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Intervention effect and mechanism of curcumin in chronic urinary tract infection in rats

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ABSTRACT

Objective: To analyze the invention effect of curcumin on chronic urinary tract infection in rats and explore its possible mechanism of action.

Methods: The experimental animals were randomly divided into three groups, normal, model and curcumin group. Chronic urinary tract infection models were built for model group and curcumin group by injecting coliform fluid into the cavity of bladder. From the first day of modeling, rats in the curcumin group were injected with 150 mg/kg curcumin, while rats in normal group and model group were given no other treatment. The treatment lasted for 14 d. The white blood cell counts in blood and urine, bacterial colony count in urine and renal tubular functional indexes of rats in all groups at day 1, 7, and 14 after treatment were detected. Urine β2-microglobulin (β2-MG), urinary N-acetyl-β-D glucosaminidase (NAG) levels were used to detected the inflammatory cytokines in serum after treatment including the contents of IL-6, IL-8, IL-10 and monocyte chemoattractant protein-1 (MCP-1), and real-time PCR was employed to determine the expression of mRNA of toll-like receptor 2 (TLR-2) and TLR-4 in renal tissues and bladder tissues of all groups after treatment.

Results: The white blood cell counts at day 1 and 7 after treatment in rats of model group and curcumin group were significantly higher than those of normal group at the same time points, while the white blood cell counts of the curcumin group were significantly lower than those of model group ($P < 0.05$). The urine white blood cell counts in rats of model group at day 1, 7 and 14 were all significantly higher than those of normal group at the same time points; those in the curcumin group were significantly lower than those of the model group at day 1, 7 and 14 at the same time points ($P < 0.05$). The bacterial colony counts of urine in rats of model group and curcumin group at day 1, 7 and 14 were all significantly higher than those of normal group at the same time points, while the counts of curcumin group were significantly lower than those of model group at the same time points ($P < 0.05$). Levels of urine β2-MG, NAG, IL-6, IL-8, IL-10, MCP-1 and expression of TLR2 mRNA and TLR4 mRNA in renal and bladder tissues in rats of model group were significantly higher than those of the normal group, while these variables of the curcumin group were significantly higher than those of the normal group but lower than those of model group ($P < 0.05$).

Conclusions: Curcumin can significantly improve the symptoms of chronic urinary tract infections, protect renal tubular function, and also decline inflammatory responses by influencing the expressions of TLR2 mRNA and TLR4 mRNA so as to exert its curative effect on chronic urinary tract infections.

1. Introduction

Urinary tract infection is a common infectious disease in the urinary system caused by pathogens invading the urinary tract and leading to urinary tract inflammatory lesions. Clinically, urinary tract infection mainly includes urethritis, cystitis and pyelonephritis [1,2]. Epidemiological data have shown that the prevalence rate of the disease is about 0.9%, and it is more prevailing in female group than males [3,4]. Urinary tract

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infection is common in women of child-bearing age and rural woman. Since urinary tract infection is characterized by repeated outbreak, long disease course, protracted and refractory treating process and difficulty for treatment, a serious of severe complications can occur with the development of the disease with poor prognosis [5,6]. Although antibiotics can alleviate the conditions to a certain extent, they cannot decline the recurrence rate and improve the prognosis of the diseases. In particular, with the application of the broad-spectrum antibiotics in recent years, pathogens of urinary tract infection have shown different degrees of antibiotic resistance, which, as a result, affects the therapeutic effect [7]. At present, it is rather difficult to treat urinary tract infection in clinic. An in-depth understanding of the pathogenesis of this disease is of great clinical significance for seeking new approaches and therapeutic drugs to prevent and treat the disease.

Chronic urinary tract infection is a kind of chronic inflammatory infectious disease. Studies have reported that many inflammatory cytokines are highly expressed in serum of patients with chronic inflammatory infectious disease [8–10]. Toll like receptor, a kind ofltrans-membrane receptor of the pathogen-associated molecular pattern, can cause inflammatory chain reaction [11]. In addition, it is found that toll like receptor has something to do with the activation of innate immunity [12] and identification of pathogenic bacteria [13]. Curcumin is the major component of *Carcuma* longa. It is a plant polyphenol with broad biological activities including immunoregulation, anti-inflammation and so on [14,15]. Curcumin has been used in clinic to treat multiple inflammatory diseases including ulcerative colitis [16] and has achieved significant efficiency. However, no report on the intervention effect of curcumin on chronic urinary tract infection has been found. Therefore, this study aimed to observe the effect of curcumin on chronic urinary tract infection and explore its possible mechanism of action by establishing rat models with chronic urinary tract infection given curcumin intervention.

2. Materials and methods

2.1. Materials

2.1.1. Reagents and drugs

Curcumin was made into 40 mg/mL suspension by Sinopharm Chemical Reagent Co. Ltd. (batch number: 8239401). The 7020 automatic biochemical analyzer (Hitachi, Japan), BCC-3000B blood-cell counter (Shanghai Huanxi Medical Instrument Co. Ltd., China), and mission U500 urine analyzer (ACOM, Hangzhou) were employed. Interleukin (IL)-6, IL-8, IL-8, and monocyte chemoattractant protein-1 (MCP-1) reagents were all purchased from Boster, Wuhan; iMark ELIASA was from BIO-RAD, USA. ABI7500 real time PCR was from Applied Biosystems, USA. The related primers were compounded by Norman Biological Technology, Nanjing. Amplification reaction kits, Trizol kits and Trizol tissue lysate were all bought from BD, USA.

2.1.2. Pathogenic bacteria

Standard *Escherichia coli* (ATCC25922) (*E. coli*), bought from the Pathogen Biology Laboratory of Experimental Center, College of Lab Medicine, Hebei North University, were made into 300 million/mL *E. coli* solution for standby application.

2.1.3. Experimental animals

Healthy SD rats (160–200 g) were bought from Hebei Experimental Animal Center with production license of SCXK (Yi) 2008-1-003. Animal feeding and the subsequent experiments were all done in Hebei Experimental Animal Center. The feeding conditions included natural sunlight, free diet, 20–23 °C room temperature. Feeding and the subsequent experiments were all met the requirements of Manipulative Technique for the Care and Use of Laboratory Animals. The study was approved by the Ethics Committee of our hospital.

2.2. Methods

2.2.1. Groups and modeling

The experimental animals were randomly divided into three groups: normal group, model group and curcumin group. Each group had 5 males and 5 females. Animals were supplemented if the experimental animals died halfway. Chronic urinary tract infection models were built for the model group and curcumin group. Detailed procedures were described as follows: rats were forbidden to drink for 24 h, and then given urethane for intraperitoneal anesthesia. Lower abdominal hairs were removed after fixing their four limbs. A 3 cm incision was made in the middle of the abdomen to expose the abdominal cavity. Then, 0.1 mL *E. coli* solution was injected intravesically. The ureter was ligatured and the incision was sutured. The abdominal cavity was closed. The experimental animals then drank and eat normally. Twenty-four hours later, the ligature string was removed.

2.2.2. Drug administration

From the first day of modeling, rats in the curcumin group were injected with 150 mg/kg curcumin, while rats in the normal group and model group were given no other treatment but free access to forage and drinking water. The treatment lasted for 14 d.

2.2.3. Sample collection and management

After the last drug administration, abdominal aorta blood and the 24 h urine were collected. Rats were sacrificed, and their mucosal tissues of the trigone of urinary bladder and the left pelvis were taken out and fixed with formalin, and cryopreserved for further detection.

2.2.4. Routine blood and urine tests and colony counts

Automatic blood-cell counter and urine analyzer were used to measure the white blood cell counts in blood and urine and colony counts in urine, respectively.

2.2.5. Detection of renal tubular function

Automatic biochemical analyzer was applied to urine β2-microglobulin (β2-MG) and urinary N-acetyl-β-D glucosaminidase (NAG). NAG was determined by glucoside, and β2-MG was detected by radioimmunoassay.

2.2.6. ELISA

ELISA was used to test the contents of serum inflammatory cytokines IL-6, IL-8, IL-10 and MCP-1. The first step was to establish standard curve. Next step was to dilute the sample. Then, the tested sample was added. After the plate was washed by cleaning mixture and dried by filter paper, biotinylated

antibody was added. Again, after the plate was washed by cleaning mixture and dried by filter paper, enzyme conjugate was added. TMB solution was added finally. The absorbances were recorded and curves were drawn.

2.2.7. Real-time PCR

Real-time PCR was used to detect the expression of mRNA of toll-like receptor 2 (TLR-2) and TLR-4 in renal tissues and bladder tissues. A total of 0.1 g renal tissue and bladder tissue was collected and ground into tissue homogenate. Kits were used to extract total RNA. Reverse transcription kits were used for reverse transcription. Fluorescent quantitative PCR was used to for amplification with the amplification conditions of pre-denaturation 95 °C, 30 s; denaturation 95 °C, 5 s; anneal 60 °C, 34 s; and annealing extension 60 °C, 1 min. Forty amplification circles were conducted. The conditions of annealing extension in the last amplification circle were 72 °C and 10 min. After that, the sample was preserved with GAPDH gene serving as the control.

2.3. Statistical management

SPSS19.0 was applied for variance analysis and *t*-test. All measurement data were expressed as mean ± SD. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of white blood cell counts in blood and urine and colony counts in urine of all groups at different time points

The white blood cell counts in blood at day 1 and 7 after treatment in rats of model group and curcumin group were significantly higher than that of normal group at the same time points, while the white blood cell counts of the curcumin group were significantly lower than that of the model group ($P < 0.05$). The white blood cell counts in blood at day 14 in rats of the two groups increased but showed no significant significance compared with that of the normal group at the same time point ($P > 0.05$). The urine white blood cell count in rats of model group and curcumin group increased firstly and then decreased. Besides, the urine white blood cell counts in rats of model group at day 1, 7 and 14 were all significantly higher than those of the normal group at the same time points; while those in the curcumin group were significantly higher than those of the normal group at day 1 and 7 and significantly lower than those of the model group at day 1, 7 and 14 at the same time points ($P < 0.05$). The bacterial colony counts in urine in rats of model group and curcumin group increased firstly and then decreased as well. The counts of the two groups at day 1, 7 and 14 were all significantly higher than those of the normal group at the same time points, while the counts of the curcumin group were significantly lower than those of the model group at the same time points ($P < 0.05$) (Table 1).

Table 1
Comparison of white blood cell counts in blood ($\times 10^9$ per/mL) and urine ($\times 10^3$ per/mL) and colony counts in urine (×10E5 CFU/mL) of all groups ($n = 10$) at different time points.

Group	Time	White blood cell counts in blood	White blood cell counts in urine	Bacterial colony counts in urine
Normal	Day 1	6.41 ± 0.48	2.21 ± 0.08	4.89 ± 0.68
	Day 7	6.52 ± 0.51	2.16 ± 0.09	5.03 ± 0.72
	Day 14	6.45 ± 0.50	2.32 ± 0.10	5.01 ± 0.69
Model	Day 1	4.34 ± 0.56*	11.14 ± 1.32*	447.32 ± 87.54*
	Day 7	3.68 ± 0.46*	16.90 ± 1.60*	887.21 ± 108.28*
	Day 14	6.18 ± 0.53	10.26 ± 1.28*	518.80 ± 90.63*
Curcumin	Day 1	5.21 ± 0.50*#	4.12 ± 0.34*#	208.35 ± 65.12*#
	Day 7	5.09 ± 0.44*#	3.87 ± 0.49*#	48.23 ± 8.10*#
	Day 14	6.30 ± 0.55	2.43 ± 0.23#	16.97 ± 3.06*#

*$P < 0.05$, compared with the normal group at the same time points; #$P < 0.05$, compared with the model group at the same time points.

3.2. Comparison of renal tubular functional indexes

Levels of urine β2-MG (135.54 ± 15.56) mg/L and NAG (51.26 ± 10.31) U in rats of model group were significantly higher than those of the normal group, (78.76 ± 6.90) mg/L and (14.42 ± 2.21) U respectively ($P < 0.05$), while the urine β2-MG (89.45 ± 7.35) mg/L and NAG (25.69 ± 3.65) U of the curcumin group were significantly higher than those of the normal group but lower than those of the model group ($P < 0.05$).

3.3. Comparison of serum inflammatory cytokines

Among three groups, levels of IL-6, IL-8, IL-10 and MCP-1 of the model group were all significantly higher than those of the normal group, while levels of IL-6, IL-8, IL-10 and MCP-1 of the curcumin group were significantly higher than those of the normal group but lower than those of the model group ($P < 0.05$) (Table 2).

3.4. Comparison of expressions levels of mRNA of TLR-2 and TLR-4 in renal tissues and bladder tissues

The expressions of TLR2 mRNA and TLR4 mRNA in renal tissues and bladder tissues of model group were all significantly higher than those of the corresponding normal group; while expressions of TLR2 mRNA and TLR4 mRNA of curcumin group were significantly higher than those of the normal group but significantly lower than those of the model group ($P < 0.05$) (Table 3).

Table 2
Comparison of serum inflammatory cytokines (pg/mL) of the three groups ($n = 10$).

Group	IL-6	IL-8	IL-10	MCP-1
Normal	15.10 ± 1.63	5.15 ± 1.09	10.10 ± 2.01	14.08 ± 2.03
Model	26.29 ± 3.68*	12.35 ± 2.73*	45.29 ± 4.11*	36.42 ± 4.14*
Curcumin	19.09 ± 2.17*#	8.19 ± 1.25*#	23.33 ± 3.08*#	19.70 ± 2.78*#

*$P < 0.05$, compared with the normal group; #$P < 0.05$, compared with the model group.

Table 3
Comparison of the mRNA expressions levels of mRNA of TLR-2 and TLR-4 in renal tissues and bladder tissues of the three groups (n = 10).

Group	Renal tissues		Bladder tissues	
	TLR2	TLR4	TLR2	TLR4
Normal	1.19 ± 0.36	1.83 ± 0.68	0.20 ± 0.04	0.18 ± 0.03
Model	3.53 ± 0.89*	4.28 ± 0.97*	0.51 ± 0.09*	0.47 ± 0.06*
Curcumin	1.76 ± 0.64*#	2.53 ± 0.88*#	0.31 ± 0.05*#	0.26 ± 0.04*#

*$P < 0.05$, compared with the normal group; #$P < 0.05$, compared with the model group.

4. Discussion

Chronic urinary tract infection is a kind of complicated, capricious and persistent chronic inflammation with non-specific clinical features. At present, the pathogenesis of this disease has not been completely clarified. By far, many pathogens causing chronic urinary infection have been identified. Among them, *E. coli* serves as the predominant pathogenic bacterium [1,2]. Therefore, the chronic urinary infection model established in this study was conducted by injecting *E. coli* solution intravesically. When urinary infection occurs, the white blood cell count in the body decreases, while white blood cell count in urine increases, which are the self-protective mechanisms of the body. Moreover, the pathogenic bacteria in urine increase significantly. Persistent inflammatory responses influence the renal tubular function. There are researches claiming that the metabolism and immune function of bodies are active when chronic urinary infection happens [5,6]. Compounded β2-MG and NAG increase remarkably. Urine β2-MG and NAG can be used as objective evaluation indexes for pyelonephritis to evaluate efficacy. For this purpose, this study chose to observe the white blood cell count, urinary white blood cell count, urinary colony count, urine β2-MG and NAG as observation indexes to evaluate the efficiency of each group.

Since pathogens are the primary pathogenic factor in chronic urinary infection, antibiotics become the preferred treatment. However, there are several problems in treating chronic urinary infection with antibiotics [17,18]. Firstly, in order to be effective, antibiotics used need to reach a certain drug concentration. However, with the poor permeability, it is difficult for antibiotics to access to the infected bladder and renal tissues smoothly. Second, microlithiasis in the urinary tract, bladder and renal pelvis would affect the therapeutic effect of antibiotics, and some pathogenic bacteria would generate biofilms for self-protection to prevent themselves from getting injured by those antibiotics. Finally, prolonged use of antibiotics would not only increase the drug resistance of pathogenic bacteria, but also cause side effects for other systems. Thus, it is not that satisfactory to use antibiotics to treat chronic urinary infection, and there is a great limitation as well.

Curcumin is a kind of pleiotropic plant polyphenol. It is found that it possesses capabilities of anti-cancer effect [19], immunity enhancement potential [20], anti-inflammatory [21] and certain antibacterial activities [22]. It is also characterized by easy absorption, low toxicity, lipid solubility and so on. Therefore, the effect of curcumin on treating chronic urinary tract infection was explored in this study by establishing animal models. The results showed that the white blood cell count of curcumin group at days 1 and 7 after treatment was significantly lower than that of the model group, and its white blood cell count in urine and urinary colony count at day 1, 7 and 14 after treatment were obviously lower than those of model group as well. In addition, the urine β2-MG and NAG levels of curcumin group were significantly higher than those of normal but lower than those of model group ($P < 0.05$). The results indicated that curcumin could significantly improve the symptoms of chronic urinary tract infection for rats and protect their renal tubular functions.

Chronic urinary tract infection is a kind of chronic inflammatory infectious disease. Many inflammatory cytokines are expressed highly in patients with chronic inflammatory infection. IL-6 is a proinflammatory factor, which can aggravate inflammatory responses. It is generated by mononuclear macrophages. Moreover, IL-6 can promote B cells to generate a large number of immunoglobulin [23]. IL-8, generated by epidermal cells, mononuclear macrophages and fibroblasts, is a chemotactic factor with the strongest effect mediating bacterial infections so as to cause inflammatory responses [24]. IL-10 participates in inflammatory responses and immunoregulation, which plays double-acting role. The increase in the IL-10 secretion would inhibit the differentiation of Th1 cells, activate Th2 cells, inhibit the release of Th1 cytokines including IL-2 and other related biological effects, which inhibits cellular immunity and then declines the antibacterial ability of the body [25]. Furthermore, there are researches discovering that IL-10 could participate in the regulation of inflammation by inhibiting the activity of macrophages [26]. MCP-1 is also a chemotactic factor possessing strong chemotaxis activity which can gather multiple inflammatory cells including mononuclear leucocytes in the lesion site [27]. MCP-1 can express chemotactic effect on T lymphocytes and mononuclear leucocytes, and induce endothelial cells and endothelial cells to express adhesion molecules. Its chemotactic effect is the molecular basis of inflammatory cell infiltration, accumulation and tubulointerstitial injuries. Besides, MCP-1 is capable of inducing macrophages to release lysosome, expressing some proinflammatory factors, generating oxygen-free radicals and so on to further intensify inflammatory responses in tissues [9]. In this study, IL-6, IL-8, IL-10 and MCP-1 were used as observation indexes to investigate the effect of the intervention of curcumin in rats with chronic urinary tract infection on the above inflammatory cytokines. The results revealed that the levels of IL-6, IL-8, IL-10 and MCP-1 of curcumin group were significantly higher than those of normal group but lower than those of model group ($P < 0.05$). The results indicated that curcumin has strong anti-inflammatory properties which can significantly reduce the levels of inflammatory factors in rats with urinary tract infection and decrease inflammatory responses.

TLRs are not only related to the recognition of pathogenic bacteria and activation of innate immunity, but also influence the activation of downstream signal molecules by signal pathway and activate NF-κB, so as to cause chain release of inflammatory cytokines and biological reactions [28]. Wittmann *et al* [29] found that TLRs play an important role in the collection of inflammatory cells. It has been found that it is the reproductive system that expresses the most TLRs [30]. In order to further study the anti-inflammatory mechanism of curcumin, two TLRs, the expressions of TLR2 mRNA and TLR4 mRNA, in the renal tissues and bladder tissues in rats of the three groups were detected in this study. The results revealed that the expressions of TLR2 mRNA and TLR4 mRNA in the

renal tissues and bladder tissues in rats of model group were significantly higher than those of the corresponding normal group, which indicated that high expressions of TLR2 mRNA and TLR4 mRNA could be activating factors of septic shock and inflammation storm; while the expressions of TLR2 mRNA and TLR4 mRNA in the renal tissues and bladder tissues in rats of curcumin group were significantly higher than those of the corresponding normal group but lower than those of model group ($P < 0.05$). It is concluded that curcumin could inhibit the expressions of TLR2 mRNA and TLR4 mRNA and reduce the over-release of inflammatory mediators so as to decline the inflammatory responses of chronic urinary tract infection in rats.

In conclusion, findings from this study suggest that curcumin could significantly improve the symptoms of chronic urinary tract infections, protect renal tubular function, and possibly decline inflammatory responses by influencing the expressions of TLR2 mRNA and TLR4 mRNA so as to exert its curative effect on treating chronic urinary tract infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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