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Original Article

Influence of mesenchymal stem cells on expression of AQP1 and AQP2 in rats with nephropathy induced by adriamycin

Yi-Hua Bai[1], Hong-Ying Jiang[1], Xi-Yan Lian[1], Jian-Song Wang[2], Jia-Ping Wang[3]

[1]Department of Nephrology, The Second Affiliated Hospital of Kunming Medical University, Yunnan, China; [2]Surgery of Urology, The Second Affiliated Hospital of Kunming Medical University, Yunnan, China; [3]Department of Radiology, The Second Affiliated Hospital of Kunming Medical University, Yunnan, China

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Abstract: This study aims to explore the therapeutic effect of bone marrow mesenchymal stem cells on adriamycin nephrosis, and the potential mechanism. The rat experimental nephropathy model was established by unilateral nephrectomy combined repeated injecting adriamycin (ADR). Thirty adriamycin nephrosis rats were randomly divided into three groups, including ADR (n=10), MSCs transplantation through peripheral veins groups (M-V, n=10), and MSCs transplantation through right renal artery groups (M-A, n=10), and there was another normal control group (N, n=10). This study lasted 8 weeks, 24 hours urine was collected through simple metabolic cage to measure urinary volume and urine protein quantitation in 24 hours. The levels of plasma albumin (ALB), sodium were measured by biochemical analysis. The expressions of AQP1-2 were measured by immuno-histochemistry assay. Kidney medulla ultramicroscopic structure was observed by TEM. The results indicated that the ALB and 24 h urinary volume have significant increased in M-V and M-A group compared to the ADR group ($P<0.05$). Furthermore, the serum sodium and urine protein quantitation in 24 hours were decreased in M-V and M-A group compared to ADR group ($P<0.05$). Protein expression of AQP1-2 had been remarkably decreased ($P<0.05$). It showed degenerative changes of kidney ultra microscopic structures of the ADR rats, while MSCs transplantation could significantly improve the damage. In conclusion, in adriamycin nephropathy rats, MSCs transplantation exerts its therapeutic effects by decrease urinary albumin excretion, increase ALB, decrease sodium and the expression of AQP1-2 in renal tubules.

Keywords: Mesenchymal stem cells, adriamycin nephropathy, urinary volume, AQP1, AQP2

Introduction

The aquaporin (AQP) is a highly conserved protein, and there are 13 subtypes of aquaporin in mammals, including seven subtypes in kidney [1, 2]. AQP plays important role in the body's water balance and homeostasis [3], and the kidney is the important organ for regulation of water balance [4]. Therefore, the poor kidney failure would lead to water retention. In this study, we used Adriamycin to induce chronic kidney disease in rats, and then the expression of AQP1 and AQP2 was detected; on the other side, the mesenchymal stem cells (MSCs) were transplanted into rats with nephropathy, and the expression of AQP1 and AQP2 was determined again and analyzed.

Materials and methods

Animals

42 male Sprague Dawley (SD) rats (150±10 g) were provided by the Experimental Animal Center of Sichuan University.

Reagents and instruments

Adriamycin (ADR) were purchased from Sigma; fetal bovine serum (FBS) were purchased from Bioengineering Research Institute, Chinese Academy of Medical Sciences; L-DMEM were purchased from Gibco; AQP1 and AQP2 antibodies were purchased from Santa; SP kit and DAB reagent were purchased from Hebei Bohai Biological Engineering Co., Ltd.; the microscope

Table 1. Comparison of serum albumin and urinary protein in SD rats after MSCs transplantation

Groups	24 h urinary volume (mL)	24 h urine albumin (mg/24 h)	serum sodium (mmol/L)	serum albumin (g/L)
N group	19.00±1.22	26.10±1.62	138.07±1.43	32.55±1.51
ADR group	14.94±1.91*	49.41±3.20*	140.88±0.87*	21.90±1.58*
M-V group	15.92±0.73*,a	39.63±2.87*,aa	139.64±1.44*,a	23.40±3.40*,a
M-A group	15.96±0.58*,a	37.33±3.21*,aa	139.87±0.74*,a	23.53±2.18*,a

*P<0.01: V.S. N group; aP<0.01, aaP<0.05: V.S. ADR group.

(BX51T-PHD-J11) was purchased from Olympus, Japan; the centrifuge (LDZ5-2) was purchased from Centrifuge Company, Beijing; the constant temperature water-bath chamber (DSHZ-300) was purchased from Jiangsu Taicang Medical Instrument factory.

Culture of MSCs in vitro

Two SD rats were chose to isolate femur and tibia in PBS, then the L-DMEM medium with 10% FBS was used to wash bone marrow cavity, and the cell suspension was collected and added Ficoll separation medium; the bone marrow cells were collected by centrifugation at 2000 rpm for 30 min, and washed with PBS by centrifugation at 1600 rpm for 10 min. Then the cells were seeded in plastic culture flasks, and cultured in 37°C, 5% CO_2 incubator. The cells from passage 4 to 6 were chosen to perform the further study.

Model establishment

All animals were performed anesthesia with 2% sodium pentobarbital (0.2 ml/100 g), the rats were supine, and the legs were fixed on the operating table and the back was exposed, then the incision was made at 1 cm from rib ridges of left kidney, and the left kidney was removed. The ADR (4 mg/kg) was intravenously injected into rats. One week later, injection of ADR through tail vein again. Then 6 weeks after the second injection, the blood urea nitrogen, serum creatinine levels were increased, which was consistent with the references [4], suggesting the modeling was successful.

Animal grouping

30 modeling rats were randomly divided into three groups: unilateral nephrectomy and injection of ADR twice group (group ADR, n=10); transplantation of MSCs via tail vein group (group M-V, n=10); transplantation of MSCs via renal artery (group M-A, n=10). Another 10 rats were set as normal control group, in which the ADR was replaced by saline. After injection of ADR twice, the model was successful. The MSCs was digested with 0.1% trypsin and counted, then 1×10^6 cells were injected into rate via tail vein and renal artery in groups M-V and M-A, respectively. Two weeks later, the 24 h urine of rats were collected and the urinary protein was detected, the abdominal aortic blood were collected and the serum albumin and serum sodium concentration were determined, the kidneys were moved for preparation.

Detection of serum albumin and urinary protein

Two weeks after transplantation of MSCs, 50 ml/kg saline was feed to rats, 30 min later, the urine was drained completely, and then 24 h urine was collected to detect the 24 h urinary protein. The blood was collected from abdominal aorta to detect serum albumin and serum sodium concentration in laboratory department of our hospital. 8 animals were chosen to conduct experiments due to some animals died during the experiment.

The expression of AQP1 and AQP2 in kidney

The rats were performed anesthesia through intraperitoneal injection of 2% sodium pentobarbital (0.2 ml/100 g), and the kidney were removed and fixed in 4% paraformaldehyde. The immunohistochemistry of AQP1 and AQP2 was performed according to the instructions provided by manufacturer. The brown granules appearing in the cytoplasm or cell membrane was considered as positive under light microscope, PBS instead of first antibody was set as the negative control. 5 high-power fields (× 400) were randomly chosen by two dependent pathologists, and the positive cell area and integrated optical density (IOD) were analyzed with Image-Proa Plus version 6.0, the average optical density was used to indicate the positive expression, the average optical density = IOD/total area of positive cells.

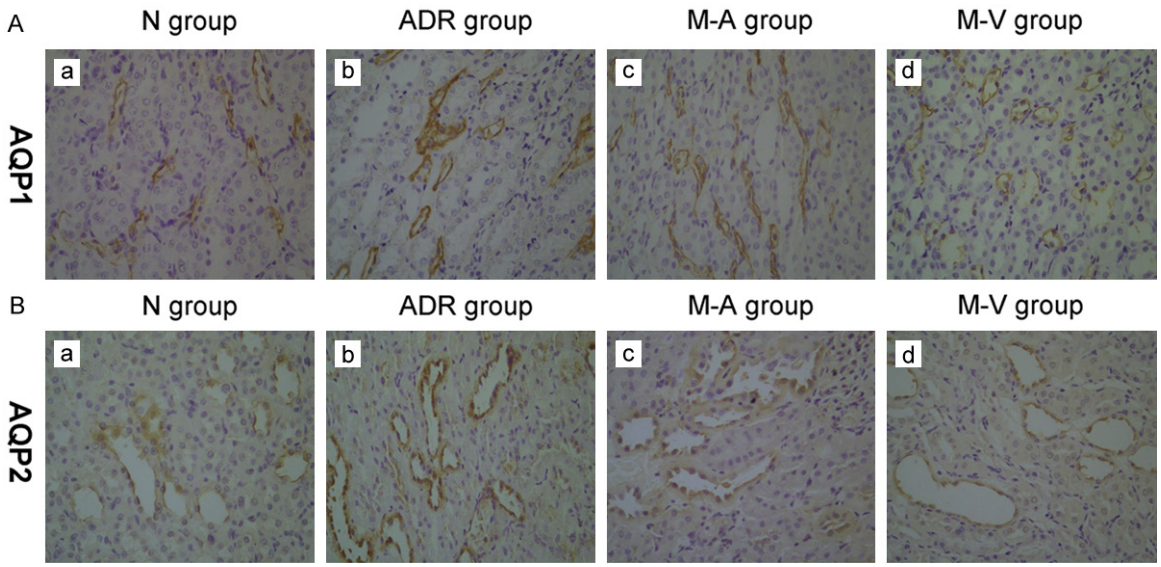

Figure 1. Immunohistochemical staining of AQP1 and AQP2 in rat kidney (magnification: × 100). A. Staining of AQP1 in rat kidney. a. N group. b. Staining of AQP1 in ADR group. c. Staining of AQP1 in M-V group. d. Staining of AQP1 in M-A group. B. Immunohistochemical staining of AQP2 in rat kidney (magnification: × 100). a. Staining of AQP2 in N group. b. Staining of AQP2 in ADR group. c. Staining of AQP2 in M-V group. d. Staining of AQP2 in M-A group.

Table 2. Average optical density of AQP1, AQP2 in rat kidney

Group	n	AQP1	AQP2
N group	8	0.24±0.02	0.21±0.02
ADR group	8	0.34±0.02*	0.33±0.02*
M-V group	8	0.33±0.01*,a	0.31±0.01*,a
M-A group	8	0.32±0.02*,aa	0.31±0.02*,a

*P<0.01: V.S. N group; aP<0.01, aaP<0.05: V.S. ADR group.

Ultrastructure of kidney

The kidney was cut into 1 mm × 1 mm × 1 mm pieces, fixed in 5% glutaraldehyde quickly, and observed under electron microscopy.

Statistical methods

All data were expressed as (x±s) and analyzed with single-factor analysis of variance (SPSS17.0), LSD method was used to group comparison. A $P<0.05$ was considered statistically significant.

Results

Influence of MSCs on serum albumin, serum sodium, 24 h urine and 24 h urinary protein

The albumin and 24 h urine in group ADR were significantly lower than that in normal control group ($P<0.01$), while the serum sodium concentration and 24 h urinary protein in group ADR were significantly higher than normal control group ($P<0.01$). The albumin and 24 h urine in groups M-V and M-A were significantly higher than that in group ADR ($P<0.05$), while the serum sodium concentration and 24 h urinary protein in groups M-V and M-A were significantly lower than that in group ADR (serum sodium: $P<0.05$; 24 h urinary protein: $P<0.01$). There was no significant difference about all these indicator between groups M-V and M-A. The details were shown in **Table 1**.

The expression of AQP1 and AQP2 in kidney

The AQP1 protein was expressed in renal proximal convoluted tubule and medullary loop, and AQP2 was expressed in renal distal tubule and collecting duct. The expression of AQP1 and AQP2 was significantly enhanced in group ADR compared to normal control group ($P<0.01$), while the expression of AQP1 and AQP2 in groups M-V and M-A was significantly lower than that in group ADR ($P<0.05$), and there was no significant difference between groups M-V and M-A. The details were shown in **Figure 1A**, **1B** and **Table 2**.

The effects of MSCs on renal ultrastructure of nephropathy rats

In normal control group, the renal proximal tubule and distal tubule had complete and

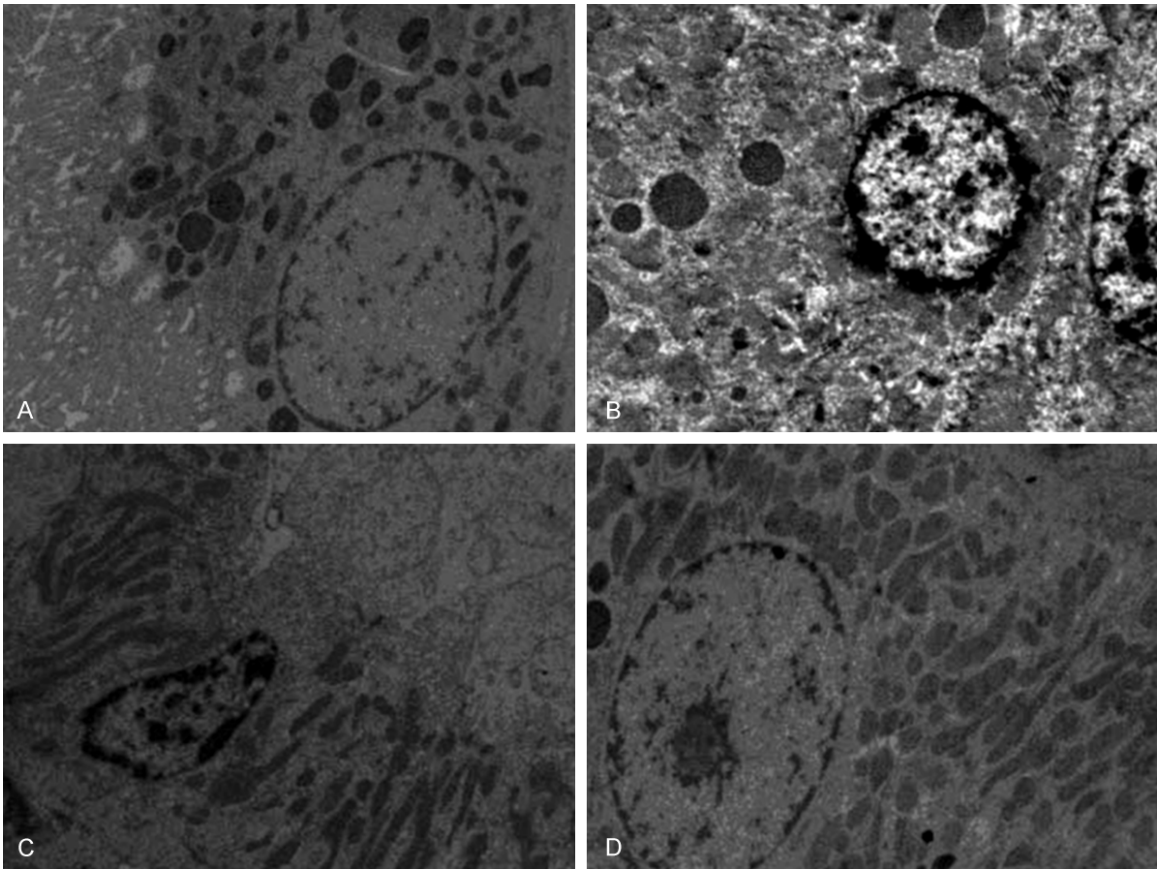

Figure 2. Observation of kidneys observed by transmission electron microscope. A. N group (magnification: × 6000). B. ADR group (magnification: × 4000). C. M-V group (magnification: × 5000). D. M-A group (magnification: × 6000).

clear ultrastructure, round nuclei, dispersed chromatin, clear basement membrane boundaries and uniform electron density; in details, the membrane of basal surface was tidiness and boundaries were clear, lots of normal mitochondria were observed in cells, and lots of microvilli existed in free surface, shown in **Figure 2A**. In group ADR, the nuclei was irregular karyopyknosis, and the nuclear chromatin showed margination phenomenon; the fold of basal membrane showed dissolution, mitochondrial was swelling, and the crest was dissolved or disappeared (**Figure 2B**). In group M-V, only a few nuclei were karyopyknosis and chromatin showed margination phenomenon; the fold of basal membrane was clear in some cells, and there was abundant mitochondria and neat crest; the mitochondrial was swelling in a few cells; lots of microvilli existed in free surface (**Figure 2C**). In group M-A, only a few nuclei had chromatin margination phenomenon; the fold of basal membrane was clear and mitochondria was abundant; a part of mitochondrial was swelling and inter-cavity was increased; lots of microvilli existed in free surface (**Figure 2D**).

Discussion

AQP1 is mainly distributed in renal proximal convoluted tubule and epithelial cells of medullary loop, which mainly mediates the water reabsorption of original urine of the two tubular; the expression of AQP1 was related to high permeability of outside of basal membrane [3]. It has been proved that the down-regulation of AQP1 and AQP2 could decrease the liquid reabsorption ability of tubular to increase diuresis [5]. AQP2 is mainly expressed in luminal side of principal cells in collecting duct, and the collecting duct is the key component of urine concentrated, lack of AQP2 caused by genetic variation could lead to insipidus in clinic [1], suggesting that AQP2 is important for maintaining the water balance and osmotic pressure. ADR is a broad-spectrum cell cycle non-specific chemotherapy drugs, which has strong cytotoxic effect to directly damage the glomerular and

tubular cells. ADR nephropathy model is one of the classical models in nephrotic areas [6, 7]. The present experiments showed that the expression of AQP1 and AQP2 was increased in ADR nephropathy rats, which caused water retention and renal edema.

In this study, we used ADR to establish the chronic kidney disease in rats; on the 8th week, we found that the serum albumin was significantly decreased and 24 h urinary protein was significantly increased in ADR model, indicating that the hypoalbuminemia was associated with loss of urinary protein. Through transplantation of MSCs with two different ways (group M-V and M-A) [8, 9], we explored the effects of MSCs on AQP expression in ADR rats. The results showed that the serum albumin of both M-V and M-A group was significantly higher than that of group ADR, and the 24 h urinary protein was significantly decreased, suggesting that MSCs can alleviate the proteinuria in ADR chronic kidney disease rats. In addition, the serum sodium concentration of group ADR was significantly higher than that of normal control group, while the 24 h urine was decreased; moreover, the expression of AQP1 and AQP2 was significantly increased in group ADR than that in normal control group, which suggested that the water and electrolyte balance disorders were related to the up-regulation of AQP1 and AQP2 in ADR rats. After MSCs transplantation, the serum sodium concentration was significantly decreased and 24 h urine was increased in both group M-V and group M-A, and the expression of AQP1 and AQP2 was reduced, suggesting that transplantation of MSCs could decrease the expression of AQP1 and AQP2 in kidney of ADR rats, thus alleviating the water and sodium retention. However, the average optical density values of AQP1 and AQP2 in group M-A were lower than that in group M-V, but there was no significant difference, as well as the serum and urine indicators. Therefore, we speculated that the different ways of MSCs transplantation would not affect the expression of AQP1 and AQP2. Under electron microscope, the ultrastructure of kidney had obvious degeneration in group ADR. After transplantation of MSCs, the ultrastructure of kidney had been improved either group M-V or group M-A, which also confirmed that MSCs have a therapeutic effect on ADR nephropathy rats [10-12].

Our study used MSCs to intervene rats with chronic kidney disease induced by ADR, then detected the expression of AQP1 and AQP2 of kidney in rats. The results showed that MSCs could improve the water and sodium balance disorders in chronic kidney diseases at a certain degree. We also found that transplantation of MSCs via peripheral vein or renal artery wouldn't affect the expression of AQP1 and AQP2, suggesting that the transplanting ways didn't relate to the improvement of water and sodium balance disorders with MSCs. However, due to short time, limited number of rats, and the water and sodium balance disorder was only a part of nephropathy. Therefore, the research still needs further study to confirm the effects of MSCs.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jia-Ping Wang, Department of Radiology, The Second Affiliated Hospital of Kunming Medical University, Dianmian Road 374#, Yunnan, China. E-mail: wangjpkm@yeah.net

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