ORIGINAL RESEARCH

Inhibition of Autophagy in Renal Cells With Human, Urine-derived, Stem-cell Exosomes to Improve Diabetic Nephropathy

Han Nie, MD; Lilan Zhou, MD; Renwei Xu, MD; Jianxin Yu, BD

ABSTRACT

Context • Clinicians can use stem cells to repair kidney injury. The kidneys' exosome secretions hold the secret to this therapeutic impact. Exosomes from urine-derived stem cells can prevent and treat glomerular damage that diabetes can cause, but the underlying process has remained a mystery.

Objective • The study aimed to investigate the protective impact of exosomes from urine-derived stem cells (USCs) against diabetic nephropathy (DN) and to determine the mechanisms involved.

Design • The research team performed an animal study.

Setting • The study took place at the Affiliated Hospital of Jiujiang University in Jiujiang, Jiangxi, China.

Animals • The animals were rats, SD male rats, weighing 200-220g, 40 animals, purchased from Weitong Lihua Experimental Animal Technology Co., Ltd. (certificate number: SCXK (Beijing) 2021-0006).

Intervention • Except for a control group, the rats in the groups had induced DN. The five groups, with 10 rats each, were: (1) the negative control group, which received 0.2 ml of PBS solution; (2) the DN group, a second negative control group, which received 0.2 ml of PBS solution, (3) the inhibitor group, an intervention group that received 20 mg/kg of autophagy inhibitor; (4) the exosomes group, an intervention group that received 100 ug/kg of exosomes; and (5) the exosomes + inhibitor group, an intervention group that received 100 ug/kg of exosomes + 20 mg/kg of autophagy inhibitor. From week 8, for four weeks the team injected the inhibitor, exosomes, and exosomes + inhibitor groups with the appropriate treatments using the rats' tail veins.

Outcome Measures • The research team: (1) examined the USCs in the exosomes of stem cells; (2) assessed the rats' weights and fasting blood glucose (FBG), using a blood glucose meter; (3) used Coomassie brilliant blue (CBB) staining to determine the amount of protein in the rats' urine and assessed their biochemical indexes; and (4) used Western blot (WB) and a quantitative polymerase chain reaction (Q-PCR) to detect autophagy and the signal transduction pathway.

Results • Human exosomes from USCs alleviated injury in the rats that DN caused by reducing urinary-protein levels, serum creatinine (SCR), blood urea nitrogen (BUN), glomerular cell accumulation, and kidney weights. In rats with induced DN, the exosomes + inhibitor significantly reduced the activation of the mTOR signaling pathway, reduced the autophagy of their kidney cells, increased the protein expression of Bcl-2 in the kidney tissues, and lessened the damage to glomerular cells.

Conclusions • Human urine-derived stem cell exosomes can significantly reduce the activation of the mTOR signaling pathway, reduce the autophagy of rats' kidney cells, increase the protein expression of LC3B in kidney tissues, and reduce the damage to glomerular cells. By blocking the mTOR signaling pathway, human urogenic exosomes can alleviate the signs and symptoms of DN. (*Altern Ther Health Med.* 2023;29(8):545-551).

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End-stage renal disease (ESRD) mostly occurs due to diabetic nephropathy (DN), a major consequence of diabetes mellitus (DM).[1] DN is a significant causal factor in pathological renal alterations. Changes in the various functions and outcomes of the kidney occur throughout DN's pathogenesis, and these comprise renal-tissue fibrosis, tubulointerstitial fibrosis, mesangial cell growth, oxidative stress, hemodynamic alterations, glomerular hypertrophy, glomerulosclerosis.[2,3]

Although medical practitioners have thought that regulating blood pressure and blood-glucose levels can aid in slowing the onset of DN, Dong et al found that these actions aren't enough to do so.[4] Current treatments often fail to target the major underlying contributors to the progression of renal disease. Chronic glomerular and tubulointerstitial fibrosis is often associated with apoptosis, oxidative damage, and

microvascular rarefaction. In fact, Ullah et al postulated that renal dysfunction better correlates with the degree of tubulointerstitial than with glomerular damage.[5]

Most current treatments target the metabolic and hemodynamic changes that diabetes causes, which can alleviate the disease's progression to a certain extent but isn't sufficient to reverse the development of DN.[6]

Autophagy

Pisitkun et al found that autophagy is a crucial component in the treatment of DN.[7] Changes in autophagy activity are essential for enhancing and regaining renal function because impaired autophagy in renal cells results in glomerular cell lesions.

Podocytes and tubular cells in particular exhibit impaired autophagy, which researchers think is a common etiology of many kidney disorders, including DN.[8] Activation of the mammalian target of rapamycin (mTOR) signaling pathway may be the specific mechanism underpinning this. Gonzales et al demonstrated stimulation of the mTOR signaling complex in type 1 and type 2 DN in both animal and human studies.[9]

Grewal et al indicated that the enhancement and restoration of the autophagy function can provide a new therapeutic target for DN.[10] Injecting mesenchymal, stem-cell-derived exosomes into the tail veins of type 1 diabetic rats led Deng X et al to discover that restoring autophagy function and blocking the mTOR signaling pathway could reduce tissue damage in diabetic kidneys, safeguard renal function, and prevent renal-tissue fibrosis.[11]

Stem Cells

In DN management, stem-cell therapy is straightforward, efficient, and secure, and physicians can use it to successfully halt the progression of DN.[12] Stem cells secrete many nutrients through their exosomes, which the body can then use to rapidly propagate stem cells to grow into mature functional cells and to promote the expansion and proliferation of other nearby functional cells.

While stem cells can self-renew and differentiate as regular cells do, they can't cause tumors. Using stem-cell therapy to treat DN is a relatively safe and effective way in the clinic at present and can effectively control the development of DN and delay its deterioration.[13] Grewal et al and Deng et al found that stem-cell therapy was safe and feasible for renal injury.[10,11]

Some studies have found that stem cells can dramatically improve kidney function and play a role in kidney repair, and clinicians view stem-cell therapy for DN as a potential treatment. For example, Zhu et al confirmed that stem cells can exhibit good renal-repair ability in kidney injury due to ischemia-reperfusion, kidney transplantation, and other forms of kidney damage.[14] A stem-cell transplant into the kidney can successfully restore renal function, reduce levels of the urine proteins serum creatinine (Scr) and blood urea nitrogen (BUN), repair renal fibrosis, and reduce inflammation. The question remains as to whether they also have good repair ability in the context of kidney injury due to diabetes.

Exosomes

Yeagy and Cherqui found that the mechanism for intercellular signal transmission involves exosomes generated from cells.[15] Mesenchymal stem cells can produce numerous tissue cells that can differentiate in multiple directions.[16] Their therapeutic effects mainly depend on the factors they release, including the exocrine body.[17]

Urine protein is an important manifestation of kidney damage. When the urine protein in the body increases, the glomerular effective rate decreases, and the urine volume consequently increases.[18] The exocrine body of human urinary stem cells can promote the development, reproduction, evolution, and recovery of kidney cells, help recover damaged glomerular cells, increase the glomerular filtration rate, reduce urine output, and thus ultimately reduce the production of urine protein.

Clinicians can use the nutrients that the exosomes from stem cells secrete to treat disease. Stem-cell exosomes from human urine are more similar and comparable to other stem cell exosomes than other exosome types. Exosomes from urinary stem cells are more extensively available and can create new fields of medical research due to their low rejection rates, high differentiation potential, and sufficient sources, thereby laying a solid foundation for their future clinical use.

DN Induction

The streptozotocin (STZ)-induced diabetic model is well-established and commonly used to induce DN. Ebrahim et al created a rat model of type 1 DN using a single injection of high-doses STZ and found that a subsequent intravenous injection of mesenchymal stem cells could reduce blood sugar and urine protein and maintain the normal structure and function of the kidneys of rats with DN.[19]

Current Study

The study aimed to investigate the protective impact of exosomes from urine-derived stem cells against diabetic nephropathy (DN) and to determine the mechanisms involved.

METHODS

Animals

The research team performed an animal study, which took place at the Affiliated Hospital of Jiujiang University in Jiujiang, Jiangxi, China. The animals were SD male rats, weighing 200-220 g, 60 animals, purchased from Weitong Lihua Experimental Animal Technology Co., Ltd. (certificate number: SCXK (Beijing) 2021-0006). For a week prior to induction of DB, all rats were fed in an adaptable manner.

The institutional review board (IRB) of the Affiliated Hospital of Jiujiang University approved the study's protocols for conducting experiments on animals.

Procedures

Materials and equipment. The research team purchased: (1) a low temperature ultracentrifuge (Xiangyi L535R, Hunan Xiangyi Laboratory Instrument Development Co., Ltd; Changsha City; Hunan Province; China); (2) an inverted microscope (OLYMPUS CKX41, OLYMPUS, Tokyo, Japan); (3) a fluorescent quantitative PCR instrument (BIO-RAD1855200); (4) Dulbecco's Modified Eagle Medium (DMEM) basic medium (A4192101, Gibco); (5) fetal bovine serum (10099, Gibco); (6) a reverse transcription polymerase chain reaction (RT-PCR) detection kit (RP1100, Solarbio Life Science, Beijing, China); (7) a bicinchoninic acid (BCA) kit (article No. PC0020, Solarbio); (8) phosphate-buffered saline (PBS) from P1020; Solarbio Life Science, Beijing, China); (9) a 5% CO2 incubator from (3121, Thermo, Waltham, Massachusetts, USA); (10) autophagy inhibitor (MCE, New Jersey, USA,); (11) Coomassie brilliant blue (CBB) stain (Nanjing built C035-2-1; manufacturer, city, state, country); (12) automatic blood biochemical instrument (SMT-120VP, Smart, Chengdu, Sichuan, China); TRIzol reagent (15596018; Invitrogen, Waltham; Massachusetts; USA); protein-expression kits for detection of p-mTOR, ab109268; autophagy-related 5 (Atg5), ab221604; light chain 3 beta (LC3B), ab222776, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), b8227, in renal tissues (all Abcam, Cambridge, UK)

Extraction and identification of stem cells and exosomes (Exo). The research team recruited healthy human volunteers to obtain fresh, midstream urine and used adherence screening to evaluate the urinary stem cells (USCs). The team: (1) drew the urine out of sterile, closed vessels and performed centrifugation at 400 g for 10 min; (2) then added 20 ml of PBS and discarded the supernatant; (3) after resuspension and sedimentation, centrifuged the resulting solution at 400 g for 10 min; (4) added 5 ml of DMEM medium into each tube and cultured it in the CO_2 incubator at 37°C; (5) observed cell adhesion after 5-7 days; (6) discarded the cells that weren't attached to the wall; (7) performed screening on the resulting generation of P0 USCs; and (8) with the help of flow cytometry, used third-generation USCs to identify the unique surface markers of the urine-derived stem cells.

The team: (1) collected the extracellular bodies of the human USCs (USC Exo) using ultracentrifugation; (2) then collected the third generation of USCs; (3) completed three PBS washes, (4) applied 0.25% trypsin for digestion, (5) centrifuged 2000 g of the sample at 4°C for 20 minutes; (6) passed the supernatant through a 0.22-m filter membrane and ultracentrifuged 100 000 g at 4°C for 90 min to remove the cell fragments, (7) then produced USCs Exo precipitate; and (8) removed the supernatant and added PBS to dissolve the precipitate.

Induction of DN. The research team randomly chose 60 rats to induce DN, gave them a single intraperitoneal injection of 60 mg/kg of 1% STZ solution that the team had made the previous night. The team also randomly chose 10 rats as the control group. After the model was successfully established, 10 mice were selected for each group, and the unqualified mice were eliminated, the research team determined that 40 of the 60 rats had induced DN.

Groups. Except for a control group, the rats in the groups had induced DN. The five groups, with 10 rats each, were: (1) the negative control group, which received 0.2 ml of PBS solution; (2) the DN group, a second negative control group, which received 0.2 ml of PBS solution, (3) the inhibitor group, an intervention group that received 20 mg/kg of autophagy inhibitor; (4) the exosomes group, an intervention group that received 100 ug/kg of exosomes; and (5) the exosomes + inhibitor group, an intervention group that received 100 ug/kg of exosomes + 20 mg/kg of autophagy inhibitor. From week 8, for four weeks the team injected the inhibitor, exosomes, and exosomes + inhibitor groups with the appropriate treatments using the rats' tail veins.

Blood-glucose levels. The team measured the fasting blood glucose (FBG) for the groups with induced DN, using blood-glucose test sheets. The team drew the blood from the tip of the rats' tails at 72 hours after the STZ injection and again on the seventh day after it. The two blood-glucose checks yielded average readings and both were higher than 16.7 mmol/l.

At week 8, the team collected the urine of the rats over a period of 24 h. The collected urine volume was 50% greater than the urine volume that the research team had collected at baseline, and the average 24-hour urine-protein level was greater than 30 mg/24-hour. These results indicated successful construction of the type 1 diabetic model.

Urine volume, blood glucose, and protein levels. The research team: (1) collected blood samples from the tips of the tails of the five groups at the same time each week to gauge FBG levels; (2) kept track of the rats' weights; and (3) measured the rats' 24-hour urine-output volume and urine-protein concentration.

Postintervention, the team: (1) kept the rats in a metabolic cage for adaptive feeding (Figure 3) for one day, after which they underwent a 24-hour fast without water; (2) collected their 24-h output of urine and recorded its volume; (3) after 12 additional weeks of feeding, had the rats fast for 12 hours; (4) after measuring body weights and testing blood glucose, put the rats to sleep with an intraperitoneal injection of 10% chloral hydrate; (5) took blood from their abdominal aortas; and (6) weighed the left and right kidneys and put the left kidney's tissues into tissue fixative for subsequent use while quickly freezing the right kidneys in liquid nitrogen for further use.

Urine output. For 24-hour urine output, the research team collected the urine, spun it for 10 minutes at 2000 rpm, and used CBB staining to test the supernatant for urine protein.

Blood biochemical indexes. The research team measured the biochemical indexes in the serum of the rats using the biochemical instrument.

Renal morphology and function. The research team: (1) after removing the left and right kidneys from the rats after their deaths, quickly flushed them until no blood residue was left; (2) after drying the kidneys with absorbent paper, weighed

both kidneys using an electronic balance and calculated the kidney index according to the formula: kidney index = sum of bilateral kidney weights (g)/body weight (g) × 100%.

The team: (1) took the left kidney out of the tissue fixative and dewaxed, hydrated, and embedded it in paraffin, cut it into 4-m posterior slices, and finally stained it with hematoxylin and eosin (HE) and periodic acid–Schiff (PAS); (2) after staining, comprehensively evaluated the renal morphology, glomerular lesions, and tubular lesions of the rats in all groups; (3) using a high-power microscope, randomly selected 30 nonrepetitive visual fields for each rat to identify the extracellular matrix (ECM) index.

RT-PCR. The research team: (1) isolated total RNA from kidney tissue using the TRIzol reagent, as directed by the kit's manufacturer, and produced cDNA using reverse transcription; (2) used the RT-PCR kit to determine the relative expression levels of mTOR, "5' adenosine monophosphate (AMP)-activated protein kinase" (AMPK), B-cell lymphoma 2 (Bcl-2), and protein kinase B (AKT) in renal tissues.

The internal reference was GAPDH. The team managed the reaction system and its surroundings in accordance with the guidelines and determined the level of relative mRNA expression using 2-ΔΔct.

Western blot (WB). The research team isolated the total protein from kidney tissues using the BCA technique, as per the instructions provided by the kit's manufacturer. The sample was 20 μg/well concentrated. The first antibody was rabbit anti-mouse, and the second was sheep anti-rabbit. The team detected the protein expressions of p-mTOR; Atg5, and LC3B in renal tissues. The team used GAPDH as the internal reference protein.

Outcome Measures: The research team: (1) examined the USCs in the exosomes of stem cells; (2) assessed the rats' weights and fasting blood glucose (FBG), using a blood glucose meter; (3) used Coomassie brilliant blue (CBB) staining to determine the amount of protein in the rats' urine and assessed their biochemical indexes; and (4) used Western blot (WB) and a quantitative polymerase chain reaction (Q-PCR) to detect autophagy and the signal transduction pathway,

Outcome Measures

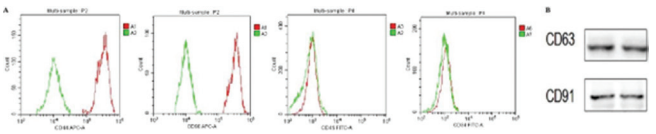
Stem cells and stem-cell exosomes. The research team used flow cytometry to examine the USCs in the exosomes of stem cells.

Exosomes, blood sugar, and weight. When the research team had successfully established DN induction, they checked the DN, exosomes, inhibitor, and exosomes + inhibitor groups' weights and FBG levels at the same time every seven days.

Biochemical tests. The research team measured the biochemical indexes in the rats' serum after 4 weeks of continuous administration of the treatments.

Renal-specific Gravity and ECM. The research team divided the glomerular lesions they found with a high-power microscope into three grades for the ECM index: 0%-25% of the glomerulus = 0 points, 25%-50% = one point, 50%-75% = 2 points, and more than 75% = 3 points. The team then calculated the average score.

Figure 1. Identification of Urine-derived, Stem-cell Exosomes. Figure 1A shows that CD44 and CD90 were positive, but CD34 and CD45 weren't. Figure 1B shows that the WB found that CD63 and CD91 were highly expressed in the exosomes of the USCs.



Abbreviations: CD, cluster of differentiation; USCs urine-derived stem cells; WB, Western blot

Autophagy. The team used RT-PCR and Western blot to identify the specific surface markers of the USCs Exo.

Statistical Analysis

The research team analyzed the data using SPSS 19.0 (IBM, Armonk, NY, USA). The team: (1) expressed measurement data as means ± standard deviations (SDs), assessed group differences using the *t* test, and used one-way analysis of variance (ANOVA) to compare groups, and (2) employed Tukey's multiple comparison test for the correlation analysis, following the ANOVA. $P < .05$ indicated significant differences.

RESULTS

Stem Cells and Stem-cell Exosomes

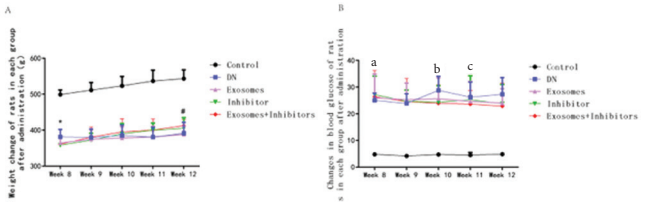
The USCs CD44 and CD90 were positive but CD34 and CD45 weren't (Figure 1A). The transmission electron microscopy revealed that the exosomes from the USCs were vesicle-like objects with various diameters between 40 and 100 nm. The WB showed that the USC Exo expressed the markers of the exosomes, and the proteins CD63 and CD91 were highly expressed in the exosomes (Figure 1B).

Exosomes, Blood Sugar, and Weight

Figure 2A shows that the weights of the rats in the DN group and the three intervention groups were significantly lower than that of the control group at week 8 after the model had properly formed ($P < .05$). The rats' weights in the exosomes, inhibitor, and exosomes + inhibitor groups gradually increased after one week of treatment. The exosomes + inhibitor group had significantly higher weights than those of the DN group at week 12 ($P < .05$).

Figure 2B shows that the blood glucose levels of the DN group and the three intervention groups were significantly higher, 16.7 mmol/L, than that of the control group ($P < .05$). The blood glucose of the exosomes group, at week 11, and of the exosomes + inhibitor group, at weeks 10 and 11, were significantly lower than those in the DN group ($P < .05$). This drop in blood glucose began in the exosomes, inhibitor, and exocrine body + inhibitor groups at week 9.

Figure 2. Exosomes' Control of Blood Sugar and Maintenance of the Rats' Weights. Figure 2A shows the rats' changes in body weight, and Figure 2B shows the changes in the rats' blood glucose.



[a]$P < .05$, indicating that at week 8 after the model had properly formed, the weights of the rats in the DN group and the three intervention groups were significantly lower and their blood-glucose levels were significantly higher than those of the control group
[b]$P < .05$, indicating that the exosomes + inhibitor group's weights were significantly higher at week 12 and the group's blood-glucose levels were significantly lower at weeks 10 and 11 than those of the DN group
[c]$P < .05$, indicating that the blood-glucose levels of the rats in the exosomes group were significantly lower than those of the DN group at weeks 10

Abbreviations: DN, diabetic nephropathy.

Biochemical Tests

Figures 3A and 3B show that the DN, exosomes, inhibitor, and exosomes + inhibitor groups at week 8 had significantly greater urine volumes and urinary-protein contents than the control group (all $P < .05$), but no significant differences existed among those group's urine volumes at that point. However, the urine protein of the exosomes, inhibitor, and exosomes + inhibitor groups had decreased and the urine-protein volume showed a downward trend at one week after the treatments. The urine-protein release in the exosomes and the exosomes + inhibitor groups was significantly lower than that of the DN group at two weeks after the start of therapy (both $P < .05$).

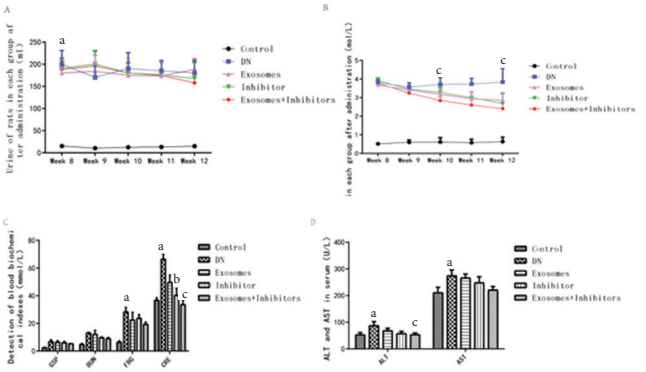
Figures3C and 3D show the biochemical indexes in the rats' serum. Postintervention, the DN group's FBG and Cre recombinase (CRE) values were significantly higher in those of the control group (both $P < .05$). The long-term, diabetes scenario had impaired the DN group's renal function. Postintervention, the CRE values of the inhibitor and the exosomes + inhibitor groups were significantly lower than those of the DN group ($P < .05$).

The DN group's ALT and AST levels were significantly higher than those of the control group postintervention (both $P < .05$). The exosomes + inhibitor group's ALT was significantly lower than that of the DN group postintervention ($P < .05$).

Renal-specific Gravity and ECM

Figure 4 shows that the DN group's renal-specific gravity and ECM rating were significantly higher than those of the control group postintervention ($P < .05$). Postintervention, the renal-specific gravity of the inhibitor and exosomes + inhibitor groups were significantly lower than that of the DN group (both $P < .05$), and the ECM rating of the exosomes + inhibitor group was also significantly lower than that of the DN group ($P < .05$).

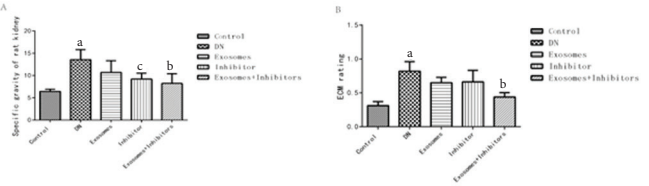
Figure 3. Detection of Urine Volume, Urine Protein, and Blood Biochemical Indicators in Rats. Figure 3A shows the changes in the rats' urine volume; Figure 3B shows the changes in levels of the rats' urinary protein; Figure 3C shows the rats blood biochemical indexes; and Figure 3D shows the AST and ALT detection in the blood.



[a]$P < .05$, indicating that the DN group's FBG, CRE, ALT, and AST were significantly higher than those of the control group postintervention
[b]$P < .05$, indicating that the inhibitor group's CRE was significantly lower than that of the DN group postintervention
[c]$P < .05$, indicating that the exosomes + inhibitor group's CRE and ALT was significantly lower than that of the DN group postintervention

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CRE, Cre recombinase; FBG, fasting blood glucose; GSP, glycated serum protein; DN, diabetic nephropathy.

Figure 4. Exosomes' Effective Reduction in Renal-specific Gravity and in the ECM rating. Figure 4A shows the specific gravity of the rats' kidneys, and Figure 4B shows the ECM rating.



[a]$P < .05$, indicating that the DN group's kidney-specific gravity and ECM rating were significantly higher than those of the control group
[b]$P < .05$, indicating that the exosomes + inhibitor group's kidney-specific gravity and ECM rating were significantly lower than those of the DN group
[c]$P < .05$, indicating that the inhibitor group's kidney-specific gravity was significantly lower than that of the DN group

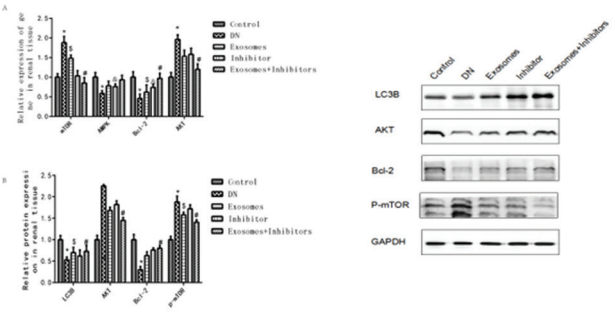
Abbreviations: DN, diabetic nephropathy; ECM, extracellular matrix.

Autophagy

RT-PCR. Figure 5A shows that postintervention, the DN group's expression of AMPK and Bcl-2 was significantly lower and its expression of mTOR and AKT was significantly higher than those of the control group (all $P < .05$). The exosomes group's expression of mTOR was significantly higher and of Bcl-2 was significantly lower than those of the DN group postintervention (both $P < .05$).

The inhibitor group's expression of AMPK and Bcl-2 was significantly higher than those of the DN group postintervention (both $P < .05$). The exosomes + inhibitor group's expression of mTOR and AKT was significantly lower

Figure 5. Exosomes' Reduction in Impairment of Renal-cell Autophagy. Figure 5A shows the autophagy gene, as detected using RT-PCR, and Figure 5B shows the gene, as detected using the Western blot.



*$P < .05$, indicating that the RT-PCR showed that the DN group's expression of mTOR and AKT were significantly higher and of AMPK and Bcl-2 were significantly lower than those of the control group and that the Western blot showed that the DN group's expression of LC3B and BCl-2 were significantly lower and of p-mTOR was significantly higher than those of the control group

$^{\$}P < .05$, indicating that the RT-PCR showed that the exosomes group's expression of mTOR was significantly higher and of Bcl-2 was significantly lower than those of the DN group and that the Western blot showed that the exosomes group's expression of LC3B was significantly higher and of p-mTOR was significantly lower than those of the DN group

$^{\&}P < .05$, indicating that the RT-PCR showed that the inhibitor group's expression of AMPK and Bcl-2 was significantly higher than those of the DN group

$^{\#}P < .05$, indicating that the RT-PCR showed that the exosomes + inhibitor group's expression of mTOR and AKT were significantly lower and of Bcl-2 was significantly higher than those of the DN group and that the Western blot showed that the exosomes + inhibitor group's expression of LC3B and Bcl-2 was significantly higher and of mTOR and AKT was significantly lower than those of the DN group

Abbreviations: AKT, protein kinase B; AMPK, 5' adenosine monophosphate (AMP)-activated protein kinase; Bcl-2, B-cell lymphoma 2; DN, diabetic nephropathy; LC3B, light chain 3 beta; mTOR, mammalian target of rapamycin; RT-PCR, reverse transcription polymerase chain reaction.

and its expression of BCL-2 was significantly higher than that of the DN group postintervention (all $P < .05$).

Western blot. Figure 5B shows that postintervention, the DN group's expression of LC3B and Bcl-2 was significantly lower and its expression of p-mTOR was significantly higher than those of the control group ($P < .05$). The exosomes group's expression of LC3B was significantly higher and of p-mTOR was significantly lower than those of the DN group postintervention (both $P < .05$).

The exosomes + inhibitor group's expression of LC3B and Bcl-2 was significantly higher and of AKT and p-mTOR was significantly lower than those of the DN group postintervention.

DISCUSSION

In comparison to the control group in the current study, the weights of the DN, exosomes, inhibitor, and exosomes + inhibitor groups had all considerably decreased by week 8. The exosomes, inhibitor, and exosomes + inhibitor groups all had FBG levels that were significantly higher than those of the control group for the same week. The study found that exosomes, when rats directly receive them, can effectively control blood-sugar levels and that the combined use of exosomes and autophagy inhibitors was significantly superior to either treatment in isolation. Exosomes can promote the absorption of autophagy inhibitors by the body, and hence, better promote the interplay of the two.

The kidney HE staining in the current study revealed that the rats in the DN-induction groups had distinctive pathological alterations as compared to the control group. The DN group had a significantly higher renal-specific gravity and ECM rating than the control group did, showing that injury to the glomerulus had occurred and that the concentration of glomerular cells had risen.

The DN group in the current study had significantly higher urine protein and volume by the eighth week than the control group did. These findings suggest that the renal rate of filtration at week 8 for the DN group had started to decline, and the rate of urine production had increased. After one week of treatment, the urine output of the exosomes, inhibitor, and exosomes + inhibitor groups didn't change significantly, but the urine-protein contents were lower than that at week 8, and the urine protein content of the exosomes group, inhibitor, and exosomes + inhibitor group decreased gradually, being lower than that of the DN group.

The urine protein in the current study showed a trend toward a decline, with a lengthening of the delivery period, and was lower than that of the DN group. The exosomes + inhibitor group's renal-specific gravity and ECM rating were significantly lower than those of the DN group. As of week 9, the autophagy inhibitor group's urine-protein concentration started to decrease, and its renal-specific gravity and ECM rating were both lower than those of the DN and exosomes groups.

The RT-PCR results for the rat's kidney tissues in the current study revealed that the expression mTOR and AKT in the exosomes + inhibitor group was significantly lower than that of the DN group, although its BCL-2 relative expression was higher than that group. The inhibitor group's expression of Bcl-2 was also significantly higher than that of the DN group. The Western blot showed that the exosomes + inhibitor group's p-mTOR expression was significantly lower than that of the DN group.

In rats' kidney tissue, stem-cell exosomes can efficiently boost AKT and BCL-2 levels, decrease mTOR protein expression, limit the stimulation of the mTOR pathway, prevent the development of renal fibrosis, enhance glomerular hypertrophy and accumulation, and promote renal permeability.

CONCLUSIONS

Human urine-derived stem cell exosomes can significantly reduce the activation of the mTOR signaling pathway, reduce the autophagy of rats' kidney cells, increase the protein expression of LC3B in kidney tissues, and reduce the damage to glomerular cells. By blocking the mTOR signaling pathway, human urogenic exosomes can alleviate the signs and symptoms of DN.

DATA AVAILABILITY

The data used to support this study is available from the corresponding author upon request.

AUTHORS' DISCLOSURE STATEMENT

The Science and Technology Plan of Jiangxi Health Commission (GJJ190906) and the project of Jiangxi Provincial Department of Health (NO.202131092) funded the study. The authors declare that they have no conflicts of interest related to the study.

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