Antifibrotic Role of Captopril after Ureteral Injury

Jiahua Pan Wei Xue Qi Chen Yonghui Chen Haige Chen Yiran Huang

Department of Urology, Renji Hospital Affiliated to Shanghai Jiaotong University, School of Medicine, Shanghai, PR China

Key Words

Ureteral injury · Fibrotic scarring · Cytokine · Captopril

Abstract

Objectives: To evaluate the antifibrotic role of captopril during ureteral scarring in a New Zealand rabbit model. **Materials and Methods:** The tissue expression and the fluctuation of EGF, TGF- β , FN, Col Ia1, Col Ia2 and Col III of the impaired ureter and the contralateral normal ureter were investigated by RT-PCR. The histological changes of the specimens were studied. When the sensitive markers had been selected, 10 New Zealand rabbits were randomly assigned to a captopril group and a control group. The specimens were harvested 2 weeks after the injury and then the histological examination and RT-PCR were performed. **Results:** By RT-PCR screening, EGF, TGF- β , FN, Col Ia1 and Col Ia2 were found to be significantly related to ureteral scarring ($p < 0.05$) confirmed by histological examination. The peak level of EGF, TGF- β and Col Ia1 appeared at 2 weeks after the injury, while for FN and Col Ia2 it was at 3 and 4 weeks after the injury. An obvious reduction of fibrotic scarring was observed in the captopril

group. The expression of EGF, FN and Col Ia2 in the captopril group was significantly lower than in the control group ($p < 0.05$) after the treatment. **Conclusions:** EGF, TGF- β , Col Ia1, Col Ia2 and FN seemed to have an important role in the ureteral scarring after injury. Captopril might partially inhibit the fibrotic process by blocking the EGF, Col Ia2 and FN pathway so that it could be a promising treatment after ureteral injury.

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Introduction

The advent of ureteroscopy has greatly changed the traditional approaches to the diagnosis and treatment of upper urinary tract stones and tumors. Nowadays, ureteroscopic surgery accounts for more than 40% of all endourologic procedures. However, according to epidemiologic studies, the incidence of ureteral injury during ureteroscopic surgery was about 1.5%, of which about 30% of patients developed postoperative ureteral stricture. At present, such ureteral injury complicating upper urinary tract obstruction is a significant impediment to further

the development of the ureteroscopic procedures. Pathological examination proved that fibrotic scarring is the major pathological characteristic responsible for upper urinary tract obstruction. For urethra stricture, Shiraz et al. [1] have reported that early administration of ACE-I gel in the urethra after urethral incision could significantly reduce the recurrence rate of urethral stricture. However, to our knowledge, up till now, no effective clinical intervention has been found to mitigate or stop such a fibrotic process after ureteral injury. In this study, with the RT-PCR technique, we tried to screen and select sensitive markers of ureteral fibrosis after injury in animal model and then tried to use captopril to restrain the fibrotic process of the injured ureter.

Materials and Methods

Establishing Animal Models of Ureteral Injury

The adult male New Zealand rabbit weighing 2 kg (*Oryctolagus cuniculus*) was chosen as the experimental object. Anesthesia was achieved and an abdominal incision was made. After getting into the abdominal cavity, we dissected the left paracolic gutter to access the left kidney and left upper ureter. A full-thickness vertical incision of the ureteral adventitia, muscularis and mucosa of about 0.5 cm was made in the left upper ureter. A hemostat clamp injury was made in the incision part for 1 min to exacerbate the damage. After that, we closed the left paracolic gutter followed by the abdominal cavity. Daily penicillin potassium 800,000 U was given intramuscularly during the first 3 days after the procedure to prevent infection.

Histological Examination

The stricture part of the left upper ureter and a segment of contralateral normal ureter of the animal model were prepared for light-microscopic examination after paraffin embedding. The hematoxylin-eosin stain was used for the histological examination.

Screening the Fibrosis Markers after Ureteral Injury

Five adult male New Zealand rabbits were taken to establish the ureteral injury animal model. The impaired ureter segment (about 2 cm) was harvested 2 weeks after the injury, while the opposite ureter was taken as the control. By real-time quantitative reverse transcriptase PCR (RT-PCR), with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference, we evaluated the expression of EGF, TGF- β , keratin growth factor (KGF), type I collagen $\alpha 1$ (Col Ia1), type I collagen $\alpha 2$ (Col Ia2), type III collagen (COL III) and fibronectin (FN) in the impaired ureter segment and the controls. The SPSS 18.0 was used to perform the paired t test to find out the sensitive markers of ureteral fibrosis.

The primers of fibrotic cytokines of New Zealand rabbits were as follows: EGF 5'-TCTCCTGTTCTCTCTGGGTA-3', 3'-CTGACGGTGAAGAGGTAAGC-5'; TGF- β 5'-AAGGGCTACCACGCCAACTT-3', 3'-CCGGGTTGTGCTGGTTGTAC-5'; KGF 5'-TGAAGTGTCCAGCCCTGAG-3', 3'-TGCCTCGTTTATCA-

ATCCTCA-5'; Col Ia1 5'-CCCCAAGGAGAAGAAGCACG-3', 3'-TGGACATGAGGCGCAGGAAC-5'; Col Ia2 5'-ATGGATGAGGAACTGGCAACT-3', 3'-GCCATCGACAAGAACAGTGTAAAGT-5'; Col III 5'-AAGCCCCAGCAGAAAATTG-3', 3'-TGGTGGAAACAGCAAAAATCA-5'; FN 5'-CTCACCCGAGGCGCCACCTA-3', 3'-TCGCTCCCCTCTCTCCAACG-5'; GAPDH 5'-CCTGCCGCCTGGAGAAAGCT-3', 3'-ACGACCTGGTCCTCGGTGTA-5'.

The RT-PCR parameters were as follows:

RNA Extraction: The ureteral tissue is homogenized in 1 ml Trizol, we then add 200 μ l of chloroform and then leave the EP tube standing upright for 10 min after mixing. Transfer the supernatant (13,000 rpm, 15 min) to a new EP tube; add an equal volume of isopropanol to the new EP tube; centrifuge for 15 min (13,000 rpm) after standing 10 min; discard the supernatant and wash once with 75% ethanol; centrifuge at 13,000 rpm for 10 min; discard the supernatant and let ethanol evaporate for 5–10 min; add 60 μ l water to the dissolved RNA precipitation and measure the RNA concentration by OD₂₆₀.

Reverse: digest genomic DNA – take 1 μ g RNA, add DNase 1 μ l, buffer 2 μ l and add H₂O up to 20 μ l. After digestion at 37°C for 1 h, add 2 μ l stop buffer to inactivate DNase at 65°C for 10 min. Following the instructions of the PROMEGA kit, reverse at 37°C for 1 h and inactivate the reverse transcriptase at 95°C for 2 min.

Quantitative PCR: using the TAKARA Premixed Perfect Real-Time PCR Kit.

Fibrosis Markers' Volatility after the Ureteral Injury

15 male adult New Zealand rabbits (weighing 2 kg) were randomly assigned to 3 groups.

The impaired ureter segment and the contralateral normal ureter segment were harvested at 2, 3 or 4 weeks after the surgery (group A: 2 weeks after injury; group B: 3 weeks after injury; group C: 4 weeks after injury). The tissue expression EGF, TGF- β , Col Ia1, Col Ia2 and FN were observed in different groups in order to find out their high-expression period during the ureteral fibrosis after injury.

Biologic Function of Captopril for the Prevention of Ureteral Fibrosis

10 male adult New Zealand rabbits (weighing 2 kg) were randomly assigned to a captopril group and a control group. Ureteral injury animal models were built as described. Captopril 10 mg/kg/day was given orally in the intervention group while the placebo was administrated in the control group. The ipsilateral impaired ureter segment (area of about 2 cm) and contralateral normal ureter of 2 cm were harvest 2 weeks after injury. By RT-PCR, with GAPDH as an internal reference, we measured the expression of EGF, TGF- β , Col Ia1 and Col Ia2 of the impaired ureter segment and the contralateral ureter segment in both the captopril and control groups. Histological examination was carried out at the same time to compare the fibrotic scarring between the 2 groups. The SPSS 18.0 was used to perform a t test to compare the different expression level of the fibrotic markers between the captopril group and the control group to evaluate the efficacy of captopril in the prevention of a ureter fibrotic process after ureteral injury.

Fig. 1. Normal ureter (a) and ureter with fibrotic scar after injury (b) in an animal model.

Results

The animal model was established successfully. Two New Zealand white rabbits died because of anesthetic accidents. A laparotomy performed 2 weeks after ureteral injury showed obvious retroperitoneal urinary extravasation and different degrees of ureteral narrowing. In some animal models, the impaired ureter turned out to be stiff and thickened. The renal pelvis and ureter were dilated with the renal cortical attenuated. Histological analysis of the stricture parts of the impaired ureter revealed scar formation with disordered collagen deposition and inflammatory infiltrate in the smooth muscle layer and ureteral serosa. The fibroblasts could be found in the collagen matrix (fig. 1).

For screening of the ureteral fibrotic markers by RT-PCR, EGF, TGF- β , FN, Col Ia1 and Col Ia2 proved to be significantly higher in the impaired ureter segment than in the contralateral normal ureter segment ($p < 0.05$), suggesting that these 5 markers might be largely involved in the fibrotic process after ureteral injury in New Zealand rabbits (fig. 2). For the dynamic observation of the expression of different fibrotic markers, EGF, TGF- β and Col Ia1 were highly expressed at 2 weeks after the injury (0.37, 0.12, 22.02), while the peak levels of Col Ia2 and Fn appeared at 3 and 4 weeks after the injury. Therefore, in New Zealand white rabbits, the main period of ureteral fibrosis appeared to be during the first 2 weeks after ureteral injury.

As for the antifibrotic role of captopril, in the experimental group, the elevation of EGF ($p = 0.005$), Fn ($p = 0.022$) and Col Ia2 ($p = 0.009$) were significantly decreased compared to the control group, while the expression of TGF- β ($p = 0.18$) and Col Ia1 ($p = 0.58$) did not show a statistical difference between the 2 groups (fig. 3).

Meanwhile, an obvious reduction of fibrotic scarring was observed in the captopril group compared to the control group at histological examination. There was less collagen deposition in the smooth muscle layer and ureteral serosa, which decreases the thickness of the obstructed ureteral wall in the experimental group (fig. 4).

Discussion

Subject to the protection of the back muscles and retroperitoneal fat tissue, ureteral trauma is quite rare. Ureterscopy or other iatrogenic practice currently account for the majority causes of ureteral injuries. In open surgery, the ureteral injury incidence rate was 0.05–30% [2], especially in gynecologic pelvic surgery and lower abdominal surgery. However, in the endourologic procedure, the injury rate was 1.4–2.3% [3].

After the ureteral injury, a fibrotic process occurred, resulting in ureteral stenosis or blockage, which may further affect renal function. According to some previous reports, about 30% of the ureteral injury caused by the endourologic procedure could finally develop a ureteral stricture. For these cases, a fibrotic scar formation was the main pathologic finding. Pathological scars include hypertrophic scars and keloids, characterized fibroblasts aggregation, extracellular matrix, proteoglycans and collagen deposition. In the late stage of ureteral fibrosis, the ureteral stricture could be found due to collagen fiber retraction. Therefore, the intervention of scar formation and fibrosis process after injury seems to be extremely important. In this study, we have successfully constructed a ureteral injury animal model in adult male New Zealand rabbits and confirmed the phenomenon of ureteral fibrotic scar formation associated with upper urinary

Fig. 2. RT-PCR screening of ureteral fibrosis biomarkers.

Fig. 3. Elevation of ureteral tissue biomarker after the treatment.

Fig. 4. Antifibrotic effect after treatment with captopril (E₁, E₂, E₃, E₄, E₅: mild ureteral fibrosis after injury in the experimental group; C₁, C₂, C₃, C₄, C₅: severe ureteral fibrosis after injury in the control group).

tract obstruction in such an animal model. That this animal model could be an experimental platform might serve future studies on the pathological process after ureteral trauma.

In daily clinical practice, surgery, pressure therapy, radiation therapy and hormone therapy are the most common treatments of pathological scars. Laser therapy and cryotherapy are mainly used for surface wounds and are not suitable for ureteral pathologic scars. In recent years, with the in-depth study of the pathogenesis of pathologic scar, some of the cytokines (such as TGF- β , EGF and KGF) might be considered as therapy target to interfere with the fibrotic process after the ureteral injury. TGF- β is highly expressed in fibroblasts and it is the closest cytokine to the scar formation [4, 5]. Studies have proved that TGF- β is highly expressed in the ureter hypertrophic scar [6]. The role of TGF- β is to guide angiogenesis in a mature wound, to stimulate fibroblast proliferation and extracellular matrix secretion and to induce a fibrotic reaction. In animal experiments, some groups have found [7] that by inhibiting TGF- β expression in the wound, scar formation can be reduced and wound healing can be promoted. EGF is also one of the main factors involved in hypertrophic scar formation [8, 9]. EGF is secreted by platelets, keratinocytes and macrophages. It is a strong mitogenic factor for the epidermal cell and can promote epidermalization and stimulate the synthesis of fibrous tissue. In addition, in vitro experiments have shown [10] that the EGF receptor (EGFR) played a very important role in the fibroblast signaling pathway. When binding EGFR, EGF could switch on the

gene transcription to promote cell division and proliferation of fibroblasts and start further cell migration in wound healing. In the studies of open wounds, it has been proven that EGF can accelerate fibroblast proliferation, contributing to wound healing and scar tissue development [11]. However, there is no basic or clinical research to study the relationship between EGF regulation and pathological scar formation. In addition, other cytokines such as KGF, collagen I, collagen III and FN all participate in the fibrotic process and scar tissue building after the injury. In this study, we first select TGF- β , EGF, KGF, Fn, Col Ia1, Col Ia2 and Col III as prescreening indicators. By the RT-PCR technique, we confirmed that EGF, TGF- β , Col Ia1, Col Ia2 and FN played an important role in ureteral scar formation.

At the same time, since fibrous scar formation and maturation require a relatively long pathological process, we decided to observe the cytokine and collagen expression dynamically at 2, 3 and 4 weeks after the ureteral injury in order to find out the main period of scar formation. Our results confirmed that the peak expression of EGF, TGF- β and Col Ia1 appeared at 2 weeks after the injury. However, Fn and Col Ia2 are highly expressed at 3 and 4 weeks after the injury, respectively. Thus, the tissue fibrosis and scar formation occurred mainly during the first 2 weeks after injury. However, after 2 weeks, the pathological process of fibrosis was not completely stopped and different cytokines are still involved in end-stage fibrous scar formation. Thus, after 2 weeks, with the scar tissue maturation, the efficacy of such an intervention might be notably limited.

In pulmonary fibrosis [12], liver fibrosis [13], Crohn's disease, urethral stricture, and congestive heart failure, angiotensin II was proven to promote collagen I production, accumulation of inflammatory cells and fibroblast growth by the increased expression of TGF- β . At the same time, it could reduce collagenase activity and reduce collagen degradation. In addition, in hypertrophic scars, the fibroblasts expressed angiotensin II receptor type 1 and 2, and angiotensin II regulates the synthesis of collagen of fibroblasts through activation or inhibition of these two receptors. The type 1 receptors can enhance the fibrotic role of angiotensin II, while type 2 receptors can inhibit its function. Moreover, the ratio of angiotensin II and its receptor also affected the scar formation and maturation [14]. The angiotensin-converting enzyme inhibitors (ACE-I) can inhibit the angiotensin-converting enzyme and block the conversion of angiotensin I to angiotensin II, thereby reducing angiotensin II levels within the fibrotic tissue, and then reducing TGF- β expression, resulting in an antifibrosis function [15–17]. Shiraz et al. [1] have reported in patients with urethral stricture, after urethral incision, early administration of ACE-I gel in the urethra could significantly reduce the recurrence rate of urethral stricture. Currently, angiotensin-converting enzyme inhibitors represented by captopril have become an important treatment of congestive heart failure, liver fibrosis, pulmonary fibrosis and other fibrotic diseases. But till now, there have been no in vitro or animal experiments using angiotensin-converting enzyme inhibitors for the prevention and treatment of ureteral hypertrophic scar formation after ureteral injury.

In this study, by the RT-PCR method, we selected EGF, TGF- β , Col Ia1, Col Ia2 and FN as observation markers to test the effectiveness of captopril's antifibrotic role. We give the experimental group oral administration of compound captopril 10 mg/kg/day for 2 weeks after the ureteral injury. The results showed that in the experimental group, EGF, Fn and Col Ia2 at the injury side increased significantly lower than the control group while no significant difference has been found in TGF- β and Col Ia1 between the experimental and control groups. Thus, ACEI could partially block the process of fibrosis after ureteral injury and might reduce the concurrent incidence of upper urinary tract obstruction due to ureteral stricture or stenosis. However, through our experiment, ACE-I had a limited influence on TGF- β and Col Ia1 expression, which might suggest that these 2 cytokines could be also regulated by other mechanisms in addition to the angiotensin pathway. Their tissue expression could not be downregulated by simply blocking the serum level

of angiotensin II. Thus, further studies have to be carried out to clarify the integrity of the ureteral fibrosis regulatory pathway in order to provide clues for further clinical intervention.

Conclusion

Among numerous cytokines, EGF, TGF- β , Col Ia1, Col Ia2 and FN play an important role in ureteral scar formation. Their peak expression appeared at 2 weeks after injury. However, after 2 weeks, the pathological process of fibrosis was not completely stopped and different cytokines were still involved in the end-stage fibrous scar forming. Captopril could partially block the process of fibrosis after ureteral injury by reducing EGF, Col Ia2 and FN expression so that it could be a promising treatment to decrease scar formation after ureteral injury. Further studies have to be carried out to clarify the integrity of the ureteral fibrosis regulatory pathway in order to provide clues for further clinical intervention.

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Disclosure Statement

There is no conflict of interest to be declared.

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