International Urology and Nephrology (2019) 51:2073–2081
https://doi.org/10.1007/s11255-019-02253-8

NEPHROLOGY - ORIGINAL PAPER

Losartan accelerates the repair process of renal fibrosis in UUO mouse after the surgical recanalization by upregulating the expression of Tregs

Jie Song[1] · Yangyang Xia[2] · Xiang Yan[3,4] · Jia Luo[2] · Chunming Jiang[2] · Miao Zhang[1,2] · Guo-Ping Shi[4] · Wei Zhu[1,2]

Received: 16 January 2019 / Accepted: 5 August 2019 / Published online: 10 August 2019

Abstract

Obstructive nephropathy is a common cause for chronic kidney disease. Surgery, which is adopted to promptly relieve the obstruction, is the most important method to save damaged kidneys. However, earlier studies have shown that renal function will continue to deteriorate until the terminal stage after the obstruction' relief. The aim of this study is to explore the renal fibrosis and investigate the effect of losartan on renal fibrosis after the obstruction' relief using an improved mouse model of relief for unilateral ureteral obstruction (RUUO). Experiments carried out using C57BL/6 mice ($n = 30$) were randomly divided into RUUO + Losartan group, RUUO group and sham group. Using an improved mouse RUUO model, this study revealed that the mouse kidney for 3- or 7-day unilateral ureteral obstruction undergoing the RUUO surgery was still in a state of injury and fibrosis, while losartan could effectively ameliorate renal fibrosis by upregulating the expression of CD4 + CD25 + Foxp3 + regulatory T cells (Tregs) in kidney after the surgery of RUUO.

Keywords RUUO · Mouse model · Renal fibrosis · Losartan · Tregs

Introduction

Obstructive nephropathy (ON) exerts as the vital pathogenesis factor in CDK which is induced by the dysfunction of urinary flow and eventually contributes to the process of kidney damage. The surgery by relieving obstruction has been acknowledged to suppress the pathological process of kidney injury. The pathological process of renal obstruction plays an important role in the pathogenesis of CKD [1, 2]. However, prolonged kidney fibrosis will fundamentally contribute to the deterioration of kidney after the obstruction was removed. In the pathological process of CDK, the ureteral obstruction resulted in various pathological alterations such as increased pressure of ureter, decreased blood flow of kidney. These pathological alterations effectively activate the renin–angiotensin system (RAS) which will eventually upregulate the expression of angiotensin II (Ang II). The increased expression of Ang II aroused numerous pathological processes such as upregulated expression of transforming growth factor β1 (TGF-β1), renal interstitial cell infiltration and renal tubular apoptosis [3–5]. Furthermore, the activation of the RAS is the leading pathological accelerator which results in the pathogenesis of renal fibrosis.

The RAS system exerted as an important regulation system which effectively maintains and balances blood pressure, water, electrolytes and the homeostasis. The components of RAS consist of renin, angiotensinogen, angiotensin-converting enzyme (ACE) and Ang II plays vital role in correcting water–sodium balance, promoting cell growth, stabilizing the cardiovascular microenvironment and regulating organ inflammation. Earlier studies have confirmed involvement of RAS system in promoting renal interstitial inflammatory infiltration thus causing fibrosis

Jie Song and Yangyang Xia have contributed to the work equally and should be regarded as the joint first authors.

✉ Wei Zhu
zhuwell@163.com

1 Department of Nephrology, The Drum Tower Clinical College of Nanjing Medical University, Nanjing 210008, China

2 Department of Nephrology, Drum Tower Hospital, Medical School of Nanjing University, Nanjing 210008, China

3 Department of Urology, Drum Tower Hospital, Medical School of Nanjing University, Nanjing 210008, China

4 Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

[5]. The underlying mechanism of RAS inhibitors related to ameliorating renal fibrosis has been studied previously using the model of unilateral ureteral obstruction (UUO) [6]. However, the UUO model could not effectively simulate the pathological process after the clinical recanalization, and the second operation was not utilized to remove the obstruction. In contrast, the RUUO model is an appropriate simulation of clinical recanalization of ureteral obstruction, which is more conducive to the study of renal damage and repair after the release of obstruction.

Losartan is an angiotensin receptor 1 (AT1) antagonist that inhibits the activation of RAS system effectively. Many studies have revealed the relationship between RAS and immune system [7, 8]. In a mouse model of multiple sclerosis, the compounds of ACEI could upregulate the expression of Tregs [7]. Sakaguchi et al. [9] study indicated that Tregs exert the most well-characterized CD4+ CD25+ cell with the function of regulating inflammation process and play a pivotal role in the immunoregulatory and inhibitory. In recent years, many studies have shown that Tregs ameliorate the renal fibrosis by regulating immune-inflammatory responses significantly [10, 11].

This study was aimed to investigate the pathological alteration of renal fibrosis in mice for 3- or 7-day UUO and the pharmacological effect of losartan by utilizing the HE and Masson staining method and immunohistochemistry following RUUO. TGF-β1 was an important mediator of interstitial fibrosis thus the expression level of TGF-β1 in urinary was related to the severity of interstitial fibrosis [12]. Therefore, the content of TGF-β1 in urinary was detected by ELISA. To further investigate the protecting function of losartan against the renal fibrosis, the level of Tregs in kidney was detected by Western blot. Exploiting an improved mouse model of RUUO, we probed the specific mechanism of losartan which can improve the renal fibrosis in mice after RUUO surgery.

Materials and methods

Animal subjects

Adult male C57BL/6 mice, aged 6 weeks and weighting 20 g, were obtained from Nanjing Medical University (Production license number: SCXK (Su 2014-0002) and raised in Nanjing Drum Tower Hospital Experimental Animal Center (Use the license number: SYXK (Su 2014-0052). Animals are adapted for 1 week before surgery.

Experimental instrument

Surgical microscope was purchased from Jiangsu Zhenjiang Yihua Optical Instrument Co., Ltd (Zhenjiang, China). Micro-tweezers, micro-shear, with needle suture (model 4-0, 7-0, 10-0, round needle), Teflon tube (inner diameter 0.3 mm, outer diameter 0.6 mm) and syringe (1 ml, 5 ml, 20 ml, insulin needle) were purchased from Shanghai Pudong Jinhuan Medical Supplies Co., Ltd (Shanghai, China). Microvascular clamp (6 mm, medium) was purchased from Ningbo Medical Needle Co., Ltd (Ningbo, China).

UUO operation

The mouse was intraperitoneally anesthetized (0.4 ml/100 g) by injection of 10% chloral hydrate and placed in the supine position on the operating table. The median position of the lower abdomen was selected as the surgical incision. The hair near the incision area was removed with scissors. Skin and muscle are cut layer by layer to gain access to the peritoneal cavity. Forceps was used to expose the left ureter by displacing the intestines towards the right side of the abdominal cavity. The ureter is isolated with forceps. The left ureter near the bladder was ligated twice with 7/0 black braided silk suture. The ureter is cut between the online knots with scissors. The upper line retains a certain length of thread. The abdominal cavity was washed with saline and sutured layer by layer with a 4/0 black braided silk suture. 10% chloral hydrate was purchased from Nanjing Boqiao Biotechnology Co., Ltd (Nanjing, China).

Reimplantation of the obstructed ureter

The RUUO model in which the obstructed ureter was reimplanted into the bladder after UUO [13] effectively simulate the clinical pathological state of ON. Some improvements were utilized on the basis of this method in present study. Three or seven days after UUO, the obstruction was removed using the reimplantation or reimplantation + catheter way. Preoperative preparation was the same as the previous described. The abdominal cavity was opened from the original incision thus left ureter was re-separated. A 20-ml syringe needle was utilized to penetrate the bladder from the anterior wall to the posterior wall while the thread attached in the left ureter was fixed in the hole of the needle. The syringe needle was withdrawn from the bladder in the opposite direction. The end of the ureter was fixed in the anterior wall of the bladder using microvascular clamp. The ureter was sutured on the posterior wall of bladder intermittently with 10/0 black braided silk suture. After suturing, the half of the ureteral wall which located in the 5 mm below the ligature was cut thus urine outflowing was visible. Under the guidance of the insulin needle, a diameter of 6–9-mm PTFE tube was pushed into the ureteral cavity in which the mouth of tube was exposed 1–2 mm. The end of the ureter and polytetrafluoroethylene tube was put back to the bladder as a whole, after that, the anterior wall of the bladder was

sutured and the abdominal cavity was rinsed with the raw salt water. At last, the incision was closed while postoperative treatment was executed (Fig. 1a–f).

Experimental protocol for grouping and administration

Thirty mice were randomly divided into RUUO + Losartan group ($n = 10$), RUUO group ($n = 10$) and sham group ($n = 10$). Seven days after UUO, the obstruction was released by the method of reimplantation + catheter in RUUO + Losartan and RUUO group. For losartan treatment, mice in RUUO + Losartan group were fed water containing 0.1 mg/ml (30 mg/kg per day) losartan starting at the moment of recanalization until day 7 after surgery [14–16]. Mice in other two groups were treated with water (5 ml orally per day). The standard of successful unilateral ureteral obstruction recanalization used in our study is marked no hydronephrosis, no expansion of the ureter. The mice were decapitated at 7-day post-RUUO or 14 days after UUO and the bilateral kidneys were segregated. The kidney was fixed at 10% formalin or stored in a refrigerator at − 80° C. Losartan potassium tablets were purchased from Merck Sharp & Dohme Pty (Nanjing, China).

Renal volume

The renal volume is estimated according to the following equation: $V = \pi LTA/6$.

Histology and morphometric evaluation

The kidney tissue fixed with 10% formalin solution for 24 h was made into 3-μm paraffin sections. HE and Masson staining were performed with conventional methods. The kidney histological sections were quantitative analyzed according to Image Pro Plus 6.0.-1). (1) HE staining of renal tubular injury score: The percentages of histological changes in the kidney tissue were scored using a semi-quantitative scale designed to evaluate the degree of tubular necrosis as follows

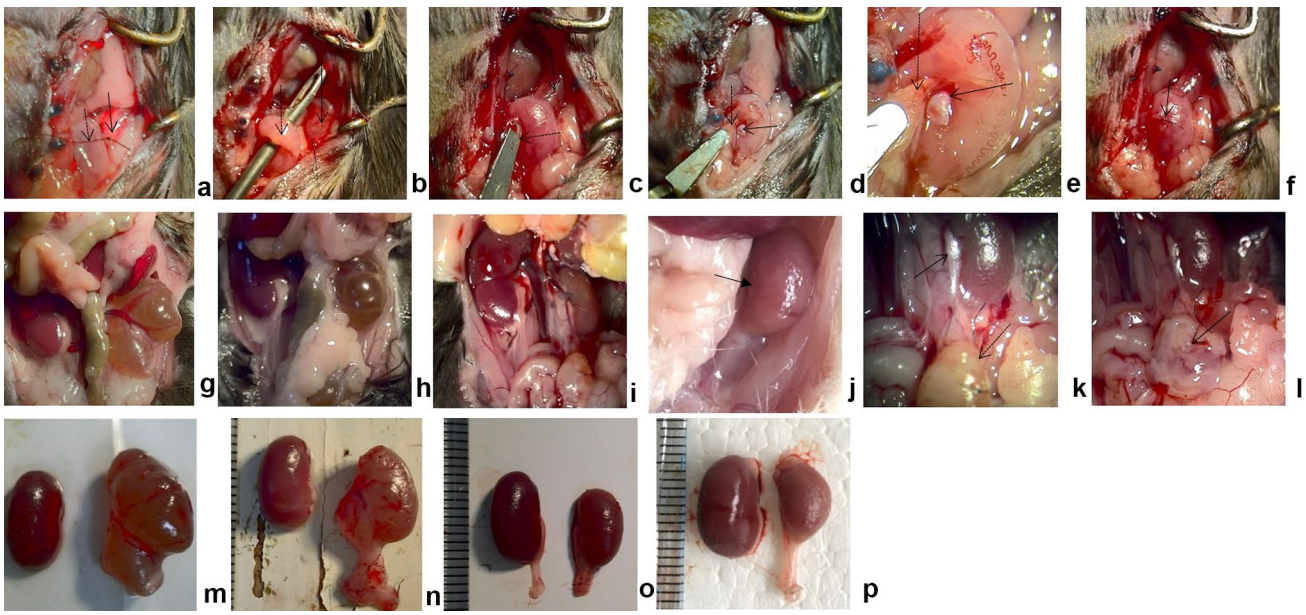

Fig. 1 Surgery pictures. **a** The ureter was found and free under the instructions of the thread left in the first operation. The solid arrow indicates the enlarged ureter. The dashed arrow indicates the filled bladder. **b** The needle of the 20-ml syringe is pushed through the bladder from the anterior wall to the posterior wall. The solid arrow indicates the enlarged ureter. The dashed arrow indicates the bladder. **c** The thread at the end of the ureter is put into the hole of the needle. The syringe needle is withdrawn out of the bladder in the opposite direction. The end of the ureter on the anterior wall of the bladder was fixed by microvascular clamp. The solid arrow indicates the enlarged ureter. The dashed arrow indicates a vascular clamp. **d** Under the guidance of the insulin needle, a diameter of 6–9-mm PTFE tube was pushed into the ureteral cavity with tube mouth exposed 1–2 mm. The solid arrow indicates the PTFE tube. The dashed arrow indicates the ureteral wall. **e** The details of **d**. **f** The end of the ureter and polytetrafluoroethylene tube were put back to the bladder as a whole. The anterior wall of the bladder was sutured. Arrows indicate the anterior wall of the bladder. **g**, **m** The kidney at 14-day post-UUO (figure right) and CUK (figure left). **h**, **n** For 7-day UUO, the kidney at 7-day post-RUUO by reimplantation way (figure right) and CUK (figure left). **i**, **o** For 7-day UUO, the kidney at 7-day post-RUUO by reimplantation + catheter way (figure right) and CUK (figure left). **j**, **p** For 3-day UUO, the kidney at 7-day post-RUUO by reimplantation + catheter way (figure right) and CUK (figure left). The solid arrow indicates no hydronephrosis. **k** For 7-day UUO, the kidney at 7-day post-RUUO by reimplantation + catheter way. The solid arrow indicates no hydronephrosis. The dashed arrow indicates the bladder. **l** The tube was seen in the bladder wall and smoothly, when the bladder in **k** was cut open. The arrow indicates the mouth of the catheter

[17]: 0 = normal kidney; 1 = minimal necrosis (5% involvement); 2 = mild necrosis (5–25% involvement); 3 = moderate necrosis (25–50% involvement); 4 = severe necrosis (50–75% involvement); and 5 = most severe necrosis (> 75% involvement). (2) Masson staining semi-quantitative analysis: Semi-quantitative analysis was calculated according to the percentage of collagen positive area. Each section was randomly selected from the 10 non-overlapping skin at the junction of the visual field (Magnification: 400×) to take the average. Microscope (Nikon Eclipse E100) and digital Camera (NIKON DS-U3) were purchased from Japan. Hematoxylin–Eosin (H&E G1005) and Masson staining (G1006) were purchased from Wuhan Saiwei Biotechnology Co., Ltd (Wuhan, China).

Immunohistochemistry

The expression level of α-SMA in mice kidney was assessed in paraffin-embedded kidney sections (4-μm thick). Immunohistochemical staining was performed as described previously [18]. The results were conducted quantitatively using Image Pro Plus 6.0.-1 system and ten high-power fields (400× magnification) were analyzed separately for each immunohistochemical reaction in renal tissue. EDTA (pH 8.0) antigen repair solution (G1206), DTA (pH 9.0) antigen repair solution (G1203) and Citric acid (PH6.0) antigen repair solution (G1202) were purchased from Wuhan Saiwei Biotechnology Co., Ltd (Wuhan, China). Microscope (CIC, XSP-C204) was purchased from Japan.

TGF-β1 measurements

During the recanalization procedure, fresh urine sample in the left ureter is extracted with the insulin needle after it guides the tube into the ureter. These samples were stored at − 80 °C until they were analyzed. Concentrations of mice TGF-β1 were measured by enzyme-linked immunosorbent assay (ELISA; Ray Biotech, Norcross, USA). Samples were run in duplicate, and the results were read on automated microplate reader.

Western blot analysis

In brief, kidneys were extracted in lysis buffer, and protein concentrations were detected using BCA method. Loading buffer (25 mM Tris, 0.1% SDS, 0.25 M glycine) was supplemented to the samples and boiled at 96 °C for 15 min. Proteins were separated on SDS-PAGE gels and then transferred to PVDF membrane using a transfer buffer (25 mM Tris, 39 mM glycine, 20% methanol) in humid environment. Furthermore, the membranes were blocked with 5% nonfat milk for 2 h, incubated with the Foxp3 primary antibodies overnight at 4 °C. Afterwards, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h. The immunoblotting was determined using the ECL system as instructed by the manufacturer. All kits are purchased from Wuhan Saiwei Biotechnology Co., Ltd (Wuhan, China).

Statistical analysis

SPSS23.0 and GraphPad Instat 6.0 software was aimed for statistical analysis. The measurement data are demonstrated as mean ± standard deviation (SD). Comparisons between multiple groups were performed by a one-way analysis of variance (ANOVA) test followed by Tukey's multiple comparison test appropriately. $P < 0.05$ was considered statistically different.

Results

A reliable RUUO mouse model can be gained using the method of reimplantation + catheter

Comparing with the sham group, the kidneys after 14 days UUO (UUO kidney) were characterized by hydronephrosis, thinning of renal parenchyma and expanded renal pelvis significantly (Fig. 1g, m). When the obstructed kidneys were released by reimplantation way (RUUO kidney), hydronephrotic is not obvious compared to UUO kidney. A certain degree of hydronephrosis of RUUO kidney suggested that the ureter still has some degree of obstruction (Fig. 1h, n). When the obstructed kidneys were released by reimplantation + catheter way (improved kidney), the volume of kidneys was smaller than contralateral unobstructed kidney (CUK), and there was no obvious hydronephrosis in the renal pelvis (Fig. 1i, o, k). When the mouse bladder is cut, the catheter was seen in place smoothly (Fig. 1l), which suggested that the ureter is completely recanalized. The volume of the RUUO kidney was smaller than that of the UUO kidney (139.793 ± 2.78 vs 412.536 ± 31.12 mm^3, $P < 0.05$), which was higher than improved RUUO kidney (139.793 ± 2.78 vs 85.019 ± 12.36 mm^3, $P < 0.05$) (Fig. 2a).

The RUUO model can be established better with an obstruction time of 3 days

In this study, the process of ureteral obstruction was sustained for 3 days. Three days after UUO, the obstruction was relieved by reimplantation + catheter way. Seven days after RUUO, the mice were dissected and analyzed. The results showed that kidneys for 3- or 7-day UUO, the volumes were smaller than CUK, and there was no hydronephrosis (Fig. 1j, p). Histology of the normal kidney reveals glomeruli surrounded by the compact tubules of the tubulointerstitium

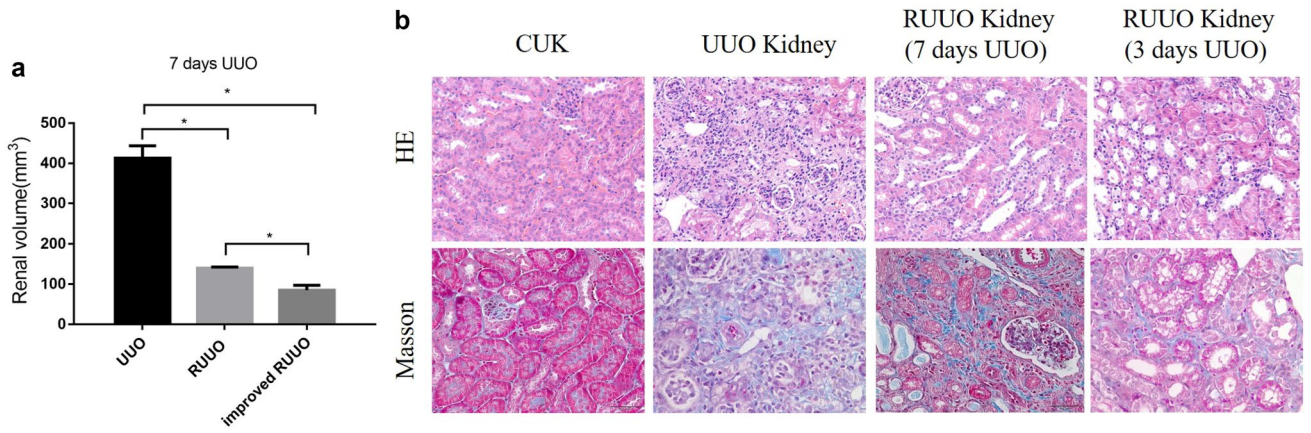

Fig. 2 Renal volume and representative histology. **a** The mice for 7-day UUO were killed at 14-day post-UUO or at 7-day post-RUUO. The length of the kidney *L*, the width *T* and the thickness *A* were measured by the vernier caliper. The renal volume is estimated according to the following equation: $V = \pi LTA/6$. The data were expressed as mean ± SD, $n = 10$. *$P < 0.05$ for the indicated comparison. **b** The healthy interstitium of the normal kidney is comprised of normal compact tubules. The pathological changes of severe fibrosis were observed in UUO kidneys. The pathological changes of RUUO kidneys undergoing 7-day UUO showed renal tubular disorder, brush border loss, lumen expansion and a distinct blue staining area. By contrast, renal tubular damage and blue area in Masson staining is markedly reduced in RUUO kidneys for 3-day UUO. CUK: contralateral unobstructed kidney

with many tubules having a discernible lumen evident. The pathological changes of severe fibrosis were observed in UUO kidneys. The pathological changes of RUUO kidneys undergoing 7-day UUO showed renal tubular disorder, brush border loss, lumen expansion and a distinct blue staining area. By contrast, decreased renal tubular damage, reduction of blue area in Masson staining, and a gradual return toward normal histology were apparent in RUUO kidneys for 3 days UUO (Fig. 2b). However, no matter the obstruction time is 3 or 7 days, renal fibrosis still exists at 7 days after RUUO by comparing with CUK (Fig. 2b).

Losartan protects against renal fibrosis after RUUO

HE staining revealed minor renal tubular disorder, brush border loss, lumen expansion, tube formation and the damage and fibrosis still can be observed in RUUO group, and sham group showed clear renal tubular structure and intact epithelium (Fig. 3). Compared with RUUO group, pathology changes in RUUO + Losartan group were mild. Tubular damage score in RUUO group was significantly higher than that of RUUO + Losartan group (3.9 ± 0.74 vs 2.4 ± 0.48, $P < 0.05$) (Fig. 4a).

As for Masson, there was no obvious blue staining areas in sham group, while RUUO group displayed obvious blue areas, mainly distributed in renal interstitium. RUUO + Losartan group had reduced blue areas compared with RUUO group (Fig. 3). The proportion of collagen area in RUUO + Losartan group was lower than that in RUUO group (5.33 ± 0.64% vs 9.60 ± 1.49%, $P < 0.05$) (Fig. 4b).

Immunohistochemical staining revealed that the expression of α-SMA in sham group was low and mainly expressed in the small vessel wall as well as in the periphery of glomerulus and renal tubule. The expression of α-SMA in RUUO group, mainly in the renal tubular interstitium, was higher than that of RUUO + Losartan group (Fig. 3). The mean IOD of α-SMA in RUUO + Losartan group was lower than that of RUUO group (0.0138 ± 0.0011 vs 0.0203 ± 0.0012, $P < 0.05$) (Fig. 4c).

The mean urine TGF-β1 concentration in RUUO group was significantly higher than that of RUUO + Losartan group (391.5 ± 38.5 vs 209 ± 25.5 ng/l, $P < 0.05$) (Fig. 4d).

Losartan promotes kidney CD4 + CD25 + Foxp3 + Tregs accumulation

At day 7 post-RUUO, the number of Tregs in kidney was tested by Western blot. Compared to RUUO group, the expression of Foxp3 in RUUO + Losartan group increased significantly (0.5987 ± 0.2543 vs 0.0664 ± 0.0080, $P < 0.05$) (Fig. 5).

Discussion

ON exerts as a predominant risk in the incident of CKD. The pathological factors of ON mainly include the damage of renal tubular epithelial cell, the activation of RAS system, the upregulation of oxidative stress, monocyte infiltration and release of inflammatory factors. These pathological factors promote the transformation of renal tubular epithelial

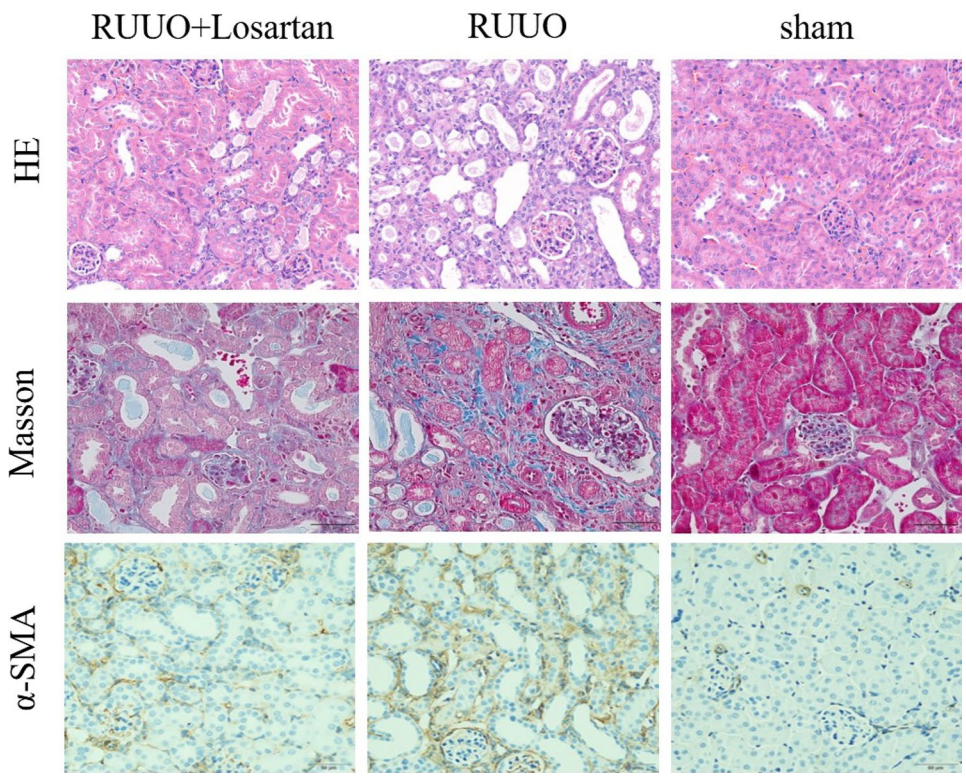

Fig. 3 Representative histological sections (magnification: × 400). HE showed minor renal tubular disorder, brush border loss, lumen expansion, tube formation in RUUO group. Kidneys in sham group showed clear renal tubular structure and intact epithelium. Compared with RUUO group, pathology changes of kidneys in RUUO + Losartan group were mild. As for Masson, there was no obvious blue staining areas in sham group while RUUO group showed obvious blue areas, mainly distributed in renal interstitium. RUUO + Losartan group had reduced blue areas compared with RUUO group. Immunohistochemical staining showed that the expression of α-SMA in sham group was low and mainly expressed in the small vessel wall as well as in the periphery of glomerulus and renal tubule. The expression of α-SMA in RUUO group, mainly in the renal tubular interstitium, was higher than RUUO + Losartan group

cells into myofibroblasts (Epithelial–Mesenchymal Transition, EMT), the maturation of renal intrinsic fibroblasts, and the synthesis and secretion of extracellular matrix (extracellular matrix, ECM) by myofibroblasts and the deposition of collagen. These reactions which were excited by pathological factors ultimately leading to renal fibrosis [19–22]. The surgery aimed to relieve the obstruction was applied in the treatment of ON extensively. Although many studies have indicated that the deterioration of renal function will prolong until the terminal stage after the obstruction was relieved [23]. In 2010, Tipu S and colleagues have found that renal function of mouse for day 1 and day 2 UUO can be restored back to baseline levels after RUUO. But renal function will deviate away from the baseline when the date of obstruction is longer than or equal to 3 days, which may degenerate into the pathological process of CKD in the later stage [24]. The present UUO mouse model largely simulated the pathological process of ON and play a vital role in investigating the pathogenesis of ON [25]. But UUO model was limited to exploring the underlying mechanism of pathological alterations because the renal parenchyma was in the damage state completely in a few weeks while few recognized and stable animal RUUO model was exploded. Therefore, few medical interventions were utilized to intervene the pathological process after the obstruction removed.

Based on previous research, there are three main methods to establish the mouse model of RUUO. Among these methods, the method that vascular and ureter folding device were widely utilized to establish mouse model [24, 26] were relatively simple to operate. However, there was no guarantee that the ureter will be recanalized completely while the renal function could not be evaluated longitudinally. The RUUO model that the obstructed ureter was reimplanted into the bladder after UUO [13] could simulate the clinical pathologic features of ON effectively. In our previous experiments, we explored this method and found that some drawbacks still in the presence of method, such as low success rate of recanalization and incomplete recanalization of ureter, so some improvements were applied on the basis of previous method. The clinical practice that the catheter was placed after ureteral surgery to prevent ureteral strictures was referred in present study. Therefore, an appropriate

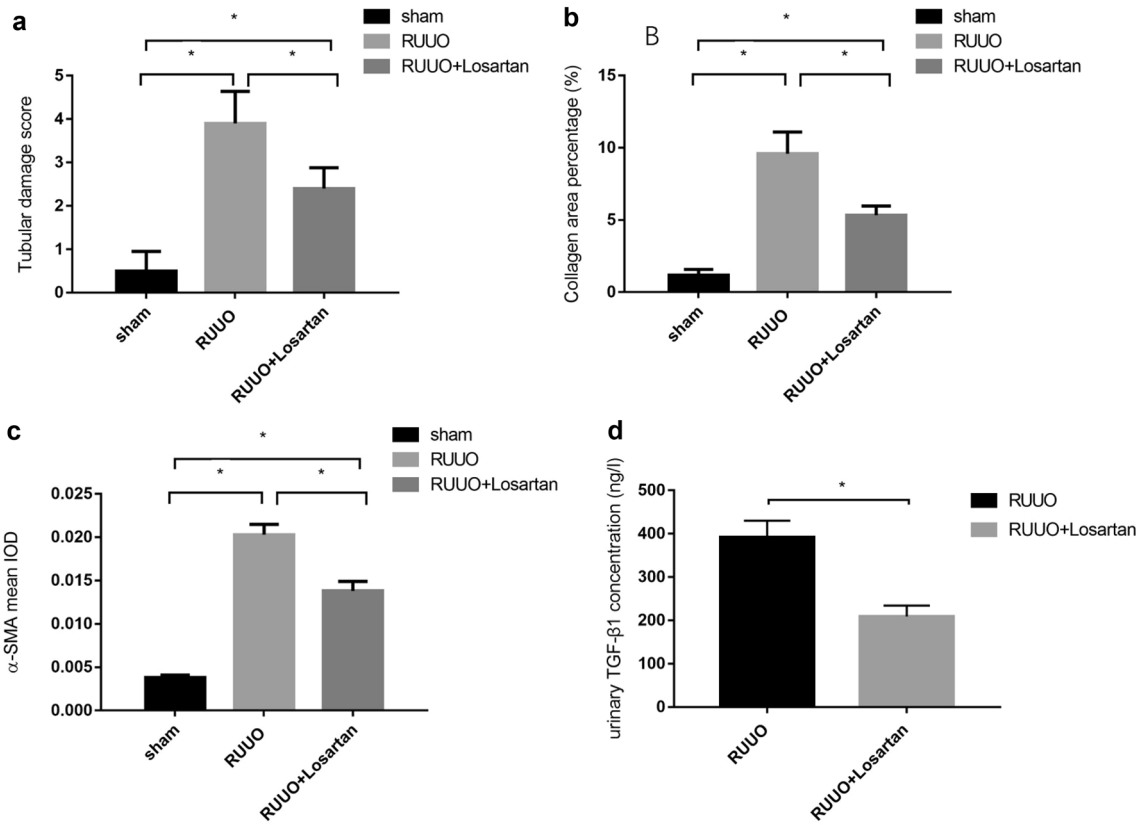

Fig. 4 Indexes of renal fibrosis were detected by HE and Masson staining and immunohistochemistry and the urinary TGF-β1 concentration was tested by ELISA, the data were expressed as mean ± SD, $n = 10$. **a** The tubular damage score in each group. **b** The proportion of collagen area in each group. **c** The mean IOD of α-SMA in each group. **d** The mean urine TGF-β1 concentration in each group. $^*P < 0.05$ for the indicated comparison

catheter was placed in the ureteral bladder connection to prevent ureteral stenosis. The results showed that no hydronephrosis occurred in the kidneys at 7 days after RUUO by comparing with UUO kidneys and CUK, indicating that the obstruction was successfully relieved. The results also indicated that on day 7 post-RUUO, the degree of fibrosis in the kidneys with obstruction time of 3 days was significantly less than the kidneys undergoing 7-day UUO. From these observations, we can conclude that a shorter duration of obstruction result in the amelioration of renal fibrosis and largely increase the survival rate of animals. However, no matter the period of obstruction lasted for 3 or 7 days, renal fibrosis still existed at 7 days after RUUO. The pathological results clearly concluded that compared with CUK, the kidneys exert the pathological changes of renal fibrosis such as tubular epithelial disorder, lumen dilatation and tubular formation at 7 days after RUUO. The results were consistent with Tipu S's study [24]. Therefore, the overall results concluded that surgery to relieve obstruction is not the terminal state for the treatment of ON while the subsequent intervention to inhibiting the pathological process of fibrosis is necessary.

Previous studies have shown the RAS involvement in renal fibrosis in UUO models [27]. The anti-fibrosis effects of ACE inhibitors (ACEI) and angiotensin receptor blocker (ARB) drugs were mentioned in many reports [5, 28]. Previous studies indicated that the RAS is one of the underlying therapeutic targets against the renal fibrosis. However, there are few researches focusing on the anti-fibrosis function of RAS inhibitor after RUUO since the immature of RUUO-modeling technology. In our previous work, we have successfully constructed mouse RUUO model which can better simulate the pathological process of ON. Therefore, the anti-fibrosis effects of RAS inhibitor were verified after the obstruction removed. The utilization of losartan might verify the practicability and reliability of improved RUUO model and explored the effective therapeutic treatment for ON.

In this study, losartan was utilized to ameliorate the renal fibrosis. To simulate clinical practice, the intervention of losartan was initiated at the same time as recanalization. The results in our study indicated that after the RUUO surgery for 7 days the collagen deposition of the kidneys in RUUO group was significantly increased compared with the sham group. The expression of a-SMA, a marker of

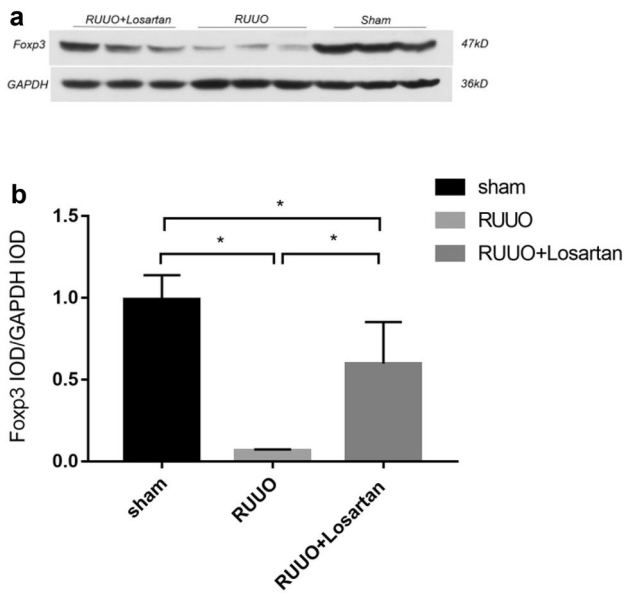

Fig. 5 The expressions of Foxp3 in kidney were detected by western blot, the data were expressed as mean ± SD, $n = 10$. **a** The bands of western blot while GAPDH were used as internal control. **b** Quantitative analysis the percentage of Foxp3 and GAPDH. $^*P < 0.05$ was considered as the statistically significant

myofibroblasts, was also significantly upregulated compared with sham group, suggesting that the kidney is in a fibrotic state. Under the intervention of losartan, our results demonstrated that on day 7 post-RUUO, the renal fibrosis indexes including collagen content and the expression of a-SMA in RUUO + Losartan group were remarkably downregulated compared to RUUO group. Based on our results and observation, we concluded that losartan has an ideal pharmacological effect on improving renal fibrosis after the obstruction' relief. The expression of TGF-β1 in the tissue will increase after activation of the RAS system [29, 30]. TGF-β1 in the kidney promotes glomerular and tubulointerstitial fibrosis by suppressing the degradation of matrix proteins and stimulating the synthesis [31]. Earlier studies have indicated that the urinary TGF-β1 can reflect activity of TGF-β1 and intrarenal production [31, 32]. In current study, under the intervention of losartan, the concentration of TGF-β1 in urine of RUUO + Losartan group was significantly lower than that in RUUO group. This result was consistent with the pathological and immunohistochemical results.

Previous studies demonstrated that Tregs downregulate the expression of proinflammatory cytokines effectively [33, 34]. Based on the previous studies, hypothesis was conducted that losartan exerts the positive function as increasing the level of Tregs in the kidney. Foxp3 was expressed in Tregs specifically, so the expression level of Tregs could be reflected by the expression level of Foxp3. Under this hypothesis, the level of Tregs was detected by Western blot in kidneys at day 7 post-RUUO. In present study, we have shown that the expression of Foxp3 in kidneys increase obviously under the intervention of losartan. Therefore, we could speculate that losartan upregulate the expression of Tregs in kidney, thereby inhibiting the process of inflammation and ultimately ameliorating the renal fibrosis in kidney. The specific mechanism was demonstrated in the further research.

In summary, the present study demonstrated that the mouse kidney undergoing 3- or 7-day UUO is still in a state of injury and fibrosis after the obstruction' relief while losartan can improve renal fibrosis by upregulating the level of Tregs in kidney after the surgery of RUUO.

Conclusion

A reliable improved mouse model of RUUO can be gained by means of using the method of reimplantation + catheter. This surgical method is an appropriate simulation of clinical recanalization of ureteral obstruction, which is more conducive to the study of renal damage and repair after the release of obstruction. Meanwhile, losartan has a better pharmacological effect on improving renal fibrosis after the obstruction' relief.

Acknowledgements This work was supported by Nanjing Municipal Health and Family Planning Commission and Nanjing Health Youth Talent. We are grateful to the Nanjing Drum Tower Hospital Animal Center.

Funding This work was supported by Nanjing Municipal Health and Family Planning Commission (ZKX17018); Nanjing Health Youth Talent (QRX17045).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Keddis MT, Rule AD (2013) Nephrolithiasis and loss of kidney function. Curr Opin Nephrol Hypertens 22(4):390–396
2. Lucarelli G, Ditonno P, Bettocchi C et al (2013) Delayed relief of ureteral obstruction is implicated in the long-term development of renal damage and arterial hypertension in patients with unilateral ureteral injury. J Urol 189(3):960–965
3. Chevalier RL (2006) Pathogenesis of renal injury in obstructive uropathy. Curr Opin Pediatr 18(2):153–160
4. Chevalier RL (2006) Obstructive nephropathy: towards biomarker discovery and gene therapy. Nat Clin Pract Nephrol 2(3):157–168

5. Mezzano SA, Ruiz-Ortega M, Egido J (2001) Angiotensin II and renal fibrosis. Hypertension (Dallas, Tex.: 1979) 38(3 Pt 2):635–638
6. Ucero AC, Benito-Martin A, Izquierdo MC et al (2014) Unilateral ureteral obstruction: beyond obstruction. Int Urol Nephrol 46(4):765–776
7. Platten M, Youssef S, Hur EM et al (2009) Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity. Proc Natl Acad Sci USA 106(35):14948–14953
8. Benigni A, Cassis P, Remuzzi G (2010) Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med 2(7):247–257
9. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25) Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol (Baltimore, Md.: 1950) 155(3):1151–1164
10. Kinsey GR, Huang L, Vergis AL, Li L, Okusa MD (2010) Regulatory T cells contribute to the protective effect of ischemic preconditioning in the kidney. Kidney Int 77(9):771–780
11. Gandolfo MT, Jang HR, Bagnasco SM et al (2009) Foxp3 + regulatory T cells participate in repair of ischemic acute kidney injury. Kidney Int 76(7):717–729
12. Border WA, Noble NA (1998) Interactions of transforming growth factor-beta and angiotensin II in renal fibrosis. Hypertension (Dallas, Tex.: 1979) 31(1 Pt 2):181–188
13. Tapmeier TT, Brown KL, Tang Z, Sacks SH, Sheerin NS, Wong W (2008) Reimplantation of the ureter after unilateral ureteral obstruction provides a model that allows functional evaluation. Kidney Int 73(7):885–889
14. Radovic N, Aralica G, Ljubanovic DG, Jelec V, Kontek M (2014) Effect of unilateral ureteral obstruction and anti-angiotensin II treatment on renal tubule cell apoptosis and interstitial fibrosis in rats. Coll Antropol 38(2):583–588
15. Radovic N, Cuzic S, Knotek M (2008) Effect of unilateral ureteral obstruction and anti-angiotensin II treatment on renal tubule and interstitial cell apoptosis in rats. Croat Med J 49(5):600–607
16. Zhang Y, Kong J, Deb DK, Chang A, Li YC (2010) Vitamin D receptor attenuates renal fibrosis by suppressing the renin-angiotensin system. J Am Soc Nephrol JASN 21(6):966–973
17. Jun C, Qingshu L, Ke W et al (2015) Protective effect of CXCR17(+)CD4(+)CD25(+)Foxp3(+) regulatory T cells in renal ischemia-reperfusion injury. Mediat Inflamm 2015:360973
18. Mei Y, Yangyang Z, Shuai L et al (2016) Breviscapine prevents downregulation of renal water and sodium transport proteins in response to unilateral ureteral obstruction. Iran J Basic Med Sci 19(5):573–578
19. Grande MT, Lopez-Novoa JM (2009) Fibroblast activation and myofibroblast generation in obstructive nephropathy. Nat Rev Nephrol 5(6):319–328
20. Klahr S, Morrissey J (2002) Obstructive nephropathy and renal fibrosis. Am J Physiol Renal Physiol 283(5):F861–F875
21. Nishida M, Hamaoka K (2008) Macrophage phenotype and renal fibrosis in obstructive nephropathy. Nephron Exp Nephrol 110(1):e31–e36
22. Bani-Hani AH, Campbell MT, Meldrum DR, Meldrum KK (2008) Cytokines in epithelial–mesenchymal transition: a new insight into obstructive nephropathy. J Urol 180(2):461–468
23. Wu AK, Tran TC, Sorensen MD, Durack JC, Stoller ML (2012) Relative renal function does not improve after relieving chronic renal obstruction. BJU Int 109(10):1540–1544
24. Puri TS, Shakaib MI, Chang A et al (2010) Chronic kidney disease induced in mice by reversible unilateral ureteral obstruction is dependent on genetic background. Am J Physiol Renal Physiol 298(4):F1024–F1032
25. Chevalier RL, Forbes MS, Thornhill BA (2009) Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. Kidney Int 75(11):1145–1152
26. Chaabane W, Praddaude F, Buleon M et al (2013) Renal functional decline and glomerulotubular injury are arrested but not restored by release of unilateral ureteral obstruction (UUO). Am J Physiol Renal Physiol 304(4):F432–F439
27. Cao Z, Cooper ME (2001) Role of angiotensin II in tubulointerstitial injury. Semin Nephrol 21(6):554–562
28. Gaedeke J, Peters H, Noble NA, Border WA (2001) Angiotensin II, TGF-beta and renal fibrosis. Contrib Nephrol 135:153–160
29. De Muro P, Faedda R, Fresu P et al (2004) Urinary transforming growth factor-beta 1 in various types of nephropathy. Pharmacol Res 49(3):293–298
30. Yang Y, Hou Y, Wang CL, Ji SJ (2006) Renal expression of epidermal growth factor and transforming growth factor-beta1 in children with congenital hydronephrosis. Urology 67(4):817–821 **(discussion 821–812)**
31. Chen X, Zhu W, Al-Hayek S et al (2015) Urinary TGF-1 has a supplementary value in predicting renal function recovery post unilateral ureteral obstruction. Int Urol Nephrol 47(1):33–37
32. Bottinger EP, Bitzer M (2002) TGF-beta signaling in renal disease. J Am Soc Nephrol JASN 13(10):2600–2610
33. Tiemessen MM, Jagger AL, Evans HG, van Herwijnen MJ, John S, Taams LS (2007) CD4+ CD25+ Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. Proc Natl Acad Sci USA 104(49):19446–19451
34. Weirather J, Hofmann UD, Beyersdorf N et al (2014) Foxp3 + CD4 + T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. Circ Res 115(1):55–67