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Original Research

Bone Marrow Mesenchymal Stem Cell-Derived Exosomes Alleviate Diabetic Kidney Disease in Rats by Inhibiting Apoptosis and Inflammation

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Abstract

Background and Aims: Previous studies have confirmed the anti-inflammation effect of bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exo). We aimed to investigate the therapeutic effect of BMSC-Exo on diabetic kidney disease (DKD), as well as the underlying mechanisms. **Methods**: SD rats were induced by streptozotocin combined with a high-fat diet to establish a diabetes disease model. BMSCs-Exo were injected via tail veins at a weekly dose of 100 μg for 12 weeks. Pathological changes in the rat kidneys were evaluated using HE, Masson, and Periodic Acid-Schiff and immunohistochemical staining. TUNEL staining and western blot were used to evaluate the expression levels of apoptosis-related proteins in the rat kidney cells. The TNF-α level was detected by PCR and NF-κB (p65) by western blotting to examine the inflammatory responses in the renal tissue. **Results**: BMSCs-Exo significantly alleviated the renal structural damage and the distribution of apoptotic cells in diabetic rats. Furthermore, BMSCs-Exo increased the expression of pro-apoptosis protein Bax and decreased the expression of apoptosis-executing protein Cleaved Caspase 9 and Cleaved caspase 3. In addition, the transcription level of TNF-α in kidney tissue and NF-κB (p65) expression was also decreased through BMSCs-Exo treatment. Besides, the levels of glucose (GLU), creatinine (Cr), and burea nitrogen (BUN) in diabetic rats were decreased by the BMSC-Exo treatment. **Conclusions**: BMSCs-Exo may alleviate diabetic kidney damage by inhibiting apoptosis and inflammation.

Keywords: diabetic kidney disease; bone marrow mesenchymal stem cells; exosome; apoptosis; inflammation

1. Introduction

Diabetic kidney disease (DKD) is one of the most common and serious complications of diabetes and is the leading cause of end-stage renal disease. Long-term hyperglycemia, hypertension, and inflammation are the main etiologies that lead to the development of DKD [1]. The pathological manifestations of DKD involve increased glomerular mesangial matrices and thickening of the basement membrane and glomerular sclerosis, along with proteinuria, decreased glomerular filtration rates, and other functional abnormalities [2]. The pathogenesis of DKD is very complex and is still not fully understood, and treatments are not fully optimized either [3]. The pathogenesis of DKD usually results from hemodynamic dyshomeostasis, metabolic disorders, and abnormal hormone synthesis [4]. The renin-angiotensin-aldosterone system, formation of advanced glycation end products, activation of transforming growth factor β1, Caspase family proteases, and reactive oxygen species (ROS) are the key etiological factors that contribute to the development of DKD. Each disease pathway contributes to renal injuries through multiple mediators or through cross-interactions with other pathways [5]. Nevertheless, there is sufficient evidence that inflammation and apoptosis play an important role in the development of diabetic nephropathy [4,6].

In classical DKD patients, standard therapy still focuses on blood glucose and blood pressure control, targeting to halt the DKD progression and regression of albuminuria. These drugs mainly include inhibitors of dipeptidyl peptidase 4 (DDP-4) and sodium-dependent glucose transporters 2 (SGLT2), and classic hypoglycemic drugs such as metformin and renin aniotension aldosterone system (RAAS) antagonists. However, the above strategies have been shown only to slow down the progression but cannot completely prevent DKD from progressing to end-stage renal failure or reduce mortality [5,7]. Thus, there is an urgent need for new therapeutic approaches to improve renal function in DKD patients, delay disease progression, and reduce mortality.

Numerous studies have shown that stem cell transplantation can successfully prevent the progression of DKD [8]. The therapeutic effect of bone marrow mesenchymal stem cells (BMSCs) on DKD has been validated [9–11]. and investigated in clinical trials [12]. The therapeutic po-

tential of stem cells rests on their paracrine function and is particularly related to exosomes [13]. Exosomes are nanoscale lipid bilayer extracellular vesicles that are actively released by mammalian cells into intercellular substances and circulation and are thus involved in various physiological and pathological processes in the body [14]. Previous work has shown that bone marrow mesenchymal stem cells derived exosomes (BMSC-Exo) and the microRNAs they contain can alleviate DKD, but the mechanism has not been fully elucidated [15,16].

BMSC-Exo has also been shown to have anti-inflammatory and anti-apoptotic effects [14]. Thus, we speculate that BMSC-Exo can alleviate DKD by inhibiting inflammation and stressing anti-apoptosis pathways. Our study may provide new evidence for the treatment of DKD.

2. Methods

2.1 Isolation, Culture, and Characterization of Primary BMSCs

We selected 2-week-old male SD rats, isolated their femurs and tibias, washed the bone marrow cavities with a complete medium, pipetted the marrow into a cell suspension, and separated mononuclear cells using density gradient centrifugation with Ficoll-Paque PREMIUM 1.084 (Cytiva, Boston, MA, USA). Cells from the tibia and femur of one rat were inoculated into two T25 cell culture flasks. The isolated and purified cells were grown in DMEM/F-12 medium (Gibco, Grand Island, CA, USA) containing 20% fetal bovine serum (Gibco, CA, USA) and 1% penicillin-streptomycin solution (Gibco, CA, USA) in a humidified cell culture incubator at 37 °C with 5% CO_2. After 24 hours, the non-adherent cells were aspirated, and the culture medium was replaced with a complete medium. Thereafter, the complete medium was replaced every 48 h. The primary cells were subcultured when they grew above 80%–90% confluency. Third-generation BMSCs were used for subsequent experiments.

In addition to the morphological observations under the light microscope, the P3 generation cells were induced to differentiate into adipogenic, osteogenic, and chondrogenic lines, and were subsequently characterized by oil red O staining, alizarin red staining, and allicin blue staining, respectively. The expression of cell surface markers CD29 (positive), CD44 (positive), and CD34 (negative) was detected by flow cytometry (**Supplementary Fig. 1**).

2.2 Extraction and Identification of BMSC-Exo

After the BMSCs grew to 50%–60% confluence, the original medium was removed, washed three times with PBS (Gibco, Grand Island, CA, USA), and replaced with DMEM/F-12 containing 5% exosome-free FBS (OriCell, Wuhan, China). The cell culture supernatant was collected after 48–72 h, and centrifuged at 500 ×g for 10 min, 2000 ×g for 15 min, and 10,000 ×g for 30 min at 4 °C. The obtained cell supernatant was transferred to an ultracentrifuge tube, centrifuged twice at 100,000 ×g for 70 min at 4 °C, and then resuspended in 50 uL PBS and stored at –80 °C. The above stock solution was diluted with 0.9% saline to a final concentration and sterilized with a 0.22 μm filter before injection.

To identify the extracted BMSC-Exo, transmission electron microscopy (TEM, Hitachi, Tokyo, Japan) was used to capture images of exosome morphology. Nanoparticle tracking technology (NTA, ParticleMetrix, Ludwigshafen, Germany) was applied to analyze the particle size distribution of exosomes, and western blotting was used to detect expressions of CD9 and CD81 on the exosomal surface.

2.3 Animal Model and Grouping

5-week-old male SD rats (150–200 g) were purchased from HFK Bioscience Co, Ltd. (Beijing, China) and kept in the SPF animal laboratory while being fed a high-fat diet (HFD). After two weeks of acclimation, streptozotocin (STZ, 35 mg/kg/day, dissolved in 0.01 M citrate buffer, PH4.5) (Sigma, Saint. Louis, MO, USA) was intraperitoneally injected into the rats in the experimental group for three consecutive days, while the rats in the control group were injected with an equal volume of vehicle. To measure the postprandial blood glucose levels by a blood glucose meter (Roche, Basel, Switzerland), blood samples were collected each morning (8:00 AM) by acupuncturing the tail vein with a 28G needle. Rats with fasting glucose continuously above 11.1mM were considered to be successful diabetic models [17]. A total of 32 rats were divided into 4 groups and given different treatments up to 18 weeks. Excluding modeling failure and death, the remaining 20 rats were finally included in the follow-up experiments: the control group (NC group, n = 5), a diabetes group (DM group, n = 5), a metformin treatment group (Met group, n = 5), and an exosome treatment group (Exo group, n = 5). From the fourth week, the Met group was treated with 300 mg/kg/day of metformin by gavage, while the other three groups were given the same volume of pure water by gavage. The Exo group was given 100 μg (dissolved in 200 μL 0.9% sodium chloride solution) of exosomes through the tail vein every week, and the other three groups were given the same amount of normal saline at the same time points [17]. After 14 weeks of continuous administration, the rats were anesthetized with sodium pentobarbital (45 mg/kg body weight). Carotid blood was collected to assess blood glucose (GLU), urea nitrogen (BUN), and creatinine (Cr) levels. Each rat's left kidney was fixed in a 4% paraformaldehyde solution overnight. The right kidney was kept in liquid nitrogen for qRT-PCR and Western blot detection. The rat grouping treatment strategy is shown in Fig. 1.

2.4 Determination of GLU, BUN, and Cr

Blood samples obtained from rat carotid artery blood collection were allowed to stand at 37 °C to promote coagulation. After the blood was coagulated, it was centrifuged at

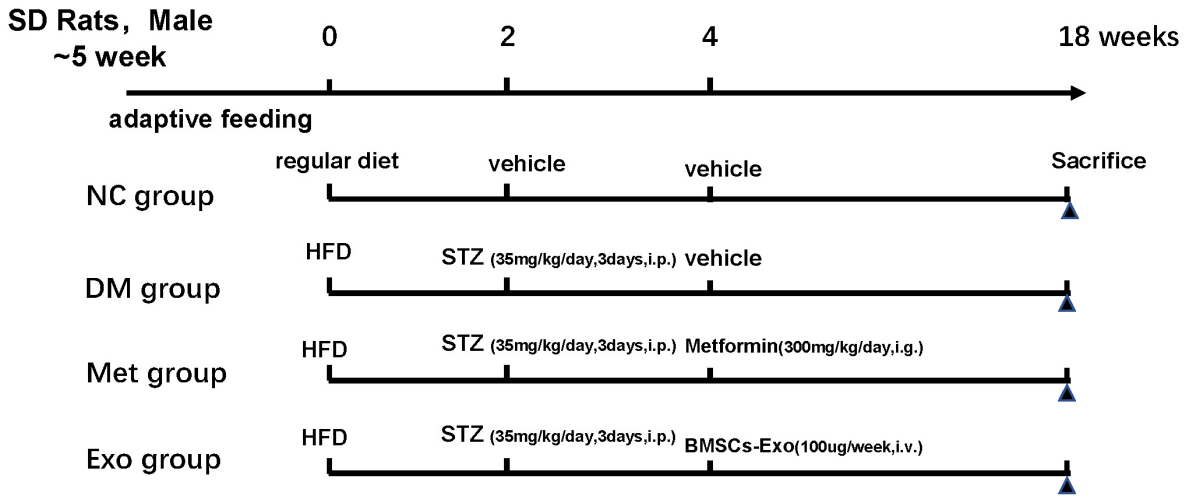

Fig. 1. Preparation of rat diabetes model and group administration. HFD, high-fat diet; STZ, streptozotocin.

2500 rpm for 10 min, and the supernatant was aspirated. We used an Indiko automatic biochemical analyzer (Thermo Scientific, CA, USA) to measure GLU, BUN, and Cr concentration.

2.5 Kidney Pathological Section Staining and Immunohistochemistry

The kidneys fixed in 4% paraformaldehyde were clarified, dehydrated, embedded in paraffin, and cut into micron-thick sections. Sections were stained with hematoxylin-eosin (H&E) and Masson and Periodic Acid-Schiff (PAS) staining to assess the severity of diabetic kidney disease. Type IV collagen immunohistochemical staining was performed on the remaining sections. The sections were first incubated with Collagen IV polyclonal antibodies (1:200, Bioss, Beijing, China) at 4 °C overnight, washed with PBS for 5 min 3 times, and then incubated with biotin-labeled secondary antibodies for 1 hour at room temperature. The stained sections were scanned with an Aperio Scan Scope AT Turbo scanner (Leica, Wetzlar, Germany) to obtain images, and Image Scope software was used to analyze glomeruli morphology, renal tubules, and collagen deposition. For each kidney tissue, we selected three different slices and five fields of view in each slice. Twenty glomeruli were randomly selected from each kidney to assess the lesion.

2.6 Kidney TUNEL Staining

Paraffin sections of left rat kidneys were stained with a TUNEL staining kit (Roche, Basel, Switzerland) to identify apoptotic cells in kidney tissue via fluorescein-dUTP labeling of DNA fragmented strands. Images were acquired using an Eclipse NI microscope (Nikon, Tokyo, Japan).

2.7 qRT-PCR of Kidney Tissue

Total RNA was extracted from right kidney tissue frozen in liquid nitrogen using TRIzol (Thermo Fisher Scientific, MA, USA). The Revertaid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA) was used for reverse transcription, and cDNA amplification was carried out using an SYBR Green Master Mix Kit (Takara, Otsu, Japan). Relative mRNA levels were calculated using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard. All primers were synthesized by Sangon Biotech and are listed in Table 1.

2.8 Western Blot

The right kidney tissue was homogenized with RIPA and centrifuged to obtain total tissue protein. The protein molecules were separated with 12.5% SDS PAGE gel electrophoresis, and the protein was transferred to a 0.22 μm PVDF membrane by a wet transfer method. A rapid blocking solution (Servicebio, Wuhan, China) was used for blocking at room temperature for 1 hour. PVDF membrane and rabbit-derived primary antibodies against against Bax (1:1000, Cell Signaling Technology, Boston, MA, USA), Caspase 9 (1:1000, CST, MA, USA), Caspase 3 (1:1000, CST, MA, USA), NF-κB (p65) (1:1000, Abcam, Cambriage, UK), GAPDH (1:5000, Proteintech, Wuhan, China) were placed in horizontal shaking incubators overnight at 4 °C. After rinsing with TBST (5 min, 3 times), they were incubated with fluorescein or Horseradish peroxidase-labeled secondary antibodies for 1 hour at room temperature. We used X-ray film to develop bands. X-ray films were digitally scanned and analyzed quantitatively using the Image J software (V1.8.0, National Institutes of Health, Bethesda, MD, USA). Three rat kidneys in each group were randomly selected for protein extraction; each sample was repeated three times, and a representative band was selected for analysis.

2.9 Statistical Analysis

Data are presented as means ± standard deviations (SD). Statistical analyses were performed using Prism

Table 1. Primer sequences in the qRT-PCR assay.

Primer sequences		Probes
TNF-α	Forward primer	5′-AAACACACGAGACGCTGAAG-3′
	Reverse primer	5′-ATCCAGTGAGTTCCGAAAGC-3′
GAPDH	Forward primer	5′-CAACTCCCTCAAGATTGTCAGCAA-3′
	Reverse primer	5′-GGCATGGACTGTGGTCATGA-3′

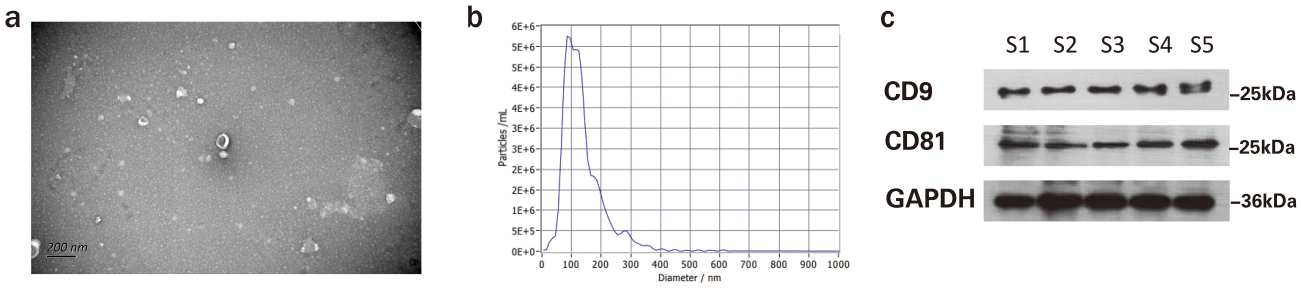

Fig. 2. Identification of BMSCs-Exo. (a) Transmission electron microscopy (TEM) shows that BMSCs-Exo have a "sauce-like" structure. (b) Analysis of BMSCs-Exo Particle Size Distribution by Nanoparticle Tracking Technology (NTA). (c) Exosome markers (D63, and CD81) are detected by Western Blot (S1-S5, 5 batches of samples).

9.0.0/121 software (San Diego, CA, USA). Significant differences between the two groups were determined using one-way ANOVA (Dunnett's *t* tests). For all comparisons, the other groups were compared with the data presented in the model group. p values less than 0.05 were considered to be statistically significant.

3. Results

3.1 Identification of Rat BMSC-Exo

TEM images revealed that BMSC-Exo had a typical saucer-like structure (Fig. 2a). NTA showed that the average particle size of the particles in the sample was 136.5 $\pm$ 68 nm, that the main particle size peak was located at 102.9 nm, and that the main peak accounted for 97.4%, which was in line with the exosome particle size distribution characteristics (Fig. 2b). Western blot detection of exosome surface marker molecules showed that the obtained samples were positive for CD63 and CD81 (Fig. 2c).

3.2 BMSC-Exo Reduces Plasma Glucose, BUN, and Cr Levels in Diabetic Rats

GLU, BUN, and Cr were notably increased in the DM group compared with the NC, Met, or Exo groups ($p < 0.01$). Treatment with Met significantly decreased these parameters ($p < 0.05$). Exo appeared to show a stronger effect than Met, but there was no statistically significant difference between the two groups ($p > 0.05$; Fig. 3, **Supplementary Table 1**).

3.3 BMSCs-Exo Alleviated Diabetic Rat Kidneys Fibrosis

To evaluate the pathological changes of kidney damage in diabetic rats, we performed PAS staining and Masson staining in addition to conventional H&E staining and detected the synthesis and secretion of collagen IV (the most important matrix collagen in diabetic kidney disease) with immunohistochemical techniques. PAS staining showed that, compared with the NC group, the glomerular basement membrane of the DM group was homogeneously thickened, the mesangial matrix was increased, and there were no Kimmelstiel-Wilson (K-W) nodules with obvious arteriolar hyalinization. The basement membrane in the renal tubular region was markedly thickened (Fig. 4a). Masson staining showed that the glomerular basement membrane, mesangial matrix, surrounding tubules, and surrounding blood vessels were stained blue in the DM group, indicating that there was obvious collagen deposition in the kidney (a manifestation of renal fibrosis; Fig. 4b). Immunohistochemistry for collagen IV showed that collagen IV in the DM group was slightly distributed in the glomerular mesangial matrix, but was mainly in the tubular cells and interstitium and that the staining was more obvious than it was in the NC group (Fig. 4c). The above-mentioned manifestations were significantly alleviated after the administration of BMSCs-Exo and metformin. Compared with the Met group, the renal pathological manifestations of the BMSCs-Exo group were lighter.

3.4 BMSC-Exo Attenuates Renal Cell Apoptosis in Diabetic Rats

To evaluate the renal cell apoptosis in diabetic rats, we performed TUNEL staining on the pathological sections of rat kidneys. The results showed that the DM group had more severe apoptosis than the NC group. Administration of Met significantly reduced apoptosis, and fewer apoptotic cells were detected with Exo than with Met (Fig. 5a). The pro-apoptosis protein Bax was detected by western blot, and

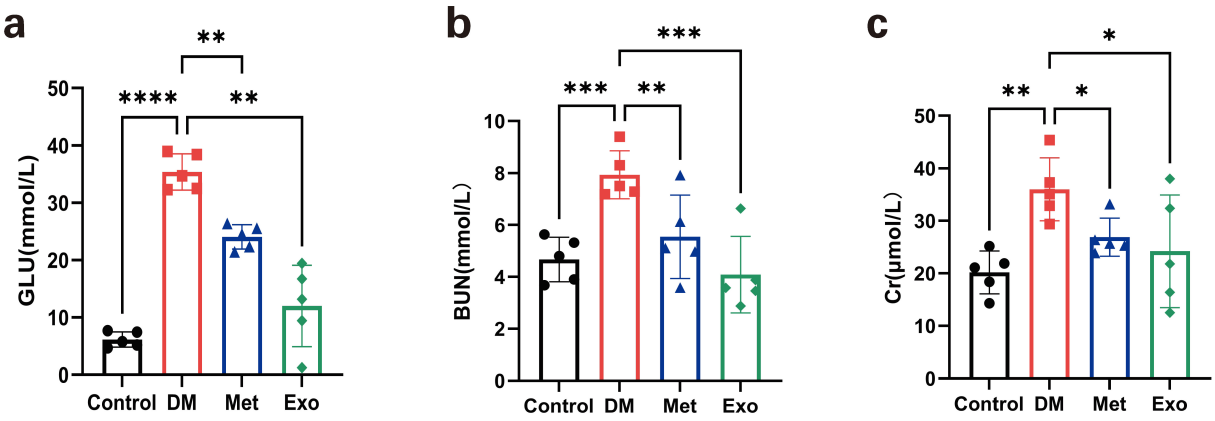

Fig. 3. BMSCs-Exo reduces blood glucose and improves renal function in diabetic rats. (a) Rats grouping and dosing schedule. (b,c) Comparison of GLU, BUN, and Cr in the NC group, DM group, Met group, and Exo group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

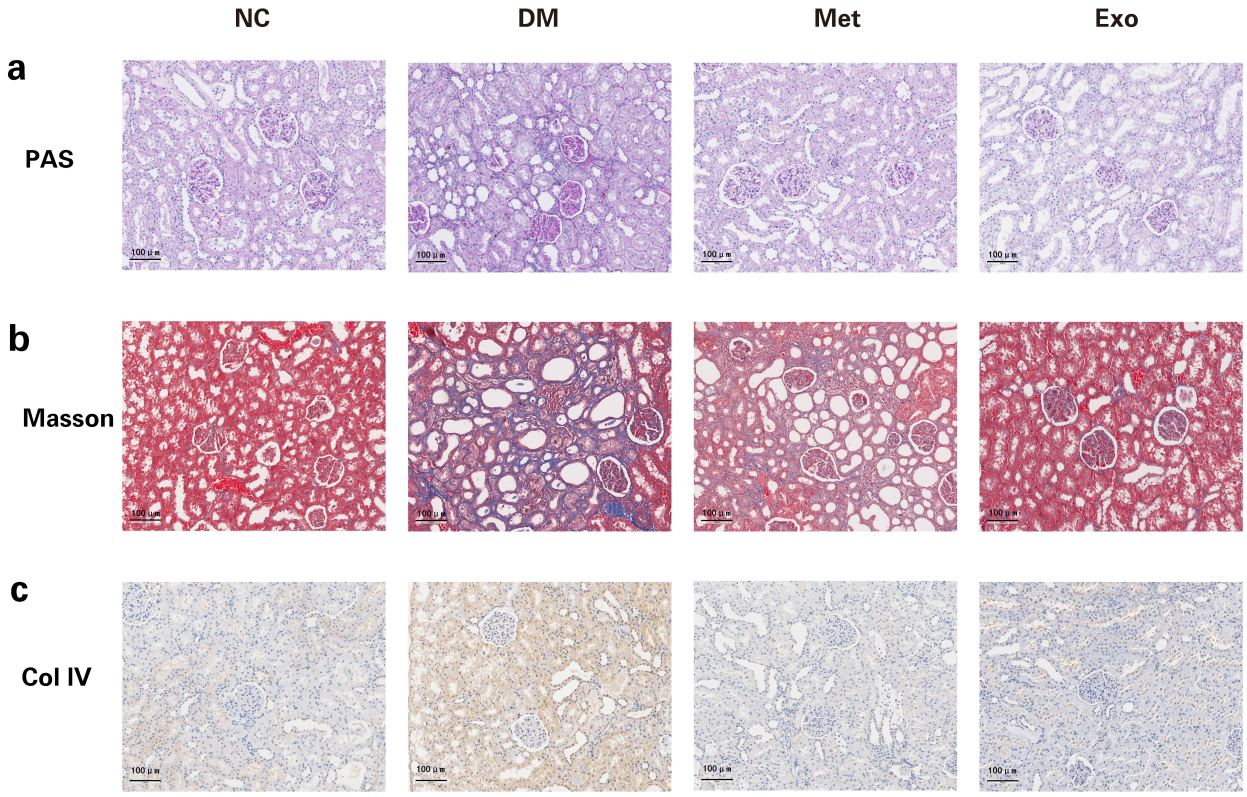

Fig. 4. BMSC-Exo alleviated diabetic kidney disease in the Exo group. (a) PAS staining of rat renal tissues (200×). (b) Masson staining of rat renal tissues (200×). (c) Immunohistochemical techniques used to detect collagen IV in kidney tissue (200×).

the results corresponded with TUNEL staining. The relative expression of Bax in the rat kidneys in the DM group was significantly elevated compared with those in the NC group ($p < 0.01$). Additionally, the gray value was significantly decreased in the Exo group (Exo vs. DM, $p < 0.05$) compared with the DM group. There was no statistical difference between the Met goup and the DM goup ($p < 0.05$). We also detected the expression levels of apoptosis-executing proteins Cleaved caspase 9 and Cleaved caspase 3, and showed that the expression level of Cleaved caspase 9 and Cleaved caspase 3 representing the activated form of Caspase, was significantly increased in the DM group compared with the NC group ($p < 0.05$). Additionally, the expressions of both the Exo group and Met group were significantly lower than those in the DM group (Exo vs. DM $p < 0.01$, Met vs. DM $p < 0.01$). There was also no statistical difference between the Met group and the Exo group ($p > 0.05$) (Fig. 5b).

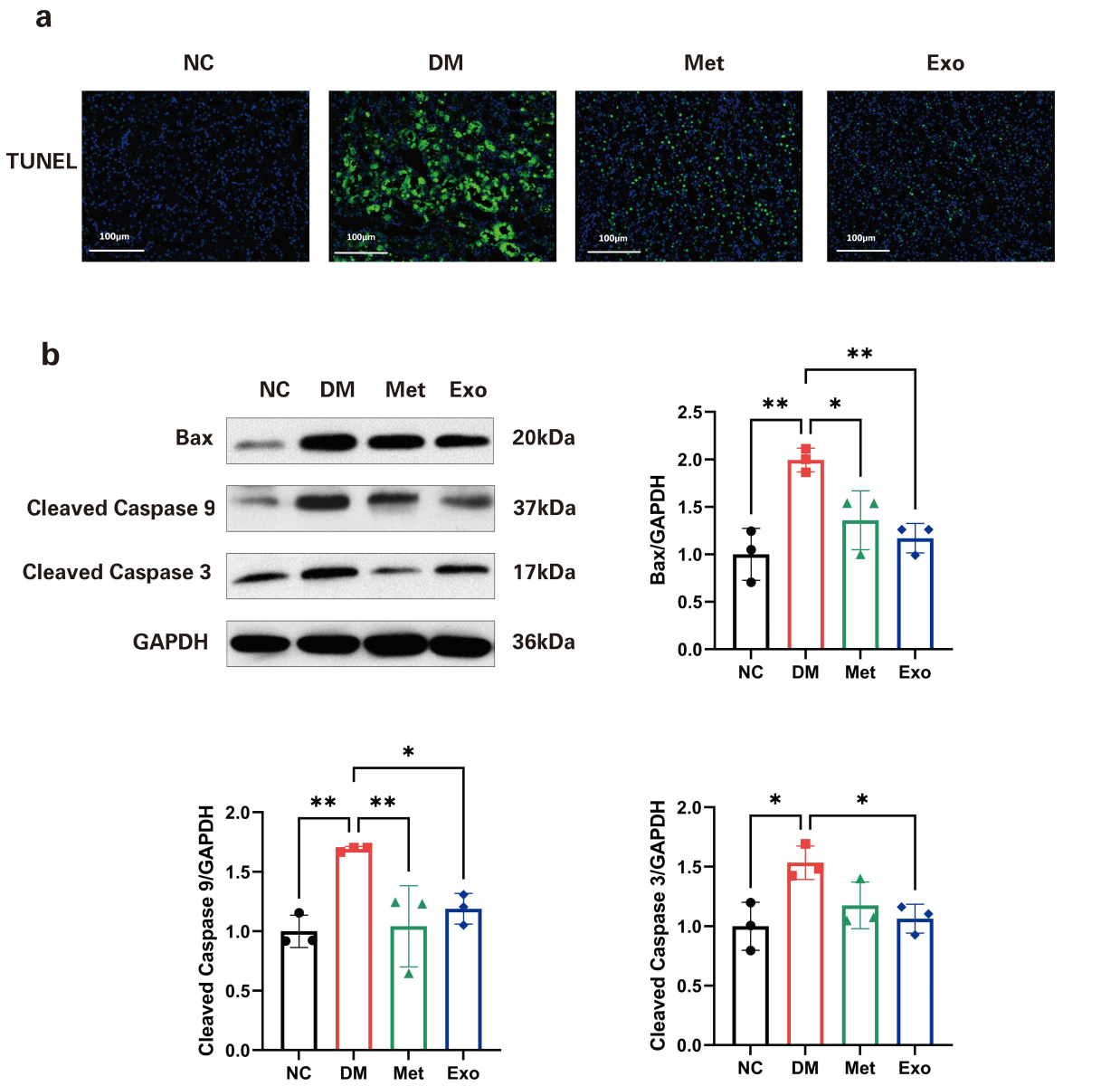

Fig. 5. BMSCs-Exo reduced apoptosis in renal tissue. (a) TUNEL staining of rat renal tissues (200×). (b) Protein expression of Bax, Cleaved Caspase 9, Cleaved Caspase 3 detected by western blot. The relative expression of Bax, Cleaved Caspase 9 and Cleaved Caspase 3 were calculated. *$p < 0.05$, **$p < 0.01$.

3.5 BMSCs-Exo Attenuates Renal Inflammation in Diabetic Rats

To observe the renal inflammatory state of diabetic rats and the interventional effects of Exo, we observed the distribution of inflammatory cells in kidney tissue using H&E staining, detected the transcription level of pro-inflammatory factor TNF-α in kidney tissue using qRT-PCR, and detected the NF-κB (p65) expression levels in kidney tissue with western blots. H&E results showed that a large number of inflammatory cells infiltrated the kidney tissue of rats in the DM group, and the distribution of inflammatory cells in the Exo and Met groups was significantly reduced (Fig. 6a). The TNF-α transcription level was significantly increased in the DM group compared with the NC group ($p < 0.01$). Compared with the DM group, the Exo group showed significant decreases ($p < 0.05$), but the Met group had no significant differences compared with the DM group ($p > 0.05$) (Fig. 6b). Finally, the expression level of NF-κB was significantly increased in the DM group compared with the NC group ($p < 0.005$), the Met group ($p < 0.05$), and the Exo group ($p < 0.05$) compared with the DM group. There was no statistically significant difference between the two groups (Fig. 6c,d).

4. Discussion

This study found that exosomes derived from BMSCs reduced GLU, BUN, and Cr levels in diabetic rats, and also reduced glomerulosclerosis and collagen deposition in di-

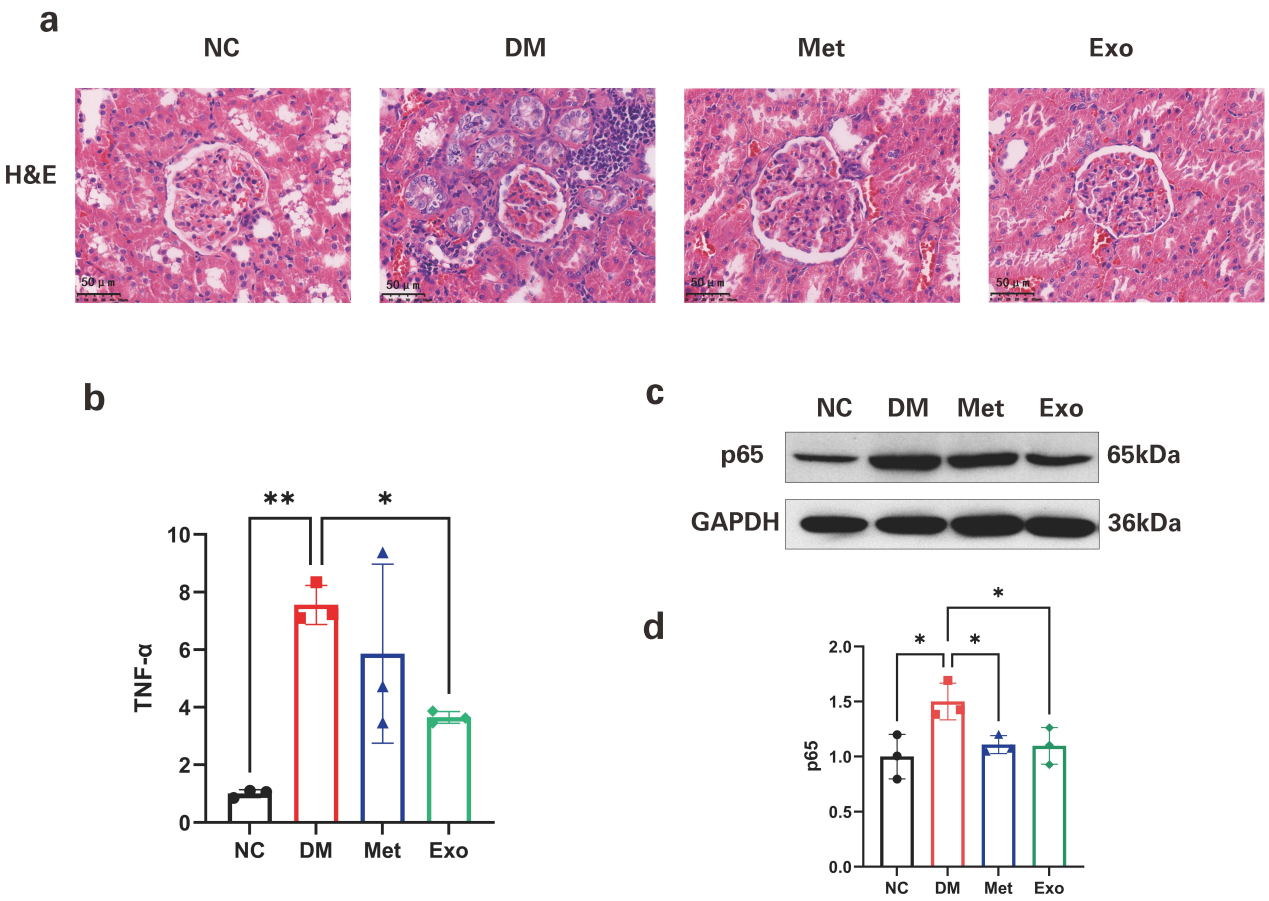

Fig. 6. BMSCs-Exo attenuates kidney inflammation in diabetic rats. (a) H&E staining showed the rat kidney tissue structure and inflammatory cell infiltration (400×). (b) Detection of TNF-α transcription levels in rat kidney tissue using qRT-PCR. (c) Western blot detection of the expression of inflammatory signaling molecule NF-κB (p65) in rat kidney tissue. (d) Quantitative analysis of the gray value of western blot bands. *$p < 0.05$, **$p < 0.01$.

abetic rats. Furthermore, we found that the inflammation and apoptosis in the kidney tissue of diabetic rats were significantly aggravated, and the BMSC-Exo could reduce the infiltration of inflammatory cells, the release of inflammatory factors, and apoptosis in the kidney tissue, and regulate apoptosis-related genes, apoptosis-related proteins, and inflammatory signaling pathways, thus improving renal function and alleviating renal damage in diabetic rats. This experiment provides laboratory evidence for the treatment of diabetic kidney disease BMSC-derived exosomes.

Obtaining reliable exosomes is the first step in this experiment. We tested the exosomes isolated from the supernatant of BMSCs in terms of three aspects: morphology, particle size analysis, and surface molecules. The characteristics are in line with Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines for the field, issued by the International Society for Extracellular Vesicles in 2018 (ISEV) [18]. This provides support for the reliability of the experimental results.

We observed that BMSC-Exo had a hypoglycemic effect that was not inferior to metformin and also had a reversal effect on serum BUN and Cr, which reflect glomerular function. Hyperglycemia is associated with the pathological progression of diabetic kidney disease and can lead to increased glycation end products (AGEs), which are the major causes of hyperglycemic kidney damage [19]. *In vivo*, AGEs can activate the local renin-angiotensin aldosterone system (RAAS) in kidney tissue, stimulate endothelial cells to release vasoactive substances, and dilate afferent arterioles, resulting in increased glomerular filtration pressures and an imbalance between the bulbs and tubes and causing disturbance of renal vascular hemodynamics. This series of processes eventually lead to a decrease in the glomerular filtration rate, which manifests in renal dysfunction [20,21]. BMSC-Exo improved renal function while lowering blood sugar. This dual-therapeutic effect could be related to the above-mentioned mechanisms. In terms of the hypoglycemic mechanism, several studies have shown that BMSC-Exo can chemoattract pancreatic tissue through the pancreas and duodenal homeobox1 pathway and promote the regeneration of pancreatic β cells [22]. BMSC-Exo can also upregulate the levels of regulatory T cells, interleukin (IL)-4, IL-10, and transforming growth factor beta (TGF-β), while downregulating IL-17 and interferon-

gamma, which ultimately improves autoimmune responses and islet regeneration [23,24]. Su *et al*. [25] demonstrated that miR-29b-3p carried by BMSC-Exo can regulate age-related insulin resistance. Together, these results suggest that BMSC-Exo exerts hypoglycemic effect by protecting pancreatic β cells, promoting insulin secretion, and reducing insulin resistance.

At the tissue level, rats in the model group only showed early manifestations of diabetic kidney disease (for example, no typical K-W nodular structure), and some studies have shown that standard diabetic animal models induced with low-dose STZ can only reflect mild renal damage [26]. It is worth noting that we observed that BMSC-Exo had significant effects on pathological manifestations known to be related to diabetic nephropathies, such as glomerular basement membrane thickening, mesangial matrix expansion, tubular basement membrane thickening, inflammatory cell infiltration, and matrix collagen deposition. Nagaishi *et al*. [27] have observed that BMSC-Exo can alleviate the renal pathological damage caused by diabetes in HFD and STZ-induced mouse models of diabetic kidney disease, which is consistent with what we observed in STZ combined with HFD-induced diabetic rat models.

Furthermore, our TUNEL results showed that the apoptosis rate of renal cells in diabetic rats was significantly increased but that these effects were alleviated by BMSC-Exo. Previous studies have confirmed that diabetes leads to increased renal AGEs and local metabolic disorders, and induces glomerular podocyte apoptosis, resulting in proteinuria [28]. Diabetic kidney disease is also accompanied by apoptosis of proximal convoluted tubule epithelial cells, resulting in renal tubular atrophy and thus a lack of connection between the glomeruli and proximal convoluted tubules [29–31]. BMSC-Exo induces autophagy through the mTOR signaling pathway to alleviate fibrosis in diabetic kidneys. The inhibition of autophagy is an inducing factor for apoptosis, suggesting that the observed BMSC-Exo-mediated reduction of apoptosis in diabetic kidneys may be achieved by inducing autophagy [32]. In diabetic kidney disease, apoptosis may result from an imbalance in the interaction between the pro-apoptotic Bax and anti-apoptotic Bcl-2 family members [33]. We also observed that Bax protein increased in diabetic rats but decreased after BMSC-Exo treatment. Caspases also play a key role in apoptotic programs [34]. We also observed that BMSC-Exo regulated the activation of initiator caspase 9 and executioner caspase 3. Thus, BMSC-Exo may improve diabetic kidney disease by regulating several stages of renal cell apoptosis.

We also observed that BMSC-Exo attenuated the inflammatory state in diabetic kidney disease kidneys. BMSC-Exo reduced inflammatory cell infiltration in renal tissue. However, only TNF-α was elevated in diabetic kidney tissue and was offset by BMSC-Exo, which is consistent with prior findings by Fan *et al*. [16]. In contrast to the inflammatory factors IL-1β and IL-6, TNF-α is mainly produced by activated macrophages [35], which indicates that the inflammatory state of diabetic kidney disease may be closely related to macrophage activation. However, why pro-inflammatory factors such as IL-1β, which are also produced by activated macrophages, are not elevated deserves further investigation. TNF-α is an activator of the canonical NF-κB pathway, which regulates the transcription of inflammation-related genes [36]. Our study found that BMSC-Exo could also reduce the expression of NF-κB in diabetic kidney tissue, suggesting that BMSC-Exo may exert a protective effect via the macrophage-TNFα-NF-κB pathway.

Our study did not verify or further explore the mechanism of action of BMSC-Exo *in vitro*. In addition, exosome contents contain proteins, lipids, and various nucleic acid molecules. Many of these key molecules need to be further screened to identify new molecular targets for the treatment of diabetic kidney disease. Besides, whether metformin and BMSC-Exo have a synergistic effect on the treatment of diabetes and related kidney damage deserves further exploration.

In summary, the proposed mechanism of BMSC-Exo in the treatment of DKD has been shown in the graphical abstract. Because DKD is caused by a combination of factors, the pathogenesis is complex. The hypoglycemic, anti-inflammatory, and anti-apoptotic pleiotropic effects of BMSCs-Exo may provide a potential new therapeutic approach for the treatment of diabetic kidney disease.

5. Conclusions

BMSCs-Exo ameliorates renal pathological damage and improve renal function in diabetic rats by reducing apoptosis and reducing inflammation.

Availability of Data and Materials

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

Author Contributions

JT, CG and XS designed the research study. LL and YZ performed the research. XZ, XY, HZ, and XW were involved in the animal feeding, model construction, and sample collection processes. ZA analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Animal Experiment Center of Beijing Anzhen Hospital ethics committee at Capital Medical University (approval No. 2022152X), which complies with the guide for the care and use of laboratory animals (eighth edition).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2809203.

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