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Matrix Metalloproteinase-2, Matrix Metalloproteinase-9, and Transforming Growth Factor Beta 1 Levels in *Escherichia coli*-Infected Rats with Acute Pyelonephritis

Juan Ji[1†], Zhaoyu Bi[1†], Ling Tian[1], Limin Zhang[1], Xing Fan[1], Jing Li[1], Song Li[2*]

[1]Department of Nephrology, Affiliated Hospital of Hebei University, Baoding 071000, Hebei Province, China

[2]Department of Urology, Affiliated Hospital of Hebei University, Baoding 071000, Hebei Province, China

[†]These authors contributed equally to the work.

***Corresponding author:** Song Li, 15830201177@sina.com

Abstract: *Objective:* To investigate the expression of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), and transforming growth factor beta 1 (TGF beta 1) in the kidney tissue of rats with pyelonephritis and their relationship with pyelonephritis by establishing a rat model of acute pyelonephritis. Methods: 80 male Wistar rats were randomly divided into a control group and an experimental group, with 40 rats each. The rats of the control group were injected with and saline and those of the experimental group were injected with 10 μg/mL *Escherichia coli* (*E. coli*) and saline (1:100); the solutions for both groups were administered every 3 days for 7 days. The expressions of MMP-2, MMP-9 and TGF beta 1 in the kidney tissues of rats in each group were observed. *Results*: The expression of MMP-9 and TGF beta 1in the kidney tissue of rat acute pyelonephritis model rats was significantly higher than those of the control group ($P < 0.01$); the MMP-9 mRNA content in the kidney tissue of the experimental group was significantly higher than that of the control group ($P < 0.05$); the TGF beta 1 mRNA content in the renal tissue of the experimental group increased significantly compared to the ($P < 0.05$); MMP-2, MMP-9 and TGF beta 1 began to express in the early stage of pyelonephritis until the complete formation of renal pelvic edema. The difference between groups was statistically significant ($P < 0.01$). *Conclusion:* MMP-9 and TGF beta 1 are important factors regulating renal tubular epithelial cell injury and inflammatory response.

Keywords: *Escherichia coli*; Acute pyelonephritis; MMP-2; MMP-9; TGF beta 1

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1. Introduction

Acute pyelonephritis is a common urologic disease and a serious threat to human health [1]. It is an infectious disease caused by *Escherichia coli* (*E. coli*) with mainly urinary symptoms, which occurs in patients with pyelonephritis [2–4]. *E. coli* is a gram-negative bacterium with 18 serotypes isolated from human Escherichia coli, the detection rate of smooth strains is only 14.4% [5-6]. The smooth strains mainly infect the epithelial cells of the kidney and genitourinary system, with the renal epithelial cells and bladder epithelial cells predominating. In addition, *E. coli* has been reported in skin and mucosal infections [7-8]. Podocytes produced by *E. coli* are an important factor in causing pyelonephritis. The strains used in the experiments were mainly derived from human and animal urine, feces, and urine smears, all of which were fresh

specimens. Pyelonephritis is mainly characterized by positive urine bacterial culture with back pain and fever. The results of treatment of urinary tract infection (UTI) with conventional antibiotics are unsatisfactory in many cases. Our study found that antimicrobial or anti-infective drugs showed no statistically significant differences in terms of bacterial clearance rate compared to conventional antibiotics, indicating that the use of antimicrobial agents is effective in the treatment of urinary tract infection.

It is currently believed that pyelonephritis is associated with inflammatory response, apoptosis, impaired renal function, and tissue fibrosis. Therefore, in this study, the role of matrix metalloproteinase (MMP) in the pathogenesis of pyelonephritis and its relationship with the aforementioned pathological changes is investigated by sampling kidney tissue specimens from rats and performing RT-PCR assay.

2. Information and methods

2.1. Materials

(1) Experimental strain: Escherichia coli was selected, numbered 1.08×10^6 CFU/mL. (2) Laboratory rats : 80 SPF-grade Wistar rats was purchased from Shanghai Pasteur Laboratory Animal Co.

(2) Reagents and consumables: Dulbecco's Modified Eagle Medium (DMEM) buffer for bacterial culture, phosphate-buffered saline (PBS) buffer, and deionized water; all reagents were prepared with ultrapure water.

(3) Antibiotics: Cephalosporin D, streptomycin, and gentamicin were purchased from Androsi Co.

(4) Main reagents: trypsin and proteinase K were purchased from Elaston, USA; pancreatic rennet and thrombin were purchased from Shanghai Bailingway Biotechnology Co. All animals were strictly sterilized and used once.

(5) The observation time was 1, 3, and 4 days after the animals were infected, and the animals were tested every 3 days until the end of week 4, and one animal was killed from each group every week for analysis. The experiment used 3×10^6 CFU/mL of *E. coli* (from Beijing Wanbang Biological Products Co., Ltd.) to infect Wistar rats.

2.1.1. Source of strains

A total of two *E. coli* vaccines, numbered S1-08.1 and S6-08.8.1 (successfully infected and isolated in rats, respectively), were obtained in this experiment.

2.1.2. Animal handling

In the experimental group, ten rats were inoculated with *E. coli* through the abdominal cavity each day on the 1st, 3rd, and 4th day. DMEM and PBS buffer solution were injected intraperitoneally, and corresponding doses of normal saline were administered into the stomach on the 1st, 3rd, and 4th day after infection to maintain blood urea nitrogen (BUN) and serum creatinine (Scr) levels. The experimental group and the control group were administered with trypsin K solution on the 1st and 3rd day respectively. The concentration of MMP-2, MMP-9, transforming growth factor beta 1 (TGF beta 1) in the serum of animals in each group by immunofluorescence labelling methods. On day 5, zinc sulfate solution administrated intragastrically to induce cell apoptosis; on day 6, an infected rat was sacrificed, and the kidney tissue of the rat was taken and stained with H & E and immunohistochemical method to detect the expression of MMP-2, MMP-9, TGF beta 1. Note: The rats were not in contact with humans and did not engage in any activities that would interfere with the experimental operation (including all operations not related to the experiment) during the course of this experiment.

2.2. Measurement method

Immunohistochemical staining was performed on the rats within 3 days after inoculation to observe the

infection and to determine the levels of MMP-2 and MMP-9.

(1) Serum specimen collection
1 mL kidney tissues were extracted on day 7 after staining and were washed in PBS, and then immersed 10% neutral buffered formalin for 5 min. After digestion with trypsin, the supernatant of the blood sample was centrifuged and transferred onto a 2 mL slide with 6-fold dilution of antimicrobial agent-streptomycin (BSP) solution. The samples were then measured using a fully automated immunohistochemistry analyzer.

(2) MMP-2
1 mL of serum specimen supernatant was immersed in PBS buffer for 5min [9]. 10μl of 2-fold diluted streptomycin solution was then added, followed by 5 μl of 2 μg MMP-2 standard solution.

(3) MMP-9
3 mL of serum samples were centrifuged and transferred to a 100 mL centrifuge tube and stored at 4℃. 2.5-fold diluted 10μl of diluted antimicrobial agent-streptomycin solution were added into the serum sample. Next, the activity of MMP-9 was measured at the wavelength of 520 nm using an enzyme label.

2.2.1. H & E staining

Two kidney tissues were extracted and immersed in saline, soaked for 10 min, then rinsed and sectioned with distilled water. The expression levels of MMP-2, MMP-9 and TGF beta 1 were determined by polymerase chain reaction (PCR) and Western blot method.

2.2.2. Enzyme-linked immunosorbent assay (ELISA)

The levels of MMP-2, MMP-9 and TGF beta 1 expressions were measured. SPSS 13.0 statistical software was applied to study the data for statistical analysis and *t*-test was used for statistical analysis.

2.2.3. Real-time fluorescence quantitative PCR (RT-PCR) method

RT-PCR reaction was performed by adding 50 μL of RT-PCR system containing total RNA [3]. In the PCR amplification process, the samples were first pre-denatured at 95 °C for 2 min, extension was then carried out at 95 °C for 40 s followed by extension at 94 °C for 30 s, and extension at 72 °C for 1 min. The samples then underwent pre-denaturation at 72 °C for 3 h followed by extension at 72 °C for 40 s. The PCR products were then analyzed by agarose gel electrophoresis. The amplified products were recovered by gel and then subjected to RT-qPCR. The PCR amplification efficiency and concentration were measured by enzyme markers. The samples were amplified at 94℃ for 20s and 95℃ for 10s, respectively, 35–45 cycles each, for 5 times. Real-time fluorescence quantification was performed for each component of the PCR reaction system. The optical density of each treated sample was measured using an enzyme standardization instrument (Eco-glucose), and the relative standard deviation (RSD) of the standard curve was calculated. The concentration of bacterial DNA was determined by real-time fluorescence quantification PCR using RPMI-1640 automatic enzyme marker (Beijing Meridian Biotechnology Co., Ltd.) and DHI (Shanghai Anke Biotechnology Co., Ltd.). The PCR results were detected by agarose gel electrophoresis analysis.

2.2.4. Western blot analysis

Kidney tissues were added onto SDS-PAGE gel containing 5% FBS, and 100 μL dsDNA was added; the sample was then incubated at room temperature for 30 min, and protein expression was detected using a Western blot kit. SDS-PAGE electrophoresis was performed on the samples. Next, 1–3μL sample was extracted and FBS and SDS-PAGE gel was added into the sample, mixed well, and then incubated at room temperature for 6 hours. The absorbance of the protein was determined using a microplate reader at 250 nm and 400 nm. The optical density, programmed cell death (PD) value and protein concentration of MMP-2,

MMP-9 and TGF beta 1 were calculated using a computer software. The concentration of MMP-9 and TGF beta 1 in kidney tissue represents the expression level.

3. Results

3.1. Expression of MMP-9 and TGF beta 1 in renal tissues

The expression of MMP-9 and TGF beta 1 was significantly higher in renal tissues of the experimental group. In addition, MMP-9 and TGF beta 1 were also expressed in renal interstitial cells, suggesting that renal interstitial cells could play a role by secreting corresponding proteins. The levels of renal matrix MMP-9 and TGF beta 1 were elevated, while SMAD2 showed a negative correlation with its mRNA expression level in renal tissues. Meanwhile, MMP-9 and TGF beta 1 promoted the proliferation of renal tubular epithelial cells and induced the transformation of epithelial-derived cells. Both MMP-9 and TGF beta 1 can stimulate the proliferation and migration of glomerular thylakoid cells, vascular endothelial cells and other renal parenchymal cells; they can also promote the proliferation of renal parenchymal cells by activating the JAK2/STAT3 signaling pathway. MMP-9 induces translocation from the renal tubular epithelium to the myofibroblast membrane by binding to SMAD2 receptors on the myofibroblast membrane. In addition, both MMP-9 and TGF beta 1 in renal tissues act through stimulation of vascular endothelial cell proliferation, migration, and angiogenesis.

3.2. Levels of TGF beta 1 in renal tissues of rats with pyelonephritis

TGF beta 1 in renal tissues was mainly secreted by renal anterior mesenchymal cells, while the level of TGF beta 1 in perinephric tissues and glomerular epithelial cells was relatively low. The results of this study showed that the expression level of TGF beta 1 in renal tissues of rats with pyelonephritis was significantly higher than that of the control group ($P < 0.05$), suggesting that the secretion of TGF beta 1 in renal tubular epithelial cells may be one of the main factors of renal inflammatory injury.

3.3. Levels of MMP-2 in renal tissues of rats with pyelonephritis

The results of this experiment also showed that the levels of MMP-2 and IL-6 in the renal tissues of rats with pyelonephritis were significantly higher than those of the control group, suggesting that the damage of kidneys of rats with pyelonephritis may be related to infection. The results of this study showed that both MMP-2 and IL-6 were expressed in renal tissues, but MMP-2 was relatively more abundant. Therefore, it was hypothesized that MMP-2 was expressed more in renal tissues than MMP-9.

4. Discussion

Pyelonephritis is a relatively common infectious disease that can lead to kidney damage. The kidneys of patients with pyelonephritis often have pathological changes such as inflammation and fibrosis, which eventually lead to decreased kidney function or even failure. MMP-9 is a cytotoxic protease that causes glomerular and tubular epithelial cell damage and apoptosis. It is involved in the development of renal inflammatory response, chronic renal insufficiency, and glomerular disease. It was found that MMP-9 is differentially expressed and varies in different types of kidney disease; patients with pyelonephritis can also show elevated or decreased MMP-9 when renal function decreases. Therefore, we speculate that MMP-9 may be one of the important molecular mechanisms in the pathogenesis of pyelonephritis. TGF beta 1 is mainly a cytotoxic polypeptide that plays an important role in angiogenesis and tissue repair. TGF beta 1 can affect normal tissue morphogenesis by regulating the TGF beta 1/SMAD3 signaling pathway. The expression level of TGF beta 1 significantly increased in the renal tissues of rats with pyelonephritis model, which suggested that TGF beta 1 in renal tissues was involved in immune cell-mediated pathological changes during the formation of renal cysts.

In this study, we investigated the relationship between the levels of MMP-9 and TGF beta 1 in the

kidney during the pathogenesis of pyelonephritis and their roles in the development of renal injury by establishing a rat model of acute pyelonephritis, which provides new ideas, methods, and techniques to explore the mechanisms of pyelonephritis and immune injury [10-15].

Main results: (1) 10 μg/mL of *E. coli* was injected intraperitoneally into rats every 3 days for 7 days, with no deaths or abnormalities during the process of infection. Serum and urine specimens were routinely examined and both kidney tissues were retained and sent to the pathology department for examination. (2) Kidney tissue specimens were stained with H&E staining, which showed that the kidney tissue was structurally intact, homogeneous, and morphologically normal; the edema of the renal pelvis and surrounding tissues was obvious but not significant. (3) MMP-9 and TGF beta 1 in serum and urine specimens were both higher than those before modeling at the second week after successful modeling, but there was no significant difference. (4) At the time of kidney tissue sampling at week 2 after successful modeling, both intrarenal MMP-9 and TGF beta 1 assays were higher than those of the control group. (5) Both serum MMP-9 and TGF beta 1 assays could be detected in the kidney of the rats. (6) The levels of MMP-9 and TGF beta 1 in the kidney of rats increased after being infected with *E. coli* after 10 μg/mL administration at the second week.

5. Conclusion

The successful modeling can be used for subsequent experimental studies. Pyelonephritis is a common urinary tract infection caused by a variety of bacteria, which is increasingly prevalent in recent years. Clinically, it can manifest as a recurrent, prolonged, and intractable disease. It is now mainly believed that pyelonephritis is associated with apoptosis and necrosis caused by the action of bacterial toxins and endotoxins. During the inflammatory phase, patients with pyelonephritis experience increased apoptosis in the kidney, increased tissue damage, and a significant decrease in renal function. Therefore, it is necessary to formulate an effective treatment method. Currently, antibiotics, diuretics and glucocorticoids are commonly used. However, the degree of renal parenchymal damage varies, and antibiotics and glucocorticoids cannot completely remove the pathogenic bacteria from the body and lead to kidney damage. Besides, long-term application of antibiotics will cause some side effects. Therefore, care should be taken to reduce the course of antibiotics or avoid misuse of antibiotics in clinical application. MMP-9 and TGF beta 1 levels are elevated in the kidneys of patients with pyelonephritis, so the development of renal injury can be influenced by altering MMP-9 and TGF beta 1 levels during the development of pyelonephritis. The present study showed that MMP-9 and TGF beta 1 levels were significantly higher in the renal tissues of rats with pyelonephritis than in the control group at the early stage of acute pyelonephritis until the complete formation of pelvic edema. In this study, we established a rat nephropathy model of pyelonephritis and investigated the expression of MMP-9 and TGF beta 1 in renal tissues and their relationship with pyelonephritis.

In summary, MMP-9 and TGF beta 1 are important factors regulating renal tubular epithelial cell injury and inflammatory response. They are expressed early in acute pyelonephritis until the renal pelvis becomes enlarged, and decreased renal function (urinary protein and creatinine clearance) with varying degrees of tubular injury can occur. Therefore, a rat nephropathy model was established in this study. By measuring the levels of MMP-9 and TGF beta 1 in renal tissues, the role of MMP-9 and TGF beta 1 levels in renal tissue in the development of pyelitis could be initially determined. Since the degree of inflammatory response in renal tissues is closely related to the degree of renal injury, this study provides a new method for understanding renal injury in patients with acute pyelonephritis.

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Disclosure statement

The authors declare no conflict of interest.

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