (PD) is a safe life-sustaining renal replacement modality, employed for the treatment of end-stage renal disease (ESRD) worldwide. According to the last registry, 11% of the global dialysis population is under PD [1–3]. In many countries, patient outcomes with PD are comparable to or better than those with hemodialysis, and PD is also more cost effective.

In spite of providing the best preservation of residual renal function and higher quality of life for patients, compared to hemodialysis, PD promotes continuous exposure of the peritoneal membrane (PM) to bioincompatible, hypertonic dialysis solutions, which can cause chronic PM inflammation. Moreover, PD patients are under the risk of infectious peritonitis. The long-term exposure to PD fluids associated with recurrent episodes of infectious peritonitis induces inflammation, neoangiogenesis, and peritoneal fibrosis (PF), which impairs its function, leading to technical failure of this modality [4, 5].

The pathophysiology of PF involves the loss of mesothelial cells and the thickening of the submesothelial area, mainly composed of an extracellular matrix (ECM) and myofibroblasts. Recent studies have proposed that mesothelial

cells represent an important source of myofibroblasts, through the epithelial–mesenchymal transition. However, a recent study showed the important role of submesothelial resident fibroblasts as myofibroblast precursors in PF [6]. Besides myofibroblasts, submesothelial infiltration by leukocytes, such as macrophages and T-cells, is also usually observed. The activation of these inflammatory cells is driven by irritative stimuli, such as the high concentrations of glucose and glucose-degradation products found in the dialysis fluid, which start to synthesize and release a number of proinflammatory factors, namely, the IL-1-β, TNF-α, IL-6, and specially, the transforming growth factor-β (TGF-β). Under TGF-β signaling, myofibroblasts, characterized by α-SMA expression, produce ECM proteins, such as collagen and fibronectin, leading to the development of PF [7, 8].

Current strategies to minimize PF in patients submitted to PD include the use of bioincompatible dialysis solutions. Additionally, clinical administration of antifibrotic drugs, such as tamoxifen, has been described in some patients as an attempt to abrogate peritoneal inflammation and fibrosis. However, these approaches are only partially effective [7, 9, 10]. Furthermore, experimental blockade of inflammation and TGF-β by the administration of valproic acid, tamoxifen, and bone morphogenetic protein-7 (BMP7) have shown positive effects in preventing PF progression in animal models. Nevertheless, further studies are required to confirm the efficiency and safety of these compounds [5, 10].

In this context, the research for alternative approaches to prevent PF, such as cell-based therapy, is of paramount importance. Previous experimental studies demonstrated that administration of mesenchymal stem cells (MSC) promotes renoprotection by preventing the development of renal inflammation and fibrosis in models of both acute and chronic kidney disease (CKD), due to its immunomodulatory effects [11, 12]. Since there are expressive similarities between the mechanisms of renal and peritoneal fibrogenesis, the aim of the present study was to analyze the potential anti-inflammatory and antifibrotic effects of adipose-derived MSC (ASC) administration in rats submitted to a combined model of uremic CKD+PF, which better reproduces the pathophysiological scenario of long-term PD.

2. Materials and Methods

2.1. Animal Model. Thirty-eight adult male Wistar rats weighing 300-350 g were obtained from the local animal facility of the University of São Paulo (USP). Animals were kept at a constant temperature of $23 \pm 2^{\circ}$C, under a 12 h light/dark cycle and had free access to tap water. All animal procedures were approved by the Research Ethics Committee of USP Faculty of Medicine (FMUSP-CAPPesq 029/2016) and were conducted in accordance with our institutional guidelines and with international regulations for manipulation and care of experimental animals. In order to mimic the clinical situation of patients on long-term PD, a combo model, characterized by the combination of PF and uremia, was employed in the present study [10]. Uremia was induced by an adenine-rich diet. Twenty-four animals were fed a 0.75% adenine-containing rat diet (Sigma Co., St. Louis, USA) for 30 consecutive days, while the 14 remaining animals were fed with standard rat chow (Nuvital Labs, Curitiba, Brazil). PF was induced in 24 animals by IP injections of chlorhexidine gluconate (CG). Body weight was assessed once a week, and tail-cuff systolic blood pressure was measured in conscious animals with an automated optoelectronic device (Visitech Systems, USA), at the end of the study period.

2.2. Experimental Protocol. After 15 days of adenine-rich diet administration, when uremia was already established, PF was induced by daily IP injections of CG. Two intravenous (IV) doses of 1×10^6 ASC each were administered to the treated group at two different moments. The first dose of ASC was given concomitantly with the first IP CG injection (15 days after the adenine-rich diet administration began). The second dose was given 6 days later, 21 days after the adenine-rich diet administration began. All animals were studied for a total of 30 days. Our experimental protocol consisted of the following groups:

(i) CKD: animals receiving adenine-rich diet for 30 days to induce severe CKD ($N = 8$)

(ii) PF: animals fed with standard rat diet, submitted to the CG-induced PF model ($N = 8$)

(iii) CKD+PF: CKD animals submitted to the CG-induced PF model 15 days after the adenine-rich diet administration began ($N = 8$)

(iv) CKD+PF+ASC: CKD+PF animals which received 2 IV infusions of 1×10^6 ASC each, diluted in sterile PBS. The first infusion was performed concomitantly with the first CG IP injection, 15 days after the adenine-rich diet administration began, and the second one was performed 21 days after the adenine-rich diet administration began ($N = 8$)

(v) Control: animals fed with standard rat diet and kept untreated for 30 days ($N = 6$).

2.3. Isolation, Expansion, and Characterization of Rat ASC. Gonadal adipose tissue from 5 healthy adult male Wistar rats was obtained after its euthanasia with an IP injection of 0.1 g of sodium thiopental. The adipose tissue samples were minced with sterile scissors and digested in a 0.075% collagenase solution (Sigma-Aldrich, USA). After centrifugation, the isolated cells were cultured under 37°C and 5% CO_2 in plastic culture flasks with Dulbecco's Modified Eagle Medium (DMEM-low glucose, Invitrogen, USA) containing 10% inactivated fetal bovine serum (FBS; Invitrogen), 100 units/mL penicillin, and 100 mg/mL streptomycin antibiotic solution (Gibco, Carlsbad, MO, USA). Culture medium was changed three times a week, and cells were trypsinized and reseeded when they reached 80% of confluence. At the 4^{th} passage, cells were characterized as MSC according to the criteria defined by the International Society of Cellular Therapy Consensus: adherence to plastic under standard conditions, positivity to specific surface markers, such as CD29, CD44, CD90, and CD105; negativity to CD45; and

ability to differentiate into mesenchymal lineages when submitted to appropriate culture medium and stimuli.

Cells were used at passages 4-6 according to flow cytometry analyses (FACSCanto™, BD Biosciences, USA). For this purpose, ASC were labeled with isothiocyanate- (FITC-) conjugated antibodies against CD31, CD29, and CD90; phycoerythrin- (PE-) conjugated antibodies against CD34, CD44, and CD105; Pe-cy5.5-conjugated antibody against CD45; and FITC- or PE-conjugated nonspecific IgG (eBioscience, San Diego, USA). The results of these analysis were presented as Supplementary Figure 1, in the supplementary data section. In parallel, the potential of ASC to differentiate into mesenchymal lineages including osteoblasts, chondroblasts, and adipocytes under in vitro conditions was evaluated. Osteogenic differentiation was induced by supplementing the culture medium with 10-8 M/L dexamethasone (Sigma), 5 μg/mL ascorbic acid 2-phosphate (Sigma), and 10 mM/L β-glycerolphosphate (Sigma). To confirm the presence of calcium deposition, cultures were stained with Alizarin Red S (Nuclear, São Paulo, SP, Brazil). To induce chondrogenic differentiation, ASC were cultured in DMEM supplemented with 10 ng/mL transforming growth factor- (TGF-) β1 (Sigma), 50 nM ascorbic acid 2-phosphate (Sigma), and 6.25 mg/mL insulin. In order to confirm the differentiation, cells were stained with Alcian Blue pH 2.5. Adipogenesis differentiation was induced by culturing ASC in DMEM supplemented with 5 μg/mL insulin, 10-6 M dexamethasone, 0.5 μM isobutylmethylxanthine, and 50 μM indomethacin. Cells were then stained with oil red O to confirm the presence of lipid droplets into the cell vacuoles (Supplementary Figure 2). For further characterization of ASC, immunofluorescence assays of cells cultured at the 4th passage were performed. For this purpose, specific primary antibodies against CD19, CD44, CD90, CD146 (Millipore, EUA), CD73, and CD45 (Santa Cruz Biotechnology, USA), as well as fluorescent dye-conjugated secondary antibodies Alexa 488, 594, and 546 (Life Technologies), were employed. Cell nuclei were counterstained with DAPI (4′6-diamidino-2-phenylindole, Invitrogen). Figure 1 shows these results.

2.4. Peritoneal Histomorphometry and Immunohistochemical Analysis. At the end of the study, after 30 days of follow-up, the animals were euthanized by IP injection of a lethal dose of sodium thiopental. Abdominal cavity was opened, and blood and tissue samples of PM were collected. Blood samples were centrifuged, and serum urea concentrations were determined using a commercially available colorimetric kit (Labtest, Brazil). Samples of the PM from the anterior abdominal wall away from the injection points were carefully dissected, immediately frozen in liquid nitrogen, and stored at -70°C for polymerase chain reaction (PCR) and multiplex analyses. Additional sections were fixed in Duboscq-Brazil solution for 45 minutes and then postfixed in buffered 4% formaldehyde solution.

PF was evaluated in sections (3 μm) stained with Masson's trichrome. At least 10 digital images at 200x magnification were taken of each rat, and the thickness (μm) of all photomicrographs was measured. Then, the mean peritoneal thickness from each rat was calculated [5]. For this procedure, we used digitized images and image analysis software (Image-Pro Plus Software 7.0, Media Cybernetics Inc., Bethesda, USA).

For immunohistochemical studies, PM sections were incubated with the following specific antibodies: anti-CD68 clone ED1 (Serotec, Oxford, UK), to detect macrophages; anti-CD3 (Abcam, Cambridge, MA, USA), to detect T-cells; and anti-α-SMA (Sigma, USA), to detect myofibroblasts. Reactions were developed using an LSAB-AP System (Dako, USA) and revealed with fast red dye (Sigma, USA). Quantitative analysis of ED1 and anti-CD3-positive cells present in the peritoneum was carried out in a blinded fashion under ×200 microscopic magnification and expressed as cells/mm^2. The α-SMA staining area (%) was calculated relative to the whole peritoneal area using Image-Pro Plus 7.0 software (Media Cybernetics, Inc., Bethesda, USA).

2.5. Gene Expression of Fibrosis Biomarkers in PM Samples. Quantitative real-time PCR (RT-qPCR) analyses were employed to assess gene expression of some of the main fibrosis-related factors, such as collagen III, TGF-β, and fibronectin. For this purpose, total RNA was obtained from PM frozen samples and converted on cDNA using a commercially available kit (Promega, USA) strictly following the instructions of the manufacturer. Reactions of qPCR were conducted using the StepOnePlus Real-Time PCR System (Thermo Fisher, USA), with the following cycle program: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C for denaturation, 20 s at 60°C for combined annealing, and 10 s at 72°C for extension.

2.6. Gene and Protein Expression of Proinflammatory Cytokines. Gene expression of cytokines such as IL-1β, TNF-α, and IL-6 were assessed by RT-qPCR, following the methods described previously. Additionally, the protein concentration of these inflammatory mediators was evaluated in the PM samples through multiplex cytokine analysis, using a commercially available kit (MILLIPLEX-EMD Millipore, Billerica, EUA), following the instructions of the manufacturer. Assays were read on the Bio-Plex Suspension Matrix System, and data were analyzed using Bio-Plex Manager version 4.0 software (Life Science, Hercules, USA). Results were expressed as pg/mg protein.

2.7. Statistical Analysis. Data are presented as mean ± SEM, and all statistical analyses were performed using the GraphPad Prism software, version 5.0 (GraphPad, San Diego, USA). One-way analysis of variance with pairwise comparisons according to Newman-Keuls formulation was used to compare the different experimental groups, while unpaired t test was used to compare different time points of each experimental group. p values equal to or lower than 0.05 were considered significant.

3. Results

3.1. ASC Infusion Ameliorates Body Weight Loss and Reversed Hypertension in CKD+PF Rats. All animals employed in the study showed similar body weight, systolic blood pressure, and serum urea concentration at the beginning of the

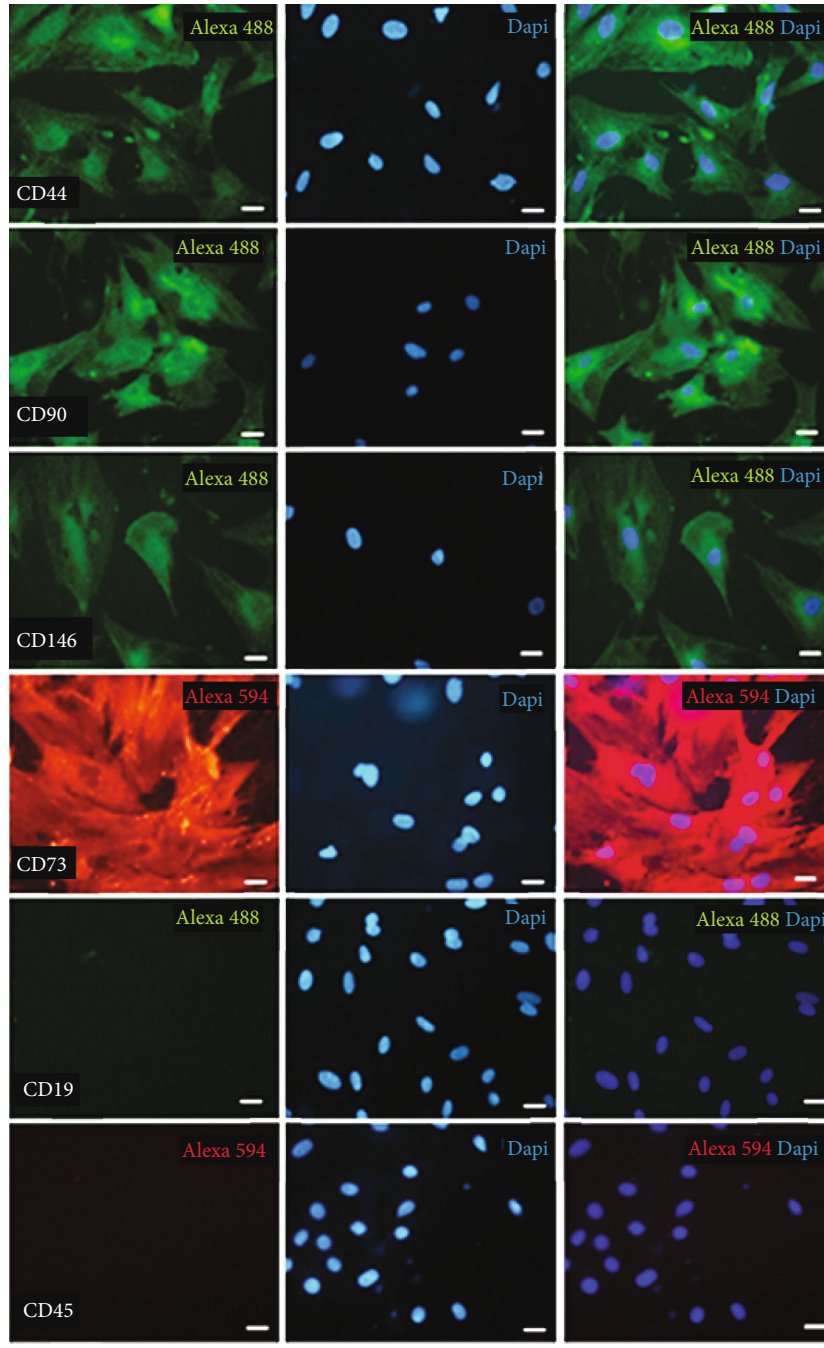

Figure 1: Cellular characterization of mesenchymal stem cell surface markers by immunofluorescence. ASC were positive for CD44, CD90, CD146, and CD73 and negative for CD19 and CD45.

protocol, before CKD or/and PF induction. As expected, the control and PF groups exhibited positive body weight gain throughout the study. Furthermore, these animals did not develop hypertension or urea retention until 30 days of follow-up. On the other hand, animals submitted to the CKD model (combined to PF or not) showed significant weight loss during the analysis period, accompanied by hypertension and uremia. As shown in Table 1 and Supplementary Figure 3, ASC treatment prevented the progression of weight loss between days 15 and 30 and reversed hypertension in the animals of the CKD+PF+ASC group.

3.2. ASC Treatment Prevented the Development of PF in Rats Submitted to CKD+PF. The establishment of PF in the animals of the different groups was assessed by histological analyses of Masson's trichrome-stained PM samples. Illustrative microphotographs of each experimental group showed severe PM thickening and collagen accumulation in both the PF and CKD+PF groups (Figure 2(a)). The quantification of these histological findings showed that the PF and CKD+PF animals exhibited threefold greater PM than those from the control or CKD rats ($p < 0.05$), as can be seen in Figure 2(b). ASC treatment significantly prevented the development of PF in

Table 1: Comparative analysis of body weight (BW), systolic blood pressure (BP), and urea nitrogen (BUN) levels in the different groups at days 01, 15, and 30.

	Control	CKD	PF	CKD+PF	CKD+PF+ASC
BW (g)					
Day 01	332 ± 3	321 ± 6	319 ± 8	310 ± 4	329 ± 5
Day 15	$360 \pm 4^{*}$	$296 \pm 6^{*\dagger}$	$356 \pm 8^{*\phi}$	$267 \pm 4^{*\dagger\phi¥}$	$279 \pm 10^{*\dagger¥}$
Day 30	$428 \pm 5^{*}$	$239 \pm 10^{*\#\dagger}$	$360 \pm 8^{*\dagger\phi}$	$243 \pm 11^{*\dagger¥}$	$277 \pm 8^{*\dagger\phi¥\$}$
BP (mmHg)					
Day 01	122 ± 4	129 ± 3	123 ± 2	127 ± 3	125 ± 3
Day 15	128 ± 5	$164 \pm 5^{*\dagger}$	$127 \pm 4^{\phi}$	$178 \pm 6^{*\dagger¥}$	$176 \pm 4^{*\dagger¥}$
Day 30	124 ± 3	$175 \pm 2^{*\dagger}$	$134 \pm 6^{\phi}$	$169 \pm 4^{*\dagger¥}$	$130 \pm 4^{*\#\phi\$}$
BUN (mg/dL)					
Day 01	51 ± 11	24 ± 13	51 ± 10	34 ± 12	60 ± 12
Day 15	55 ± 13	$178 \pm 16^{*\dagger}$	$40 \pm 10^{*\phi}$	$185 \pm 25^{*\dagger¥}$	$169 \pm 15^{*\dagger¥}$
Day 30	46 ± 14	$307 \pm 36^{*\#\dagger}$	$38 \pm 12^{*\phi}$	$287 \pm 37^{*\#\dagger¥}$	$333 \pm 24^{*\#\dagger¥}$

Unpaired t test: $^{*}p < 0.05$ vs. respective day 01, $^{\#}p < 0.05$ vs. respective Day 15. ANOVA-Newman-Keuls posttest: $^{\dagger}p < 0.05$ vs. respective control, $^{\phi}p < 0.05$ vs. respective CKD, $^{¥}p < 0.05$ vs. respective PF, $^{\$}p < 0.05$ vs. respective CKD+PF.

CKD+PF+ASC animals, which showed PM thickness similar to those observed in the control or CKD groups.

3.3. ASC Infusion Prevented PF by Reducing the Number of Peritoneal Myofibroblasts and Modulating the Expression of Genes Related to ECM Synthesis in Rats Submitted to CKD+PF. Immunohistochemistry for α-SMA, a biomarker of myofibroblasts, which are ECM producer cells, strongly related to fibrogenesis, was performed in PM samples of animals of each experimental group. As shown in Figure 3(a) and Supplementary Figure 4, animals submitted to the CKD model based on adenine overload exhibited a numerical increase in the percentage of α-SMA in the PM, compared to the control. Corroborating our previous histological findings, the groups subjected to the PF experimental model (both PF and CKD+PF) exhibited substantial peritoneal α-SMA accumulation. It is noteworthy that ASC treatment significantly prevented the increase of α-SMA percentage in the CKD+PF +ASC animals.

Similar results were obtained from RT-qPCR analyses of PM samples to assess gene expression of some of the main profibrotic genes. PM samples of untreated animals submitted to the experimental model of combined CKD+PF showed a significant overexpression of TGF-β, collagen III, and fibronectin, compared with the control group. As shown in Figures 3(b)–3(d). ASC treatment significantly reduced the overexpression of TGF-β and collagen III and normalized the expression of fibronectin.

3.4. Administration of ASC Attenuated Peritoneal Inflammation. In order to evaluate local leukocyte recruitment, peritoneal infiltration by macrophages (ED1+ cells) and T-cells (CD3+ cells) was evaluated by immunohistochemistry. Illustrative microphotographs are shown in Figures 4(a) and 4(b), and the quantification of these parameters is represented as bar graphs in Figures 4(c) and 4(d).

As shown in Figure 4(c) the number of macrophages detected in PM samples did not differ among animals from the control, CKD, and PF groups. Nevertheless, the combined CKD+PF model promoted a marked increase in PM infiltration by macrophages which was statistically significant when compared to the control, CKD, and PF groups ($p < 0.05$). ASC infusions completely prevented macrophage infiltration in the PM of animals from the CKD+PF+ASC group.

Peritoneal T-cells, likewise the macrophages, did not differ among the control, CKD, and PF groups, and meanwhile, were strikingly increased in the CKD+PF group. In accordance to data obtained with macrophage quantification, ASC treatment significantly reduced T-cell infiltration in the PM samples of animals from the CKD+PF+ASC group (Figure 4(d)).

Additionally, we analyzed both gene and protein expressions of the following inflammatory mediators: IL-1β, TNF-α, and IL6, in the peritoneal membrane of the animals in each experimental group, as shown in Figure 5.

The CKD+PF model promoted a significant increase in both gene and protein expressions of IL-1β, in which the last also had an increase in the CKD and PF groups. Animals treated with ASC presented normal gene and protein expressions of IL-1β, compared with those observed in the control group (Figures 5(a) and 5(b)). TNF-α gene was overexpressed in the PM samples of animals submitted to the combined CDK+PF model, while protein levels of TNF-α (evaluated in PM samples by multiplex analysis) were markedly increased in the CKD, PF, and CKD+PF groups. Both gene and protein expressions of TNF-α were significantly reduced in the CKD+PF animals treated with ASC (Figures 5(c) and 5(d)). Accordingly, peritoneal gene and protein expressions of IL6 were notably elevated in the animals of the CKD+PF group and were completely normalized by ASC infusions (Figures 5(e) and 5(f)).

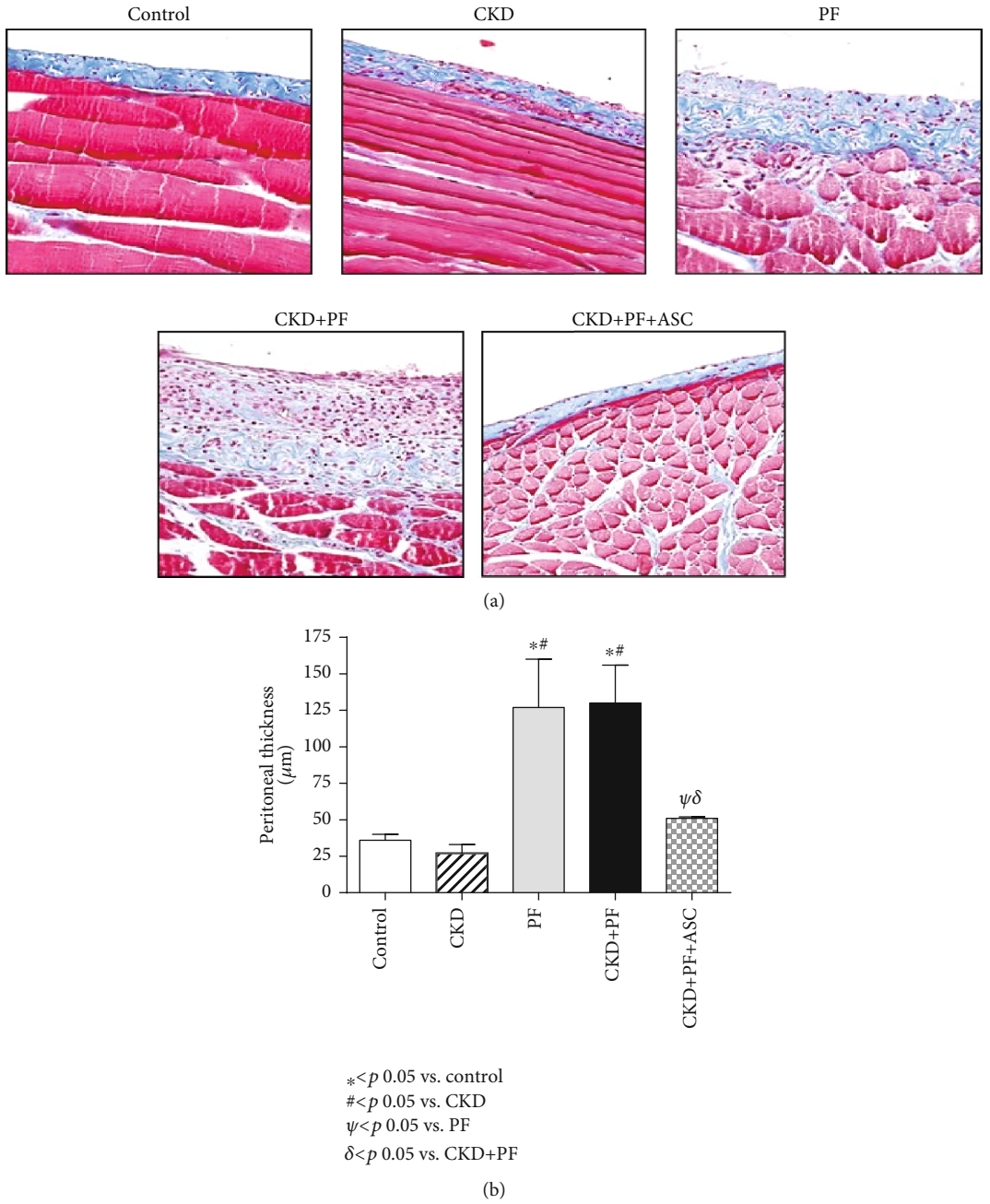

Figure 2: Histological features of peritoneal samples from the different groups stained with Masson's trichrome (×200) (a). The quantification of these findings was demonstrated in bar graphs (b). There were no morphological alterations in the mesothelial, submesothelial, or muscle cells in the control and CKD groups. IP CG injections induced marked submesothelial peritoneal membrane thickening, characterized by increased cellularity and collagen deposition, as can be seen in the PF and CKD+PF groups. Animals submitted to CKD+PF which received ASC infusions exhibited preserved peritoneal membrane.

4. Discussion

The long-term exposure of the peritoneal membrane to bioincompatible PD solutions gradually promotes local inflammation, loss of mesothelial cells, proliferation of myofibroblasts, collagen deposition, and submesothelial thickening, leading to PF, loss of ultrafiltration capacity and, eventually, to the failure of this dialysis modality [5, 7, 13]. In this study, the intravenous administration of ASC prevented the progression of PF induced by GC in uremic rats. ASC treatment also reduced myofibroblast infiltration and attenuated the upregulated expression of profibrotic and proinflammatory genes observed in untreated animals. These findings are consistent with previous reports that studied the antifibrotic effect of stem cells in experimental PF models [14, 15]. Different from those former studies, in our experiments, we used uremic animals, and the ASC were administered by intravenous route.

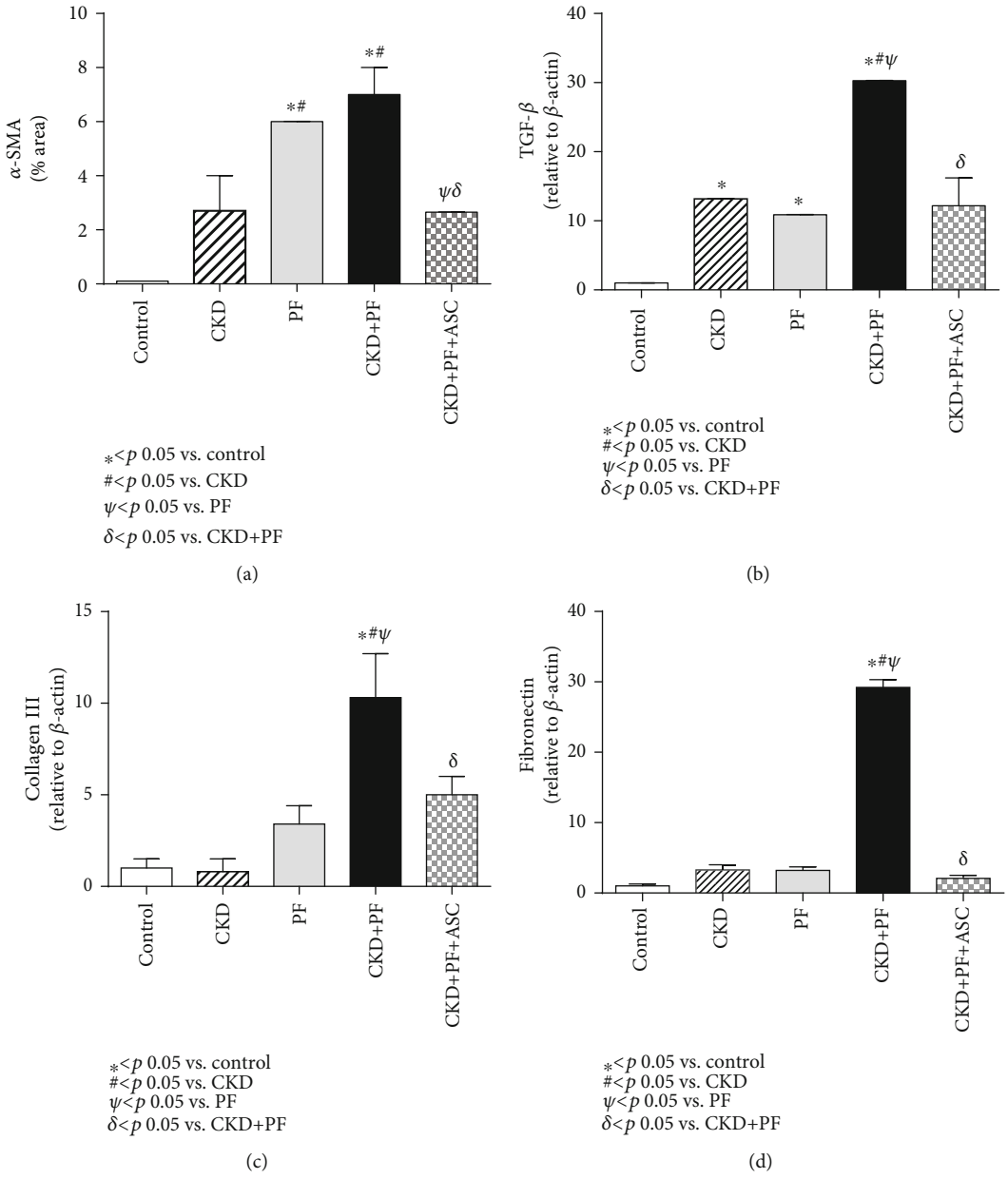

Figure 3: Comparative analysis of α-SMA expression (employed to detect myofibroblasts by immunohistochemistry) and TGF-β, collagen III, and fibronectin gene expressions (achieved by quantitative real-time PCR) in the peritoneal membrane of all groups. CG-induced PF was associated with a significant increase in α-SMA expression in both the PF and PF+CKD groups (a) that also exhibited significant overexpression of TGF-β (b), collagen III (c), and fibronectin (d) genes. ASC treatment markedly reduced the peritoneal percentage of α-SMA, as well as the expression of genes related to fibrosis.

As previously described, the combo experimental model of uremic CKD associated with PF employed in the present study resembles more closely the clinical and pathophysiological features observed in end-stage renal disease patients submitted to long-term PD. Besides the direct effects of CKD, such as body weight loss, systemic hypertension, and increased BUN, the animals of the CKD+PF group exhibited marked PM thickening, characterized by the submesothelial accumulation of collagen and α-SMA, along with peritoneal inflammation, evidenced by submesothelial macrophage and T-cell infiltration, which was statistically higher than that observed in the animals submitted only to the PF model, with no associated CKD. Additionally, local peritoneal overexpression of genes related to inflammation and fibrosis was substantially increased in the CKD+PF group compared with the PF group, thus indicating that advanced uremia aggravated the development of peritoneal inflammation in these animals [10].

The fibrous thickening and the overexpression of α-SMA, a myofibroblast marker, induced by GC injections in the PM were attenuated by ASC treatment. ASC administration also blocked the upregulation of profibrotic factors, notably,

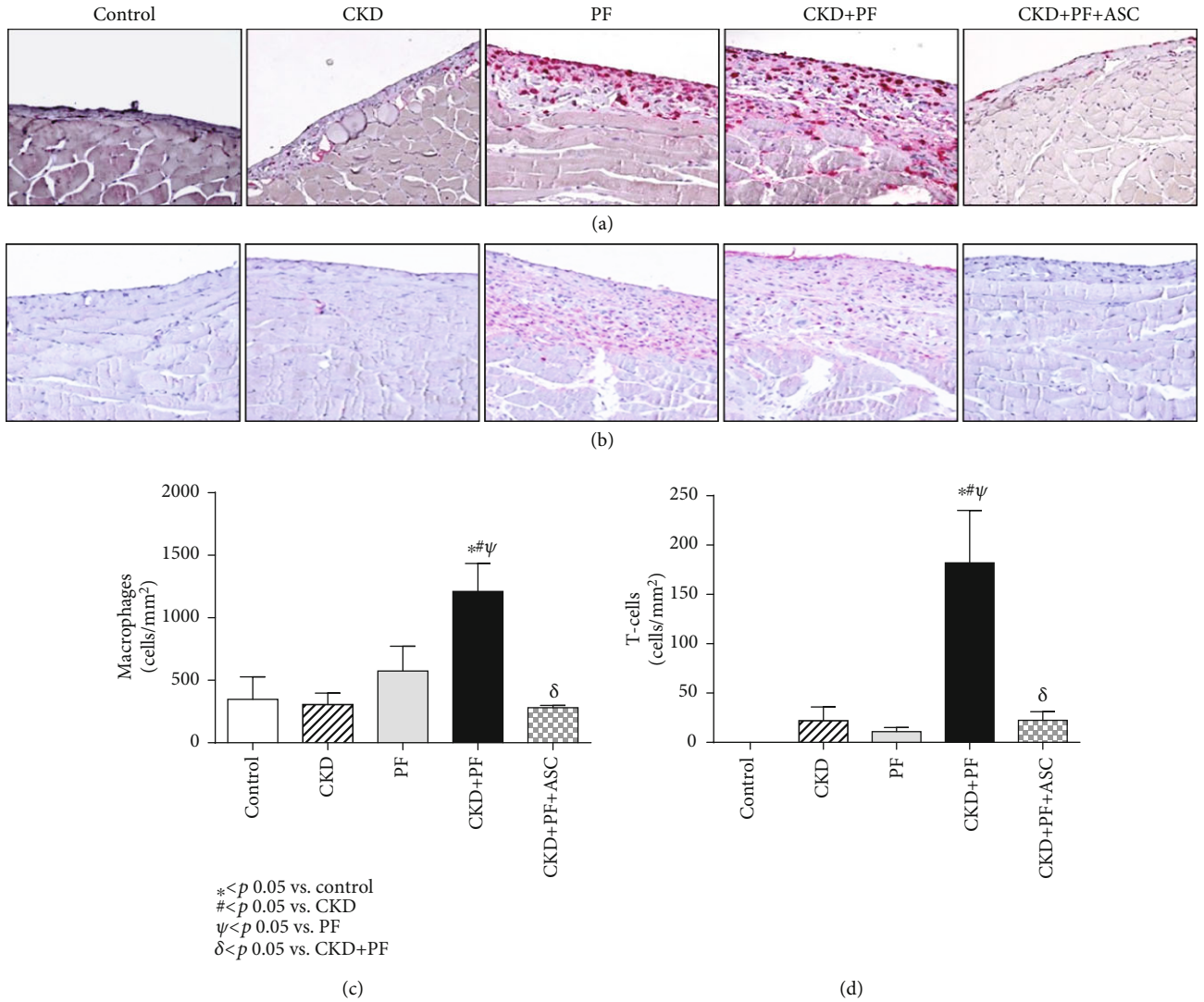

FIGURE 4: Illustrative microphotographs of peritoneal samples from the different groups submitted to immunohistochemistry for macrophage (a) and T-cell (b) detection. Associated CKD+PF induced both macrophage (c) and T-cell (d) infiltration, while ASC infusions prevented PM inflammation, completely (c, d).

TGF-β and fibronectin. Similar to our findings, Ueno et al. demonstrated that human MSC prevented PF induced by GC in nonuremic animals. Also, the coculture of human peritoneal mesenchymal cells with human MSC resulted in a significant reduction of TGF-β and fibronectin mRNA expressions compared with the levels in vehicle-treated cells [14]. Since the TGF-β signaling pathway plays a pivotal role in PF, it is possible that the antifibrotic effect of ASC observed in our study is mediated by TGF-β inhibition.

Systemic inflammation and higher levels of cytokines in the peritoneal fluid precede PF and encapsulating peritoneal sclerosis in PD patients [16]. Besides the inhibition of antifibrotic pathways, the ASC also showed a strong anti-inflammatory effect on the peritoneal membrane. Animals submitted to the combo model and treated with ASC did not present submesothelial infiltration by leukocytes, such as macrophages and T-cells. In agreement with our findings, Wang et al., using bone marrow-derived SC in a rat model of acute peritoneal adhesion, showed that intraperitoneal injections of MSC inhibited leucocyte infiltration of PM and TNF-α expression through paracrine mechanisms [17]. Thus, the inhibition of TNF-α production by ASC may account for its beneficial effect in our study.

ASC infusion also promoted significant reductions in the peritoneal gene and protein expressions of IL-1β, TNF-α, and IL-6 in the ASC-treated CKD+PF animals, compared to untreated CKD+PF rats. These findings corroborate previous reports describing MSC-induced anti-inflammatory and immunomodulatory effects. Aggarwal et al. demonstrated that purified subpopulations of human immune cells have its cytokine secretion profile altered towards a more anti-inflammatory and immunotolerant phenotype, when cocultured with MSC. According to this study, under the stimuli of MSC, dendritic cells decreased its TNF-α and IL-10 release, while Th1 lymphocytes reduced the IFN-γ expression and increased IL-4 production [18].

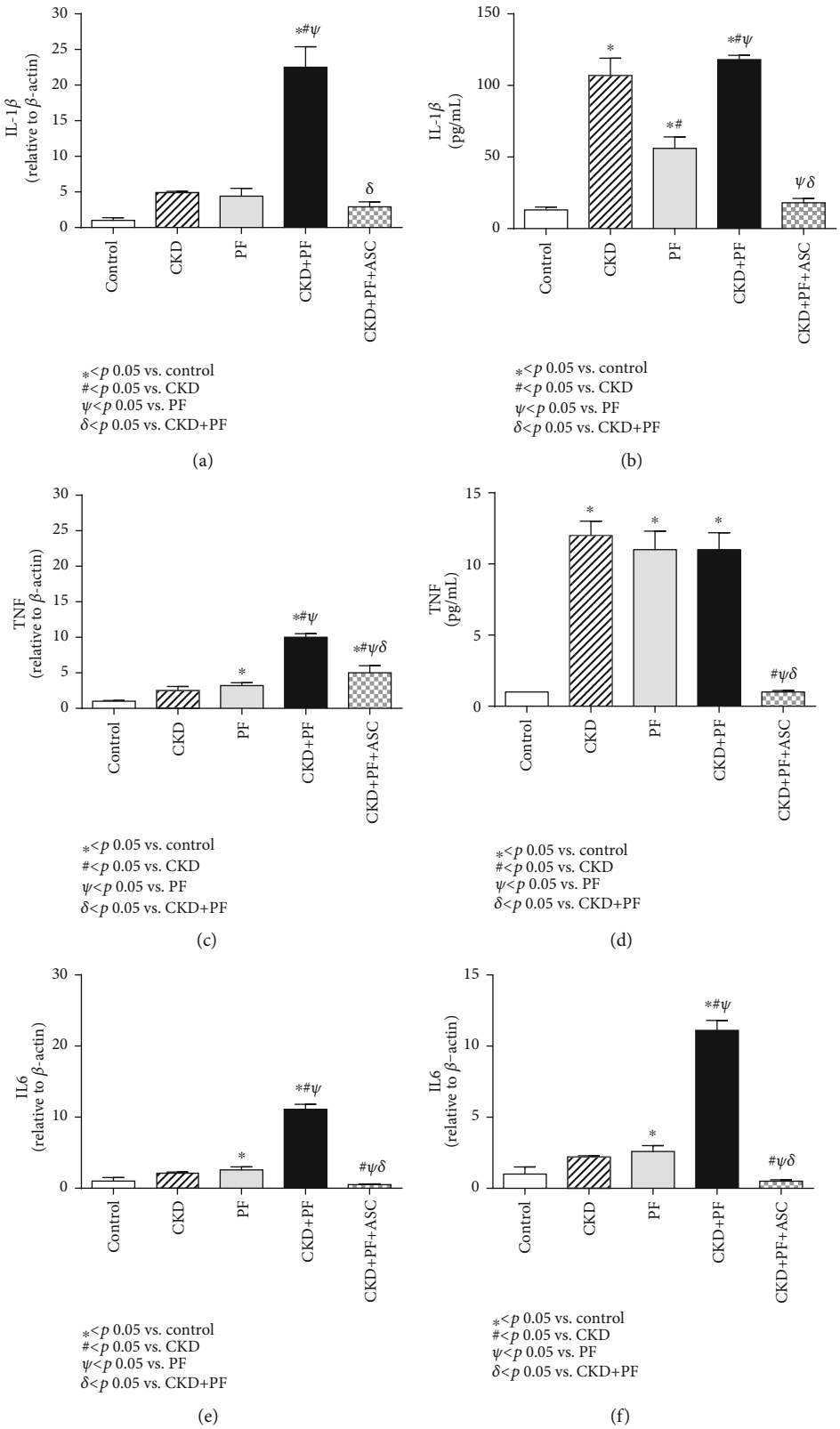

Figure 5: Comparative analysis of the gene (qPCR) and protein (multiplex) expressions of IL-1β (a, b), TNF (c, d), and IL6 (e, f) in the peritoneal membrane of the animals of each experimental group. The CKD+PF model promoted a significant increase in both gene and protein expressions of IL-1β, TNF-α, and IL6, compared to the control. ASC treatment normalized the gene and protein expressions of these three studied factors.

There are many possible mechanisms by which MSC exert its beneficial effects. The ability of MSC to migrate to the damaged tissue and differentiate into reparative cells was initially thought to occur. However, it has been recognized that paracrine factors secreted by the MSC are likely to be the main mechanism inducing tissue protection and recovery. Wang et al. showed that after IV infusion of MSC in the tail vein of rats submitted to peritoneal damage by scrapping, these cells accumulated in the lungs, liver, and spleen. No stem cells were observed in the injured peritoneum, in spite of the protective effects achieved by IV MSC infusion. These data suggest that the main biological effects of MSC infusion may be attributed more to the release of anti-inflammatory and immunomodulatory factors by these cells, than to their *in situ* differentiation [17].

Noteworthy, we found an unexpected effect of ASC treatment in blood pressure. While the uremic rats in the untreated groups (CKD and CKD+PF) showed hypertension, in the CKD+PF+ASC group, the blood pressure was similar to the control group. Future studies are needed to better understand this finding, but in an experimental model of renovascular hypertension, MSC controlled the blood pressure and suppressed the intrarenal angiotensin system [19].

5. Conclusions

In conclusion, we have shown that ASC treatment inhibited the progression of PF in a CG-induced PF model in uremic rats. ASC inhibited different and important mechanisms involved in peritoneal membrane modifications induced by PD, as the activation of the TGF-β pathway, myofibroblast proliferation, and inflammation. Our results are interesting and reinforce stem cell therapy as a perspective for the treatment of PF. However, future studies are needed before this experimental finding is translated into clinical application.

Data Availability

All data generated or analyzed during this study are included in this published article and its Supplementary Information files.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

Supplementary Material Figure 1: cellular characterization of mesenchymal stem cell surface markers by immunofluorescence. ASC were positive for CD44, CD90, CD146, and CD73 and negative for CD19 and CD45. Supplementary Material Figure 2: characterization of adipose-derived mesenchymal cells (ASC) employed in the study. ASC of Wistar rats in the passages P0 (A), P4(B), analysis of the capacity of ASC to differentiate into adipogenic (C), chondrogenic (D), and osteogenic lineages (E) under 10x magnification. Supplementary Material Figure 3: representative line graph of data shown in Table 1 of the main manuscript file. Comparative analysis of body weight (BW) (A), systolic blood pressure (BP) (B), and urea nitrogen (BUN) levels (C), in the different groups at days 01, 15, and 30. Supplementary Material Figure 4: illustrative microphotographs of immunohistochemistry for α-SMA in peritoneal samples from the different groups (×200). *(Supplementary Materials)*

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Research Article

Clinical Efficacy and Safety of Mesenchymal Stem Cells for Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a polymorphic, multisystemic autoimmune disease that causes multiorgan damage in which cellular communication occurs through the involvement of autoantibodies directed against autoantigen production. Mesenchymal stem cells (MSCs), which have strong protective and immunomodulatory abilities, are obtained not only from bone marrow but also from medical waste such as adipose tissue and umbilical cord tissue and have been recognized as a promising tool for the treatment of various autoimmune diseases and inflammatory disorders. This meta-analysis is aimed at assessing whether MSCs can become a new treatment for SLE with good efficacy and safety. Based on predetermined criteria, a bibliographical search was performed from January 1, 2000, to July 31, 2019, by searching the following databases: ISI Web of Science, Embase, PubMed, the Cochrane Library, and the Chinese Biomedical Literature Database (CBM). Eligible studies and data were identified. Statistical analysis was conducted to assess the efficacy (proteinuria, systemic lupus erythematosus disease activity index (SLEDAI), Scr, BUN, albumin, C3, and C4) and safety (rate of adverse events) of MSCs for SLE using Cochrane Review Manager Version 5.3. Ten studies fulfilled the inclusion criteria and were eligible for this meta-analysis, which comprised 8 prospective or retrospective case series and four randomized controlled trails (RCTs) studies. In the RCT, the results indicated that the MSC group had lower proteinuria than the control group at 3 months and 6 months and the MSC group displayed a lower SLEDAI than the control group at 2 months and 6 months. Furthermore, the MSC group showed a lower rate of adverse events than the control group (OR = 0.26, 95% CI: 0.07, 0.89, $P = 0.03$). In the case series trials, the results indicated that the MSC group had lower proteinuria at 1 month, 2 months, 3 months, 4 months, 6 months, and 12 months. In conclusion, MSCs might be a promising therapeutic agent for patients with SLE.

1. Introduction

Mesenchymal stem cells (MSCs) are a group of self-renewing nonhematopoietic multipotent progenitor cells that were initially discovered in bone marrow and subsequently found in many other tissues, such as umbilical cord blood, adipose tissue, skin tissue, and the periendothelial area. They can differentiate into various types of mesenchymal cells, such as osteoblasts, chondrocytes, fibroblasts, and adipocytes [1, 2]. To date, the cells have been mainly defined retrospectively based on their fibroblastic colony-forming capacity and multipotency in vitro. Therefore, these cells have been redefined as MSCs. It has been shown that MSCs have unique and powerful immunomodulatory and regenerative characteristics. The therapeutic effects of MSCs can be largely attributed to extracellular vesicles including exosomes. Exosomes from MSCs can regulate the inflammatory response, immunomodulation, angiogenesis, blood coagulation, extracellular matrix remodelling, and cell apoptosis; moreover, exosomes can also reduce the levels of creatinine (Cr) and blood urea nitrogen (BUN), as well as necrosis of proximal kidney tubules [3–5]. MSC transplantation has become one of the

treatment options for a variety of immune system diseases, such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE) [1, 6–8].

As a chronic autoimmune disease, systemic lupus erythematosus (SLE) is accompanied by multiple system damage. Immune-mediated inflammatory injury plays an important role in the pathogenesis of SLE. The disease is characterized by the production of a variety of autoantibodies represented by antinuclear antibodies, the formation of immune complexes, tissue inflammation in multiple organs (including brain, joints, blood vessels, kidneys, and skin), and high levels of serum proinflammatory cytokines [9, 10]. Lupus nephritis (LN) is one of the most serious visceral complications in SLE, occurring in approximately half of SLE patients. Clinically, LN is characterized by proteinuria, cellular casts, haematuria, and renal failure, which may lead to end-stage renal disease and the need for peritoneal dialysis, haemodialysis, or renal transplantation [11]. At present, the main drugs for treating SLE include antimalarial drugs (hydroxychloroquine (HCQ), quinacrine), corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs), immunosuppressants (cyclosporine A (CsA), tacrolimus (TAC), methotrexate (MTX), azathioprine (AZA), mycophenolate mofetil (MMF), and cyclophosphamide (CTX)), and biological agents (belimumab antibody, rituximab (RTX)) [12]. When the clinical condition is serious, high-dose immunoglobulin, plasma exchange, or haematopoietic stem cell or mesenchymal stem cell transplantation can be selected. However, the long-term use of corticosteroids or immunosuppressants may lead to serious infection and secondary malignant tumours, and the use of biological agents is also limited to a certain extent because of its high cost [13].

Previously, there were some studies focusing on the MSC in treating renal diseases, and the results were conflicting. Quimby et al. [14] conducted a study in cats with chronic kidney disease and reported that administration of MSCs was not associated with significant improvement in renal function. van Rhijn-Brouwer et al. [15] conducted a study in kidney transplant recipients and showed that MSCs have an intrinsic capacity to produce proangiogenic paracrine factors, including extracellular vesicles (EVs), which suggested that autologous MSC-based therapy is a viable option in the therapy of chronic kidney disease. Song et al. [16] reported that MSC treatment can attenuate renal interstitial fibrosis possibly through inhibition of EMT and the inflammatory response via the TGF-β1 signalling pathway.

Cell therapy has become an attractive therapeutic strategy for various types of diseases [17–20], and it has achieved certain curative effects in induction therapy in patients with SLE [13]. The purpose of this study was to evaluate the efficacy of MSCs in the treatment of SLE by meta-analysis.

2. Materials and Methods

2.1. Data Sources and Search Terms. The previous full extent of studies from January 1, 2000, to July 31, 2019, reporting the outcomes of MSC treatment for LN patients had been mined in this search strategy to determine the therapeutic promise of MSC regimen for LN as it was translated from bench to bedside. Two reviewers separately conducted the searches in the following medical databases: ISI Web of Science, Embase, PubMed, the Cochrane Library, and the Chinese Biomedical Literature Database (CBM). PubMed was searched using MeSH headings or their equivalents of "Mesenchymal Stem Cells" and "Lupus Nephritis." The entry terms for mesenchymal stem cells were as follows: Mesenchymal Stem Cells, MSC, Multipotent Stromal Cells, Mesenchymal Stromal Cells, Mesenchymal Progenitor Cells, Wharton Jelly Cells, Adipose-Derived Mesenchymal Stem Cells, and Bone Marrow Stromal Stem Cells. The entry terms for SLE were as follows: systemic lupus erythematosus, SLE, Lupus Nephritis, LN, Lupus Glomerulonephritis, Lupus Nephritides, and Lupus Glomerulonephritides. As per this method, other database searches were performed using a combination of mesenchymal stem cells and lupus nephritis terms. Any language restrictions were not applied in this meta-analysis. Additionally, the reference lists of the selected studies and reviews were also scrutinized to manually identify eligible articles.

2.2. Inclusion and Exclusion Criteria. The inclusion criteria are as follows: (1) eligible articles were required to be randomized controlled trials (RCTs) or self-controlled trials; (2) enrolled patients were diagnosed with LN disease conforming to the American College of Rheumatology (ACR) criteria and were treated with MSC therapy; (3) the presence of data on therapeutic efficacy and safety was essential.

The exclusion criteria are as follows: (1) abstracts, case reports, reviews, case-controlled trials, and editorials were excluded; (2) patient data that were not shown or were not sufficiently detailed to be pooled were excluded.

2.3. Study Selection and Data Extraction. Titles, abstracts, and, if necessary, full texts were browsed by two independent investigators. Discrepancies were resolved by them through comparing lists after reviewing the identified papers, and another investigator finalized the list of included articles.

Two investigators customized a table to extract the data independently on the basis of the surname of the first author, the publication year, patient information, the intervention, and the outcome characteristics. Any disagreement was settled by a third investigator.

2.4. Statistical Analysis. All statistical analyses were carried out by Cochrane Review Manager Version 5.3 (Cochrane Library, UK). I^2 was used to detect the heterogeneity among the included investigations. A random effects model was applied for meta-analyses, in which the P value from the heterogeneity test was less than 0.1; otherwise, a fixed effects model was used. Weighted mean differences (WMDs) were presented for continuous data, and the binary data were shown for odds ratios (ORs). 95% confidence intervals (95% CIs) were assessed for the recruited studies. Values of $P < 0.05$ were considered statistically significant.

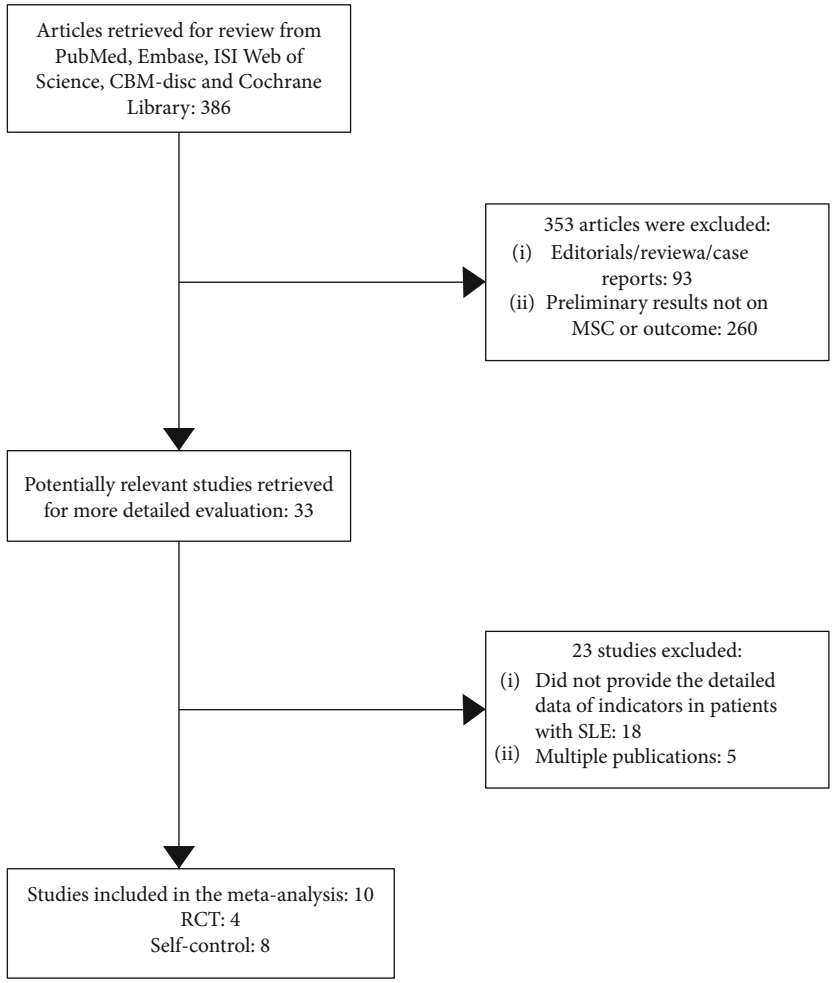

Figure 1: Flow diagram process of study selection.

3. Results

3.1. Search Results. The searches identified 386 publications, and 10 studies fulfilled the inclusion criteria and were eligible for this meta-analysis, which comprised 8 prospective or retrospective case series [21–28] and four RCT studies [27–30] (Figure 1). These eight retrospective or prospective case series included 231 SLE patients, as shown and detailed in Table 1. Furthermore, these four RCTs included 47 patients with SLE in the case group and 37 patients with SLE in the control group.

3.2. Randomized Controlled Trial

3.2.1. Proteinuria. One study [28] was included in the meta-analysis for 3 months and two [28, 30] for 6 months, and the results indicated that the MSC group had lower proteinuria than the control group (3 months: WMD = -0.92, 95% CI: -1.05, -0.79, $P < 0.00001$; 6 months: WMD = -2.00, 95% CI: -3.81, -0.19, $P = 0.03$; Table 2). However, one study [28] was included for 2 months and two studies [27, 29] were included for 12 months. The results indicated that MSC treatment resulted in lower proteinuria, but the difference was not significant (2 months: WMD = -1.74, 95% CI: -5.00, -1.52, $P = 0.30$; 12 months: WMD = -0.46, 95% CI: -1.37, 0.45, $P = 0.33$; Table 2).

3.2.2. Scr. One study [27] was included for 3 months, one [30] for 6 months, and two [27, 29] for 12 months, and the results indicated that the difference between the MSC treatment group and the control group was not notable (3 months: WMD = -2.52, 95% CI: -8.53, 3.49, $P = 0.41$; 6 months: WMD = 3.92, 95% CI: -8.55, 16.39, $P = 0.54$; and 12 months: WMD = -0.74, 95% CI: -14.04, 12.56, $P = 0.91$; Table 2).

3.2.3. Serum Albumin. One study [27] was included in the meta-analysis for 3 months, and the results indicated that the MSC group had higher serum albumin than the control group (WMD = 7.85, 95% CI: 5.93, 9.77, $P < 0.00001$; Table 2). However, two studies [27, 29] were included for 12 months, and the results indicated that MSC treatment resulted in higher serum albumin, but the difference was not significant (WMD = 0.94, 95% CI: -0.53, 2.40, P = 0.21; Table 2).

3.2.4. C3. One study [27] was included in the meta-analysis for 3 months, and the results indicated that the MSC group had higher C3 than the control group (WMD = 0.28, 95% CI: 0.16, 0.40, $P < 0.00001$; Table 2). However, two studies

Table 1: Characteristics of the studies included in this meta-analysis.

Author, year	Study type	Basic regimen	Patient characteristics	MSC	MSC dose	Infusion	Endpoint	Adverse events	Effectiveness
Yang, 2014	RCT	GC+CTX	Refractory SLE	UC-MSC	3×10^7, once	IV	SLEDAI, proteinuria, Scr, serum albumin, C3, C4	—	Yes
Zeng, 2016	RCT	GC+MMF	II-IV type of LN	UC-MSC	1×10^6/kg, 3-5 times	Renal artery	SLEDAI, proteinuria, Scr, serum albumin, C3, C4, BUN	—	Yes
Tang, 2016	RCT	GC+MMF +CTX	IV type of LN	UC-MSC	5×10^7, twice	IV	SLEDAI, proteinuria	T ($n = 2$): 1 case with upper respiratory tract infection, 1 case of right thigh abscess; C ($n = 1$): 1 case with upper respiratory tract infection	No
Deng, 2017	RCT	GC+CTX	IV type of LN	UC-MSC	2×10^8	IV	Proteinuria, Scr	T ($n = 2$): one with leucopenia and pneumonia together with subcutaneous abscess. Another with severe pneumonia; C ($n = 2$): one patient with stroke and another with ascites of unknown cause	No
Gu, 2014	Self-control	GC+CTX/MMF	Refractory SLE	BM-MSC, UC-MSC	1×10^6/kg, once	IV	SLEDAI, proteinuria, Scr, BUN	Enteritis, diarrhea, transient increase of serum creatinine, herpes virus infection. But, none of them were considered to be related to MSC infusion	Yes
Zhu, 2016	Self-control	GC+CTX/MMF	Refractory SLE	UC-MSC	5×10^7, twice	IV	SLEDAI, Scr, BUN, C4	Adverse event was not found	Yes
Li, 2016	Self-control	GC+CTX	III-IV type of LN or with type V	UC-MSC	1×10^6/kg-2×10^6 /kg, 4 times	IV	SLEDAI, proteinuria, Scr, C3, C4	Two cases of fever, 2 cases of diarrhea, 1 case of vomiting, 1 case of pruritus	Yes
Qiu, 2016	Self-control	GC+CTX/MMF	Refractory SLE	UC-MSC	1×10^6/kg, once	IV	SLEDAI, proteinuria, C3	Adverse event was not found	Yes
Bai, 2017	Self-control	GC+CTX/MMF	Refractory SLE	UC-MSC	1×10^6/kg, 3-5 times	IV	SLEDAI, proteinuria, C3, C4	One patient with headache, nausea, and vomiting during each stem cell infusion	Yes
Wen, 2019	Self-control	GC +CTX/MMF/ LEF/HCQ	Refractory SLE	BM-MSC, UC-MSC	1×10^6/kg, once	IV	SLEDAI	—	Yes

Note: RCT: randomized controlled trail; BM-MSC: bone marrow-derived mesenchymal stem cells; UC-MSC: umbilical cord-derived mesenchymal stem cells; TAC: tacrolimus; GC: glucocorticoids; CTX: cyclophosphamide; IV: intravenous; MMF: mycophenolate mofetil; HCQ: hydroxychloroquine; LEF: leflunomide; SLE-DAI: systemic lupus erythematosus disease activity index; LN: lupus nephritis; T: MSC group; C: control group.

Table 2: Meta-analysis of the efficacy of MSC in the therapy of patients with lupus nephritis (RCT).

Indicators	Time point	Studies Number	Q test P value	Model Selected	OR/WMD (95% CI)	P
Proteinuria	2 months	1	—	Fixed	-1.74 (-5.00, 1.52)	0.30
	3 months	1	—	Fixed	-0.92 (-1.05, -0.79)	<0.00001
	6 months	2	0.84	Fixed	-2.00 (-3.81, -0.19)	0.03
	12 months	2	<0.00001	Random	-0.46 (-1.37, 0.45)	0.33
Scr	3 months	1	—	Fixed	-2.52 (-8.53, 3.49)	0.41
	6 months	1	—	Fixed	3.92 (-8.55, 16.39)	0.54
	12 months	2	0.05	Random	-0.74 (-14.04, 12.56)	0.91
Serum albumin	3 months	1	—	Fixed	7.85 (5.93, 9.77)	<0.00001
	12 months	2	0.15	Fixed	0.94 (-0.53, 2.40)	0.21
C3	3 months	1	—	Fixed	0.28 (0.16, 0.40)	<0.00001
	12 months	2	0.02	Random	0.36 (-0.08, 0.79)	0.11
C4	3 months	1	—	Fixed	-0.01 (-0.04, 0.02)	0.46
	12 months	2	0.31	Fixed	-0.01 (-0.03, 0.01)	0.39
SLEDAI	2 months	1	—	Fixed	-6.25 (-9.04, -3.46)	<0.0001
	3 months	1	—	Fixed	-0.89 (-2.19, 0.41)	0.18
	6 months	1	—	Fixed	-4.25 (-6.78, -1.72)	0.001
	12 months	2	0.004	Random	-1.00 (-3.13, 1.14)	0.36
Adverse events	—	2	0.66	Fixed	0.26 (0.07, 0.89)	0.03

[27, 29] were included for 12 months and the results indicated that MSC treatment resulted in higher C3, but the difference was not significant (WMD = 0.36, 95% CI: -0.08, 0.79, $P = 0.11$; Table 2).

3.2.5. C4. One study [27] was included for 3 months and two [27, 29] for 12 months, and the results indicated that the difference was not significant between the MSC treatment group and the control group (3 months: WMD = -0.01, 95% CI: -0.04, 0.02, $P = 0.46$; 12 months: WMD = -0.01, 95% CI: -0.03, 0.01, $P = 0.39$; Table 2).

3.2.6. SLEDAI. One study [28] was included in the meta-analysis for 2 months and one [28] for 6 months, and the results indicated that the MSC group had a lower SLEDAI than the control group (2 months: WMD = -6.25, 95% CI: -9.04, -3.46, $P < 0.0001$; 6 months: WMD = -4.25, 95% CI: -6.78, -1.72, $P = 0.001$; Table 2). However, one study [27] was included for 3 months and two studies [27, 29] were included for 12 months. The results indicated that MSC treatment resulted in a lower SLEDAI, but the difference was not significant (3 months: WMD = -0.89, 95% CI: -2.19, 0.41, $P = 0.18$; 12 months: WMD = -1.00, 95% CI: -3.13, 1.14, $P = 0.36$; Table 2).

3.2.7. Adverse Events. Two studies [28, 30] were included in the meta-analysis for adverse events. The adverse events included upper respiratory tract infection, leucopenia, pneumonia, and subcutaneous abscess in the MSC group and included upper respiratory tract infection, stroke, and ascites in the control group. The results indicated that the MSC group had a lower rate of adverse events than the control group (OR = 0.26, 95% CI: 0.07, 0.89, $P = 0.03$; Table 2).

3.3. Case Series

3.3.1. Proteinuria. Two studies [21, 22] were included for the meta-analysis for 1 month, and the results indicated that the MSC group had lower proteinuria (WMD = -0.69, 95% CI: -1.02, -0.36, $P < 0.0001$; Figure 2 and Table 3). Two studies [22, 28] were included in the meta-analysis for 2 months, and the results indicated that the MSC group had better efficacy (WMD = -1.51, 95% CI: -2.40, -0.63, $P = 0.0008$; Figure 2 and Table 3). Three studies [21, 25, 27] were included in the meta-analysis for 3 months, and the results indicated that the MSC group had lower proteinuria (WMD = -1.25, 95% CI: -2.00, -0.51, $P = 0.001$; Figure 2 and Table 3). One study [22] was included in the meta-analysis for 4 months, and the results indicated that the MSC group had better efficacy (WMD = -2.04, 95% CI: -3.00, -1.08, $P < 0.0001$; Figure 2 and Table 3). Five studies [21, 22, 24, 25, 28] were included in the meta-analysis for 6 months, and the results indicated that the MSC group had lower proteinuria (WMD = −1.56, 95% CI: -2.14, -0.98, $P < 0.00001$; Figure 2 and Table 3). Two studies [21, 27] were included in the meta-analysis for 12 months, and the results indicated that the MSC group had reduced proteinuria (WMD = -1.82, 95% CI: -2.96, -0.67, $P = 0.002$; Figure 2 and Table 3).

3.3.2. Scr. Three studies [21–23] were included for 1 month, two [22, 23] for 2 months, three [21, 23, 27] for 3 months, and one [22] for 4 months, and the results indicated that

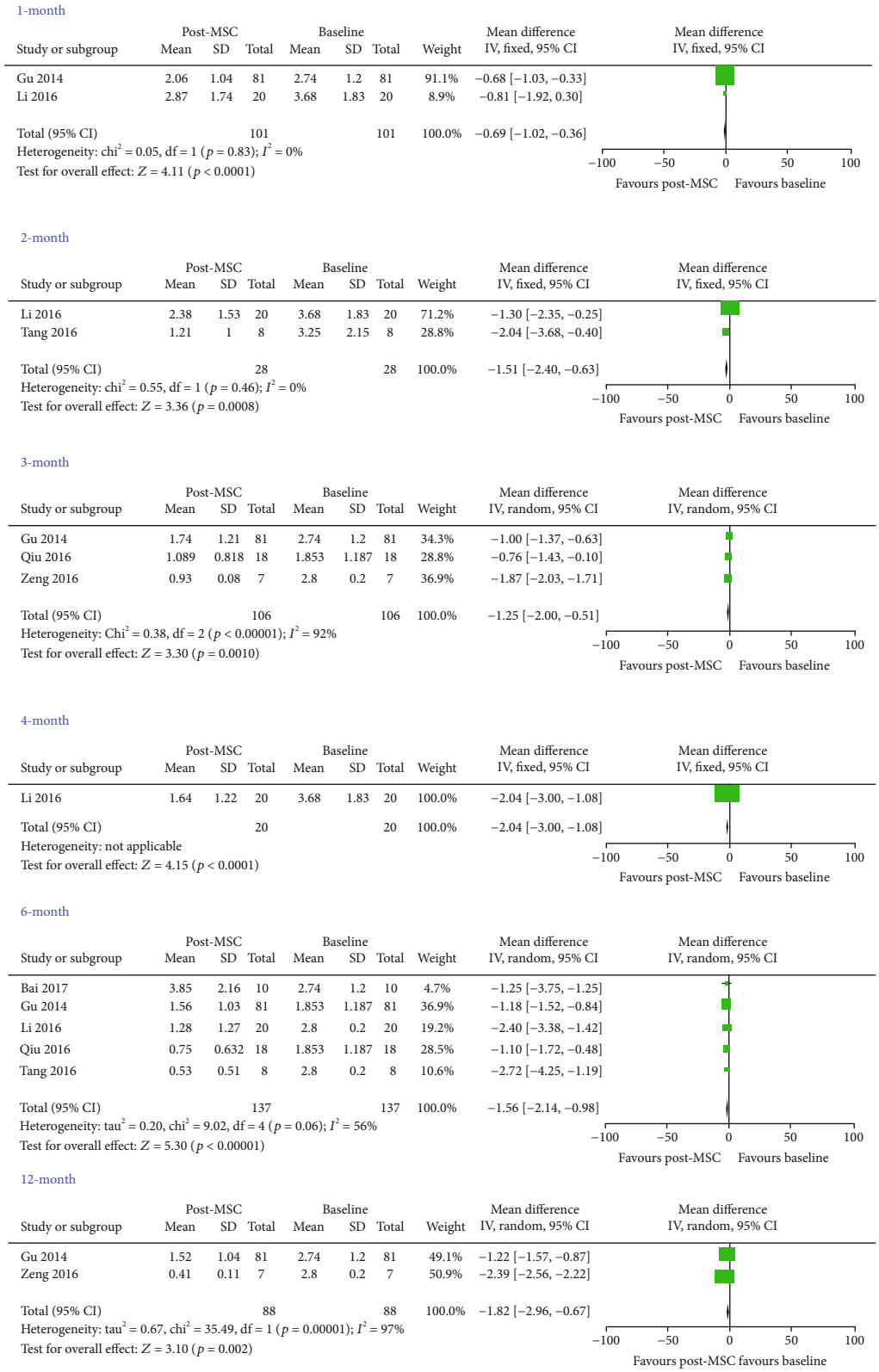

Figure 2: Assessment the efficacy of MSC on proteinuria in patients with systemic lupus erythematosus (self-controlled studies).

MSC treatment yielded a better reduction in Scr but the difference was not significant (1 month: WMD = -7.28, 95% CI: -21.97, 7.41, $P = 0.33$; 2 months: WMD = -59.18, 95% CI: -166.92, 48.56, $P = 0.28$; 3 months: WMD = −75.13, 95% CI: -187.01, 36.76, $P = 0.19$; and 4 months: WMD = -10.25, 95% CI: -25.34, 4.84, $P = 0.18$; Table 3). Interestingly, two

Table 3: Meta-analysis of the efficacy of MSC in the therapy of patients with lupus nephritis (self-control).

Indicators	Time point	Studies Number	Q test P value	Model Selected	WMD (95% CI)	P
Proteinuria	1 month	2	0.83	Fixed	-0.69 (-1.02, -0.36)	<0.0001
	2 months	2	0.46	Fixed	-1.51 (-2.40, -0.63)	0.0008
	3 months	3	<0.00001	Random	-1.25(-2.00, -0.51)	0.001
	4 months	1	—	Fixed	-2.04 (-3.00, -1.08)	<0.0001
	6 months	5	0.06	Random	-1.56 (-2.14, -0.98)	<0.00001
	12 months	2	<0.00001	Random	-1.82 (-2.96, -0.67)	0.002
Scr	1 month	3	0.32	Fixed	-7.28 (-21.97, 7.41)	0.33
	2 months	2	0.0006	Random	-59.18 (-166.92, 48.56)	0.28
	3 months	3	<0.00001	Random	-75.13 (-187.01, 36.76)	0.19
	4 months	1	—	Fixed	-10.25 (-25.34, 4.84)	0.18
	6 months	2	0.72	Fixed	-14.08 (-28.09, -0.07)	0.05
	12 months	2	0.88	Fixed	-30.00 (-38.89, -21.10)	<0.00001
BUN	1 month	1	—	Fixed	-610.6 (-835.84, -385.36)	<0.00001
	2 months	1	—	Fixed	-758.4 (-960.42, -556.38)	<0.00001
	3 months	3	<0.00001	Random	-21.31 (-46.58, 3.97)	0.10
	12 months	2	0.05	Random	-4.14 (-7.89, -0.39)	0.03
C3	1 month	2	0.69	Fixed	0.15 (0.06, 0.24)	0.0006
	2 months	2	0.70	Fixed	0.25 (0.17, 0.33)	<0.00001
	3 months	3	<0.00001	Random	0.37 (-0.01, 0.76)	0.06
	4 months	1	—	Fixed	0.33 (0.13, 0.53)	0.001
	6 months	3	0.009	Random	0.23 (0.06, 0.39)	0.006
	12 months	1	—	Fixed	0.96 (0.88, 1.04)	<0.00001
C4	1 month	2	0.51	Fixed	0.02 (-0.01, 0.04)	0.25
	2 months	2	1.00	Fixed	0.05 (0.02, 0.08)	0.0001
	3 months	2	0.04	Random	0.11 (0.07, 0.15)	<0.00001
	4 months	1	—	Fixed	0.07 (0.04, 0.10)	<0.0001
	6 months	2	0.009	Random	0.06 (-0.02, 0.14)	0.15
	12 months	1	—	Fixed	0.24 (0.22, 0.26)	<0.00001
SLEDAI	1 month	5	0.02	Random	-3.83 (-5.42, -2.23)	<0.00001
	2 months	2	0.15	Fixed	-4.38 (-6.24, -2.51)	<0.00001
	3 months	4	0.88	Fixed	-5.45 (-6.19, -4.72)	<0.00001
	4 months	1	—	Fixed	-6.35 (-8.27, -4.43)	<0.00001
	6 months	6	0.87	Fixed	-7.20 (-7.99, -6.42)	<0.00001
	12 months	3	0.51	Fixed	-8.06 (-8.79, -7.33)	<0.00001

studies [21, 22] were included for 6 months and two studies [21, 27] for 12 months, and the results indicated that MSC treatment resulted in lower Scr (6 months: WMD = -14.08, 95% CI: -28.09, -0.07, $P = 0.05$; 12 months: WMD = -30.00, 95% CI: -38.89, -21.10, $P < 0.00001$; Table 3).

3.3.3. BUN. One study [23] was included for 1 month, one [23] for 2 months, and two [21, 27] for 12 months, and the results indicated that MSC treatment yielded a lower BUN (1 month: WMD = -610.60, 95% CI: -835.84, -385.36, $P < 0.00001$; 2 months: WMD = -758.40, 95% CI: -960.42, -556.38, $P < 0.00001$; and 12 months: WMD = -4.14, 95% CI: -7.89, -0.39, $P = 0.03$; Table 3). However, three studies [21, 23, 27] were included for 3 months, and the results indicated that MSC treatment had better efficacy, but the difference was not significant (WMD = -21.31, 95% CI: -46.58, 3.97, $P = 0.10$; Table 3).

3.3.4. C3. Two studies [22, 23] were included for 1 month, two [22, 23] for 2 months, one [22] for 4 months, three [22, 24, 25] for 6 months, and one [27] for 12 months, and the results indicated that MSC treatment resulted in a higher level of C3 (1 month: WMD = 0.15, 95% CI: 0.06, 0.24, $P = 0.0006$; 2 months: WMD = 0.25, 95% CI: 0.17, 0.33, $P < 0.00001$; 4 months: WMD = 0.33, 95% CI: 0.13, 0.53, $P = 0.001$; 6 months: WMD = 0.23, 95% CI:

0.06, 0.39, $P = 0.006$; and 12 months: WMD = 0.96, 95% CI: 0.88, 1.04, $P < 0.00001$; Table 3). However, three studies [23, 25, 27] were included for 3 months, and the results indicated that MSC treatment increased the C3 levels but the difference was not significant (WMD = 0.37, 95% CI: -0.01, 0.76, $P = 0.06$; Table 3).

3.3.5. C4. Two studies [22, 23] were included for 2 months, two [23, 27] for 3 months, one [22] for 4 months, and one [27] for 12 months, and the results indicated that MSC treatment resulted in a higher level of C4 (2 months: WMD = 0.05, 95% CI: 0.02, 0.08, $P = 0.0001$; 3 months: WMD = 0.11, 95% CI: 0.07, 0.15, $P < 0.00001$; 4 months: WMD = 0.07, 95% CI: 0.04, 0.10, $P < 0.0001$; and 12 months: WMD = 0.24, 95% CI: 0.22, 0.26, $P < 0.00001$; Table 3). However, two studies [22, 23] were included for 1 month, and two studies [22, 24] were included for 6 months. The results indicated that MSC treatment increased the C4 level, but the difference was not significant (1 month: WMD = 0.02, 95% CI: -0.01, 0.04, $P = 0.25$; 6 months: WMD = 0.06, 95% CI: -0.02, 0.14, $P = 0.15$; Table 3).

3.3.6. SLEDAI. Five studies [21–23, 25, 26] were included for 1 month, two [22, 28] for 2 months, four [21, 25–27] for 3 months, one [22] for 4 months, six [21, 22, 24–26, 28] for 6 months, and three [21, 26, 27] for 12 months, and the results indicated that MSC treatment yielded a lower value of SLEDAI (1 month: WMD = -3.83, 95% CI: -5.42, -2.23, $P < 0.00001$; 2 months: WMD = -4.38, 95% CI: -6.24, -2.51, $P < 0.00001$; 3 months: WMD = -5.45, 95% CI: -6.19, -4.72, $P < 0.00001$; 4 months: WMD = -6.35, 95% CI: -8.27, -4.43, $P < 0.00001$; 6 months: WMD = -7.20, 95% CI: -7.99, -6.42, $P < 0.00001$; and 12 months: WMD = -8.06, 95% CI: -8.79, -7.33, $P < 0.00001$; Figure 3 and Table 3).

4. Discussion

In this study, the meta-analysis included two parts, one for RCT studies and one for self-controlled studies. All the included studies found that MSC treatment can achieve better efficacy, except for the investigation from Tang et al. [28]. In this meta-analysis of RCTs, the results indicated that MSC treatment can achieve better efficacy than the control treatment at 3 months, with results such as lower proteinuria, increased serum albumin, and increased serum C3. MSC treatment resulted in lower SLEDAI values at 3 months and 6 months. Furthermore, the rate of adverse events in the MSC group was lower than that in the control group. The data from the meta-analysis of RCTs indicated that MSC treatment might be a good treatment for SLE, but the sample size of the recruited investigations was small, and the results should thus be carefully examined.

Furthermore, a meta-analysis including self-controlled studies was also conducted, and the results indicated that MSC treatment can markedly reduce proteinuria and the value of SLEDAI at 1 month, 2 months, 3 months, 4 months, 6 months, and 12 months. It can also improve the values of Scr, BUN, C3, and C4 at some time points. MSCs might be a good treatment agent for SLE in the clinic. More studies with larger sample sizes should be conducted to confirm these findings in the future.

In the included studies, Zeng et al. [27] conducted an RCT and recruited 22 patients with LN, and the results indicated that MSC combined with MMF in the treatment of LN can quickly reduce urinary protein in the short term and play a protective role in renal function, which can remarkably improve the disease condition and reduce the recurrence rate. Yang et al. [29] also found that it was effective and safe for SLE refractory to UC-MSC treatment. However, the RCT from Tang et al. [28] reported that the clinical symptoms and laboratory examination results of patients in two teams were all improved, but there was no notably significant difference between the two teams. Deng et al. [30] also indicated that MSC for SLE patients has no apparent additional effect over and above standard immunosuppression from their RCT study. Interestingly, all the self-controlled studies [21–26] reported that MSC treatment had good efficacy. The sample size from these included RCTs was small, and the feasibility of the evidence might not be better than that from the self-controlled trials. However, larger sample RCTs should be conducted in the future.

In the past decades, other meta-analyses have confirmed that MSCs might be good agents to treat some diseases. Shi et al. [31] conducted a meta-analysis to detect the efficacious clinical therapy of MSC for the treatment of ulcerative colitis, including 8 animal and 7 human trials, and reported that MSC treatment reduced the disease activity index when compared with that in the control group in mice, and compared with the control group, the healing rate of patients treated with MSC was notably elevated. Fan et al. [32] performed a meta-analysis including nine investigations for MSC in the treatment of heart failure and reported that the overall rate of death was reduced in the MSC treatment group, which suggested that therapy of MSC was effective for heart failure by improving the exercise and prognosis capacity. Yubo et al. [33] conducted a study using a meta-analytical method to evaluate the therapeutic efficacy and safety of MSC therapy for patients with knee osteoarthritis and included eleven eligible studies including 582 patients with knee osteoarthritis, and the results showed that the MSC therapy could notably reduce the visual analogue scale score and increase the International Knee Documentation Committee scores compared with those of controls after a 24-month follow-up. The researchers concluded that MSC transplantation therapy was safe and had great potential to become an efficacious clinical therapy for patients with knee osteoarthritis. Our meta-analysis also reported that MSCs might be a promising therapeutic agent for patients with SLE.

However, there were some limitations in our study. The sample size of the included studies was small, and longer-term endpoints were needed. The severity of the patients' disease was inconsistent, and the basic regimen for the SLE patients was different. Furthermore, the dose of MSC administered varied from the number of repeats to the absolute dose amount to a per-kg dosage. These factors may have caused our results to be unstable.

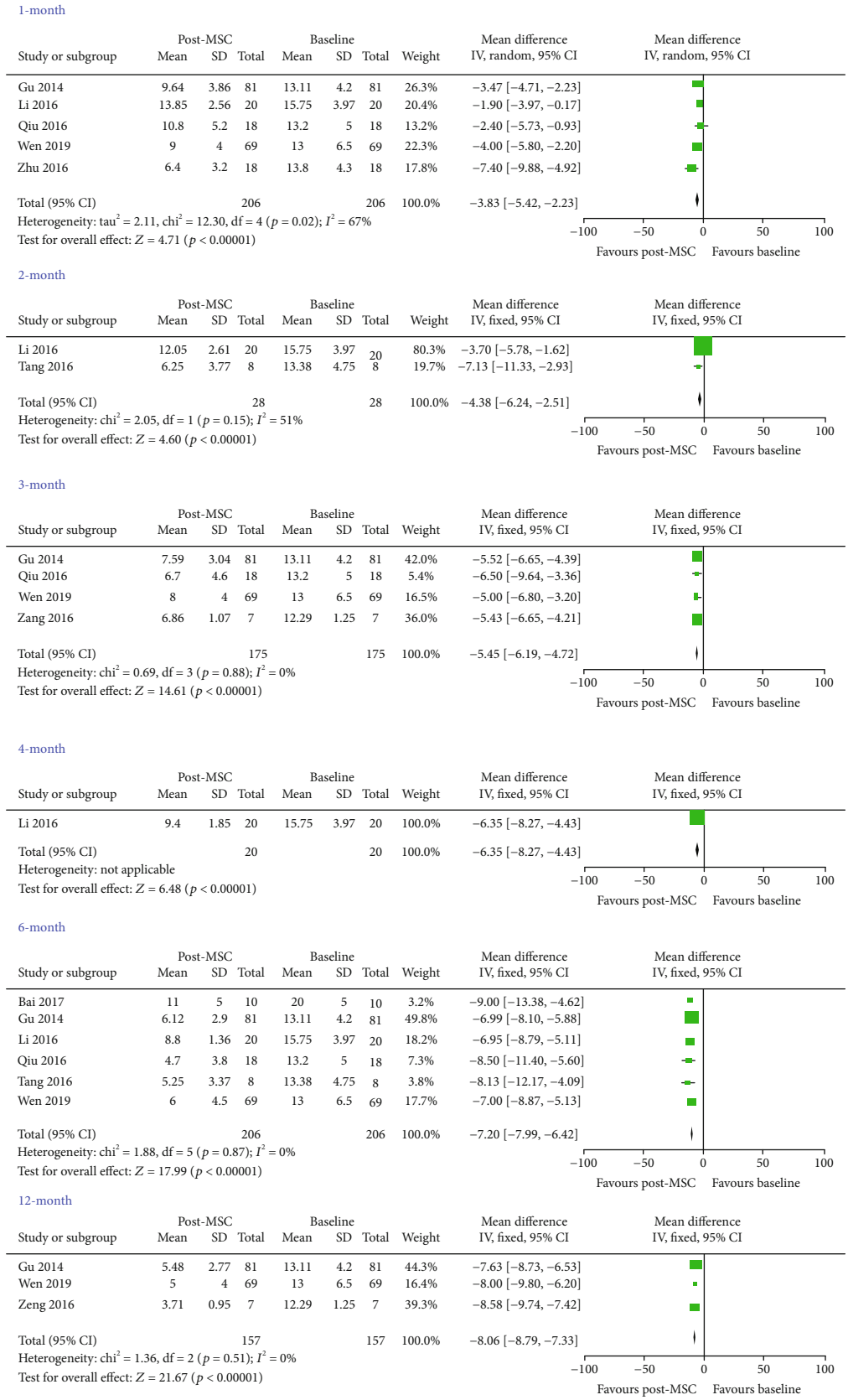

Figure 3: Assessment the efficacy of MSC on SLEDAI in patients with systemic lupus erythematosus (self-controlled studies).

5. Conclusions

The RCT results indicated that the MSC group had lower proteinuria at 3 months and 6 months, and the MSC group displayed lower SLEDAI at 2 months and 6 months. Furthermore, the MSC group showed a lower rate of adverse events. In case series trials, the results indicated that the MSC group had lower proteinuria at 1 month, 2 months, 3 months, 4 months, 6 months, and 12 months. MSCs might be a promising therapeutic agent for patients with SLE. However, more studies with longer-term end points and larger sample sizes should be designed and conducted to identify additional and robust patient-centred outcomes in the future.

Data Availability

The data supporting this meta-analysis are from previously reported studies and datasets, which have been cited. The processed data are available from the corresponding author upon request.

Disclosure

Tianbiao Zhou, Hong-Yan Li, and Chunling Liao are joint first authors.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

TBZ contributed to the conception and design of the study. TBZ, HYL, and CLL were responsible for the collection of data and performing the statistical analysis and manuscript preparation. WSL and SJL were responsible for checking the data. All authors were responsible for drafting the manuscript and read and approved the final version.

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