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Original Article

Ultrastructural pathological features of unilateral renal artery stenosis in the rats

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Abstract: Renal artery stenosis (RAS) is one of the main reasons of renovascular hypertension and its pathogenesis remains unclear. In this study, we aimed to investigate histopathological characteristics in a rat model of RAS. Sprague-Dawley (SD) male rats were randomly divided into unilateral RAS group (Model group, n = 30) and Sham group (n = 30). The left renal artery was clamped with miniature silver clip for the rats in RAS group, while it was exposed but not clamped for the rats in Sham group. After the surgery, the rats were randomly divided into ten subgroups based on the time after surgery (n = 3). Blood pressure, urinary albumin/creatinine ratio, and serum albumin and creatinine levels were measured. The kidneys were dissected for histological and electron microscopy analysis. The results showed that systolic blood pressure was significantly higher since 4 weeks after surgery compared to before surgery. There were no significant differences in urinary albumin/creatinine ratio as well as serum albumin and creatinine levels in Model and Sham groups. During the early acute renal ischemia the stenotic kidney exhibited acute tubular injury, podocyte injury and some crescent formation, and the main components of crescent are podocytes. Although renal tubules and vascular lesions gradually recover and crescent disappears, segmental lesions of podocyte appear in the late stage of RAS. These data reveal ultrastructural pathological changes during RAS, and suggest the role of podocyte lesions in chronic renal ischemia.

Keywords: Renal artery stenosis, kidney, crescent formation, podocytes

Introduction

Renal artery stenosis (RAS) is one of the main reasons of renovascular hypertension [1]. Atherosclerosis accounts for approximately 70-90% of RAS that obstructs blood flow to the renal arteries [2]. Atherosclerotic renal artery stenosis (ARAS) is a common form of RAS and predominates in older patients with hypertension. ARAS could lead to end-stage renal disease (ESRD), especially in the elderly population [3]. ARAS is a progressive disease with poor prognosis. The median survival of ARAS patients is only 25 months, and the 5-year survival rate is only 31% [4].

In clinical practice, unilateral RAS is more common. Currently, the pathogenesis of unilateral RSA remains not fully understood. Clinical studies showed that some unilateral RAS patients had proteinuria. In addition, renal biopsy of the patients with unilateral RAS showed ischemic damages and focal segmental glomerulosclerosis (FSGS) [5]. However, the detailed pathological processes underlying unilateral RAS are unclear. In this study, we aimed to investigate histopathological characteristics as well as dynamic changes in blood pressure, urine protein, renal function in a rat model of RAS.

Materials and methods

Animals

Sixty healthy Sprague-Dawley (SD) male rats (weight 170 ± 13 g) were provided by the Experimental Animal Center of Military Medical Science Institute (Beijing, China). All animals were maintained in accordance with the guidelines of the NIH (Guide for the Care and Use of Laboratory Animals, 1996) and the animal experiments were performed under approved

Table 1. Preoperative and postoperative SBP in Model and Sham groups

Time	Model group		Sham group	
	Preoperative SBP	Postoperative SBP	Preoperative SBP	Postoperative SBP
1 day	103.3±1.2	107.3±6.4	106.0±1.0	108.3±2.5
3 days	106.7±3.1	106.3±3.1	104.0±2.6	104.0±1.7
1 week	102.7±1.5	106.3±0.6	104.0±3.5	104.0±2.0
2 weeks	115.3±6.0	120.7±5.8	106.7±6.8	107.7±8.1
4 weeks	107.0±8.9	146.3±16.3*	106.7±5.5	113.3±9.1
8 weeks	112.3±2.9	149.7±11.7*	104.0±1.7	106.7±6.0
12 weeks	101.7±6.4	153.0±14.7*	103.3±3.1	107.3±3.2
16 weeks	97.7±8.1	144.3±6.4*	108.0±7.0	106.7±4.7
20 weeks	106.0±1.7	171.3±3.2*	106.3±3.1	108.7±5.5
24 weeks	103.3±9.0	162.7±14.2*	102.0±2.0	104.7±0.6

Note: *$P < 0.05$ compared with preoperative SBP.

Table 2. Comparison of urinary albumin/creatinine ratio in Model and Sham groups

Time	Urinary albumin/creatinine ratio		
	Model group	Sham group	P
1 day	1996.79±542.41	2033.69±144.48	0.915
3 days	3026.22±1556.13	3075.65±1121.21	0.967
1 week	1697.80±199.50	1666.99±410.62	0.913
2 weeks	2345.06±362.00	2312.47±212.12	0.899
4 weeks	1449.90±297.39	1537.93±241.17	0.711
8 weeks	2235.91±516.87	2194.73±746.54	0.941
12 weeks	1568.76±48.12	1465.25±122.28	0.244
16 weeks	2006.42±636.92	2317.33±1350.54	0.737
20 weeks	1514.97±275.19	1910.15±795.60	0.462
24 weeks	1346.57±217.76	1416.00±278.67	0.751

protocols of the Animal Care and Use Committee of Beijing University.

Animal grouping

The rats were randomly divided into unilateral RAS group (Model group, n = 30) and Sham group (n = 30). The rats were acclimated for one week and fasted for 12 h (with free access to water) before the surgery. The rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (60 mg/kg). Next, the abdomen was open and the left renal artery was clamped with miniature silver clip for the rats in RAS group, for the rats in Sham group, the left renal artery was exposed but not clamped. After the surgery, the rats were randomly divided into ten subgroups based on the time after surgery: one day, three days, one week, two weeks, four weeks, eight weeks, 12 weeks, 16 weeks, 20 weeks, and 24 weeks groups (n = 3).

Blood pressure monitoring

The blood pressure of the rats was measured by using a small animal noninvasive blood pressure meter (BP98A, Japan).

Determination of urinary albumin/creatinine ratio

The rats were placed individually in metabolism cages (TSE Systems, Midland, MI), and 24-h urine samples were collected for the analysis by immunoassay (Rat Albumin Enzyme Immunoassay; SPI-BIO, Montreal, Quebec, Canada). The urinary albumin: creatinine ratio was calculated.

Determination of serum albumin and creatinine levels

Blood samples were collected from the rat in Eppendorf tubes. The blood was allowed to clot and then centrifuged at 4,000 rpm for 5 min at room temperature. Serum albumin and creatinine levels were analyzed using automatic biochemical analyzer (Daytona).

Histological analysis

The kidneys were dissected from the rats and parts were fixed in 0% formaldehyde overnight at 4°C and processed for paraffin-embedding following standard procedures. Sections were cut at 2 μm thicknesses and stained by hema-

Table 3. Comparison of serum albumin and creatinine levels in Model and Sham groups

Time	Serum albumin (umol/L)			Serum creatinine (g/L)		
	Model group	Sham group	P	Model group	Sham group	P
1 day	24.00±2.00	22.67±5.86	0.728	29.57±3.62	29.73±0.40	0.941
3 days	30.00±10.15	26.00±8.19	0.623	26.10±5.57	25.10±2.23	0.787
1 week	17.67±2.52	20.00±1.00	0.21	22.70±4.93	21.93±2.38	0.82
2 weeks	28.67±4.93	23.33±3.79	0.21	23.87±2.80	24.60±3.54	0.79
4 weeks	24.67±3.06	22.67±0.58	0.33	24.40±3.34	25.80±1.54	0.55
8 weeks	22.33±1.53	24.67±4.04	0.40	25.80±4.86	26.40±2.98	0.86
12 weeks	32.00±4.36	25.67±3.06	0.11	29.40±1.65	26.67±2.51	0.19
16 weeks	24.33±2.52	22.33±4.51	0.54	26.07±0.40	23.23±2.40	0.11
20 weeks	27.67±5.69	28.67±2.08	0.79	27.53±3.73	24.20±1.45	0.22
24 weeks	23.67±3.06	23.33±1.53	0.87	28.37±2.06	25.93±2.12	0.23

toxylin and eosin (HE), periodic-acid Schiff (*PAS*) and hexamine silver.

Electron microscopy

Part of the kidneys were cut into small tissue blocks (about 1 mm^3) and fixed in 2.5% glutaraldehyde in 0.01 mol/L phosphate buffer at 4°C, followed by 2% osmium tetroxide. Next the tissues were dehydrated in a series of graded ethanol solutions and embedded in epoxy resin. Ultrathin sections were double-stained with uranyl acetate and lead and examined under a JEM1200EX transmission electron microscope (Tokyo, Japan).

Statistical analysis

Data were expressed as the mean ± standard deviation. and analyzed by SPSS 11.5 statistical software. Statistical differences were determined by *t* test or one-way ANOVA. $P < 0.05$ was considered significant.

Results

Comparison of clinical parameters between model and sham groups

First we measured systolic blood pressure *(*SBP*)* in the rats. Compared to preoperative SBP, SBP was significantly increased since 4 weeks after the surgery in Model group. In contrast, postoperative SBP showed no significant difference from preoperative SBP in Sham group (**Table 1**). These data indicate that we have successfully established rat model of RAS.

Next we compared urinary albumin/creatinine ratio in Model and Sham groups and found no significant differences at each time point after surgery ($P > 0.05$, **Table 2**). In addition, we compared serum albumin and creatinine levels in Model and Sham groups and found no significant differences at each time point after surgery ($P > 0.05$, **Table 3**).

Pathological changes in the kidneys in Model group

By histological analysis we found pathological changes in the stenotic kidneys in Model group after the surgery. On the first day after surgery, we observed vacuoles and granular degeneration in renal tubular epithelial cells, but found no edema and cell infiltration in renal interstitium. Small renal arteries showed concentric intimal hyperplasia. 3 days after surgery, we found crescent-shaped or cyclic structures in glomerulus, and about 10% of glomerulus had glomerular crescent. Vacuoles and granular degeneration were still observed in renal tubular epithelial cells but no significant renal interstitial cell infiltration was observed (**Figure 1A**). One week after surgery, no glomerular crescent was observed, and no significant interstitial and vascular abnormalities occurred. Two weeks after surgery, no significant pathological changes were observed in the stenotic kidney of Model rats under light microscope (**Figure 1B**).

Ultrastructural changes in the kidneys in Model group

To reveal ultrastructural changes in the stenotic kidneys, we performed electron microscopy

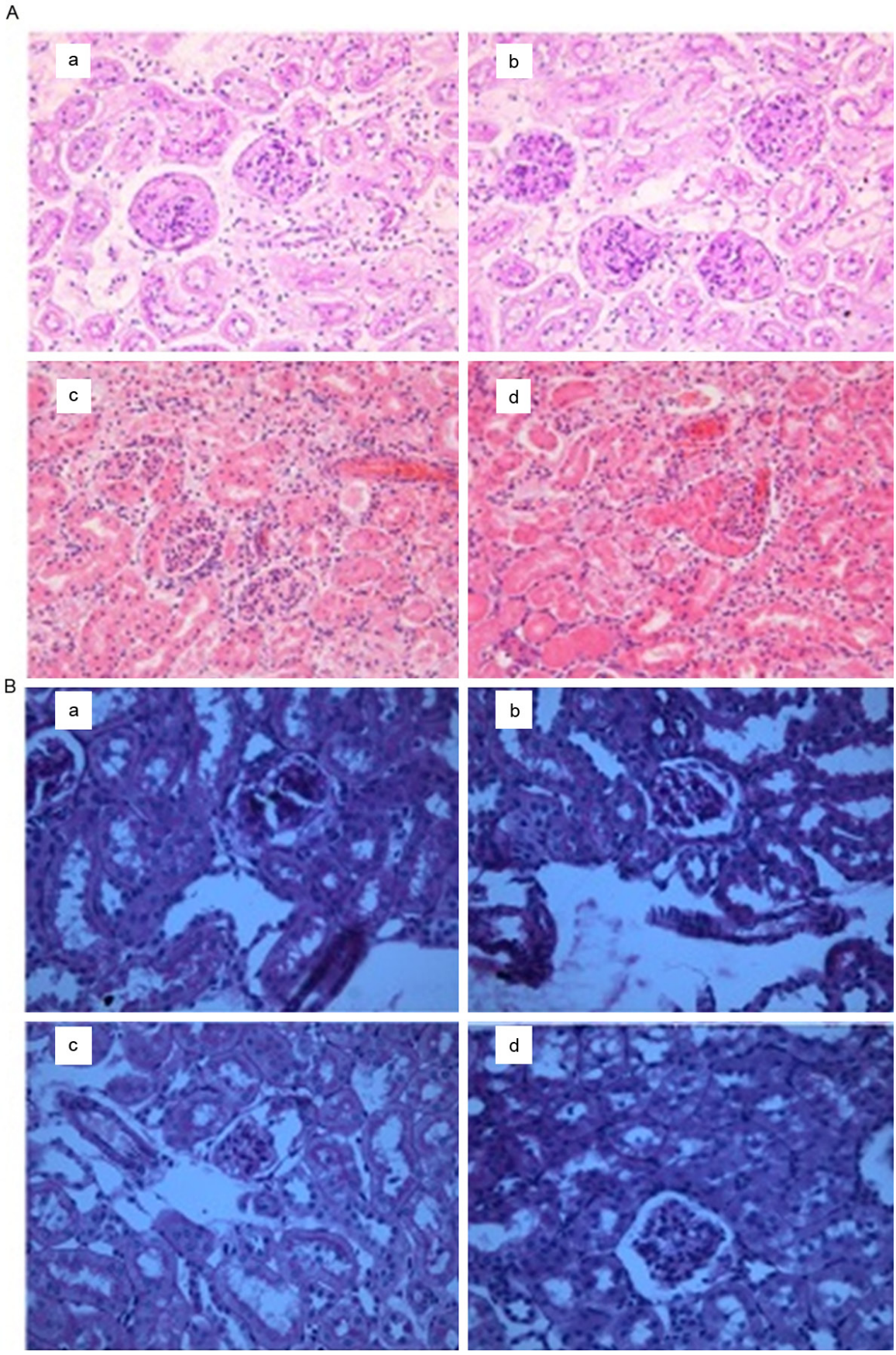
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Figure 1. A. HE staining of stenotic kidneys. a, b: Representative images of stenotic kidneys of Model group one day after surgery; c, d: Representative images of stenotic kidneys of Model group three days after surgery. B. PAS staining of stenotic kidneys. a, b: Representative images of stenotic kidneys of Model group one week after surgery; c, d: Representative images of stenotic kidneys of Model group two weeks after surgery. Magnification: × 20.

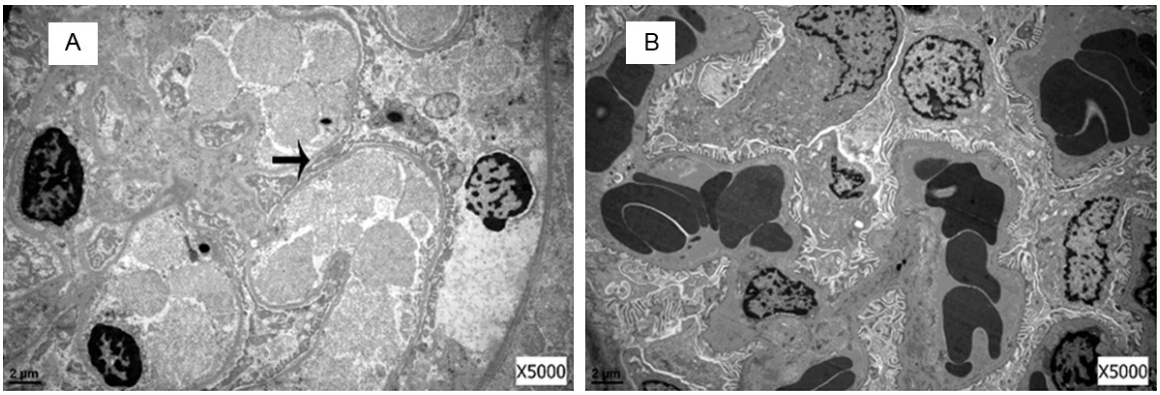

Figure 2. Electron microscopy of the kidneys one day after surgery. A: The stenotic kidneys. B: The contralateral kidney. The diffuse foot process fusion was indicated by the arrows. Magnification: × 5,000.

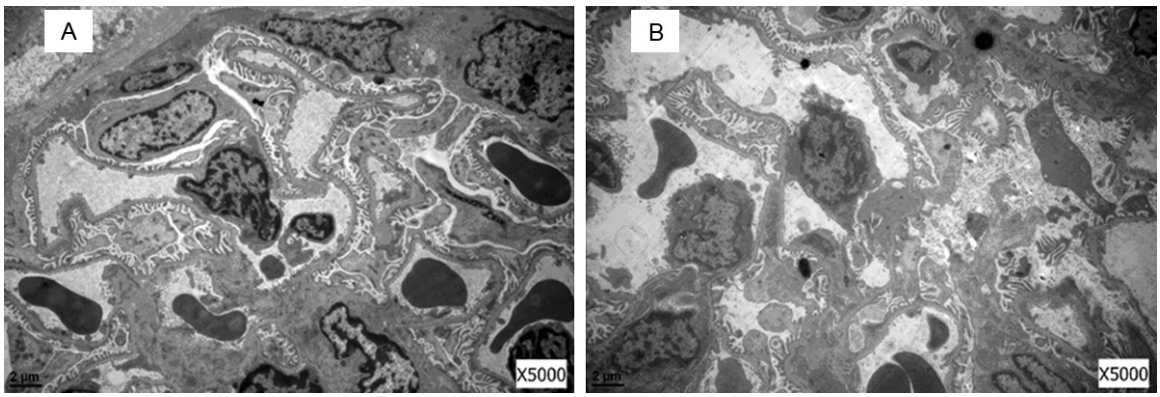

Figure 3. Electron microscopy of the kidneys one week after surgery. A: The stenotic kidneys. B: The contralateral kidney. Magnification: × 5,000.

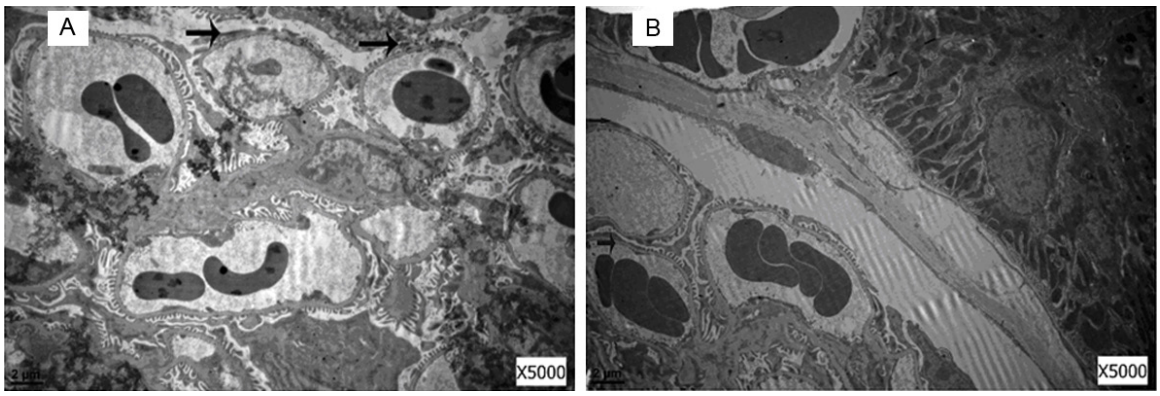

Figure 4. Electron microscopy of the kidneys 20 weeks after surgery. A: The stenotic kidneys. B: The contralateral kidney. The diffuse foot process fusion was indicated by the arrows. Magnification: × 5,000.

inspection. One day after surgery, we observed diffuse foot processes fusion and exposed glomerular basement membrane in the stenotic kidneys but not in the contralateral kidneys

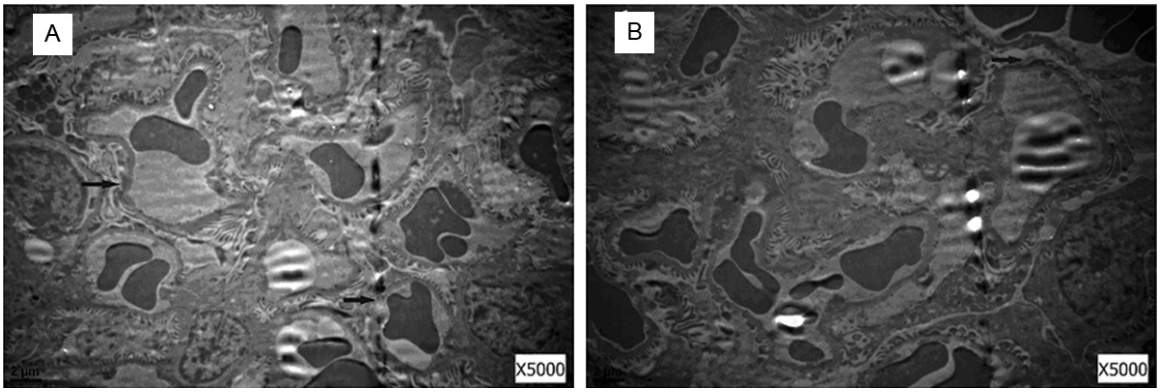

Figure 5. Electron microscopy of the kidneys 24 weeks after surgery. A: The stenotic kidneys. B: The contralateral kidney. The foot process fusion was indicated by the arrows. Magnification: × 5,000.

(**Figure 2**). One week after surgery, no obvious diffuse foot processes fusion was observed in the stenotic and contralateral kidneys (**Figure 3**). However, during 12 to 24 weeks after surgery, foot processes fusion was observed in the stenotic kidneys again, but not in the contralateral kidneys (**Figures 4** and **5**). In contrast, no ultrastructural changes were observed in both kidneys of Sham group at all time points analyzed (data not shown).

Discussion

In recent years, the prevalence of RAS especially ARAS is significantly increased and has become one of the important causes of ESRD. RAS induces a series of hemodynamic changes and causes high blood pressure. To better understand the pathological processes underlying RAS, in this study we established a rat model of unilaterial RAS and investigated histopathological characteristics as well as dynamic changes in blood pressure, urine protein, renal function.

Our results showed that systolic blood pressure was significantly higher since 4 weeks after surgery compared to before surgery, indicating that we established successfully RAS model in the rats [6]. Urinary albumin-to-creatinine ratio is an important index of urinary albumin excretion and is increasingly being accepted as a predictor of the health outcomes such as hypertension [7]. Interestingly, in this study we found that there were no significant differences in urinary albumin/creatinine ratio in Model and Sham groups. In addition, we found no significant differences in serum albumin and creatinine levels in Model and Sham groups. Perhaps longer time is needed to observe the changes in urinary albumin/creatinine ratio and serum albumin and creatinine levels in the rat model of RAS.

Renal tubular epithelial cells are characterized by the solute transport activity and the mitochondria provide the energy needed for transport. Renal tubular epithelial cells are particularly sensitive to the damaged caused by acute ischemia and hypoxia upon RAS. In this study, by histological and ultrastructural analysis we found pathological changes in the stenotic kidneys. For example, vacuoles and granular degeneration were observed in renal tubular epithelial cells within two weeks after surgery. Afterwards, renal tubular epithelial cells recovered from these damages and no vacuoles and granular degeneration were observed. These observations are consistent with the concept of adaptive response [8], in which renal blood flow redistribution and other collateral circulation help improve ischemic state in renal tubules and lessen the damages to renal tubular epithelial cells.

RAS not only causes the tubular damage, but also leads to diffuse foot process lesion and glomerular crescent. A recent study showed that renal ischemia induced podocyte effacement with loss of slit diaphragm, and this was associated with the rapid loss of interaction between slit diaphragm junction proteins Neph1 and ZO-1, and the redistribution of Neph1 and ZO-1 proteins from the cell membrane to the cytoplasm [9]. In this study, we observed ultrastructural defects in podocytes, suggesting that ischemia induced damages to podocytes may contribute to the pathogenesis

of RAS. Indeed, the mutations in mitochondrial protein-coding genes in mice and humans mitochondrial respiratory chain can lead to collapsing glomerular disease [10-12]. In addition, ischemia is known to upregulate the expression of hypoxia-inducible factor (HIFs) upregulation [13]. HIF is widely expressed in the renal cortex, medulla and renal collecting duct cells [14]. Clinical study showed that HIFs induced the proliferation and dedifferentiation of podocytes, leading to HIV-associated glomerular disease [15].

Glomerular crescent formation is a nonspecific response to the injury to glomerular capillary wall. The initiating event is the development of physical gaps in the glomerular capillary wall, glomerular basement membrane, and Bowman's capsule. These gaps allow coagulation factors to enter Bowman's space, leading to fibrin formation and crescent formation [16]. Immunohistochemical analysis showed that crescent formation involves parietal epithelial cells, macrophages and myofibroblasts, and the composition of these cells varies. When Bowman's capsule is compact, most of the cells are parietal epithelial cells. However, when Bowman's capsule ruptures the macrophages and myofibroblasts account for the major proportion [17]. In addition, podocytes are a major constituent of crescents and play important role in crescent formation by disrupting the parietal epithelial layer and attaching on its basement membrane, thus forming the bridge between the tuft and Bowman's capsule [18]. In this study, by ultrastructural analysis, we confirmed that the main components of glomerular crescent are podocytes.

In summary, by establishing rat model of RAS we demonstrated that during the early acute renal ischemia the stenotic kidney exhibited acute tubular injury, podocyte injury and some crescent formation, and the main components of crescent are podocytes. Although renal tubules and vascular lesions gradually recover and crescent disappears, segmental lesions of podocyte appear in the late stage of RAS. Therefore, the role of podocyte lesions in chronic renal ischemia deserves further study.

Disclosure of conflict of interest

None.

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