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Effect of "Fuzheng Qingretonglin" decoction on complex urinary tract infection in rats by regulating NLRP3 inflammasome pathway

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ABSTRACT

Obiective: To investigate whether "Fuzheng Qingretonglin" decoction can reduce urinary tract damage caused by complex urinary tract infection caused by drug resistant Escherichia coli by regulating Nod-like receptor pyrin domain3 inflammasome, and to explore the feasibility of this decoction combined with levofloxacin in the treatment of complex urinary tract infection caused by drug resistant bacteria. **Methods:** SD rats were divided into five groups: sham group, model group, levofloxacin group (Lev group), levofloxacin+Fuzheng Qingre Tonglin decoction group (FZ+lev group), and Fuzheng Qingre Tonglin decoction group (FZQRTL group). After the experiment, urine was taken for bacterial culture to determine the urinary tract infection of rats in each group; HE staining was used to observe the pathological changes of kidney and bladder tissues in rats; The expression of NLRP3 in kidney and bladder tissues was detected by immunohistochemistry; The expression of IL-1 β and IL-18 in serum of rats was detected by ELISA; The expressions of NLRP3, ASC and Caspase-1 were detected by Western blotting. **Results:** The positive rate of urine bacteria culture in the sham group was 0%, the positive rate of urine bacteria culture in the model group was 100%; and the positive rate of urine bacteria culture in the FZ+lev group was 37.50%, which was statistically different from that in the model group (P<0.05). A large number of inflammatory cells were observed in the kidney and bladder tissues of the model group by HE staining, while the number of inflammatory cells in the kidney and bladder tissues of the Lev group and FZQRTL group was significantly reduced compared with that of the model group. The FZ+lev group in the number and structure of inflammatory cells in kidney and bladder were similar to the sham group. The NLRP3 immunohistochemistry of kidney and bladder tissue in FZ+lev groups and FZQRTL groups was significantly different from that in model group (P<0.001). The levels of IL-1 β and IL-18 in serum of Lev group, FZQRTL group and FZ+lev group were significantly decreased by ELISA compared with model group (P<0.001). The levels of IL-1 β and IL-18 in the FZ+lev groups were significantly lower than in the Lev group and FZQRTL group, and the differences were statistically significant (P<0.05). The protein expressions of NLRP3, ASC and Caspase-1 in the Lev group, FZQRTL group and FZ+lev group were significantly lower than those in the model group (P<0.001). The protein expressions of NLRP3, ASC and Caspase-1 in the FZ+lev groups were significantly lower than in the Lev group and FZQRTL group, and the differences were statistically significant (P<0.05). **Conclusions:** "Fuzheng Qingretonglin" decoction may have a protective effect on the kidney and bladder of rats with complex urinary tract infection caused by drug-resistant E*scherichia coli* by inhibiting the activation of NLRP3 inflammatory bodies, and TCM combined with levofloxacin has a better therapeutic effect than TCM or levofloxacin alone.

1. Introduction

Complex urinary tract infection (CUTI) is one of the clinically difficult infections due to the existence of urinary tract obstruction, urinary system malformation, long-term indwelling of ureteral catheter or ureteral stent, *etc*., which often persists or occurs repeatedly. CUTI often leads to bacterial resistance due to repeated antibiotic use over a long period of time[1,2], which brings great challenges to clinical treatment. Clinical studies have shown that Chinese medicine has a certain therapeutic effect on CUTI[3,4], Our

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previous research also showed that: The self-designed prescription "Fuzheng Qingretonglin decoction" has a good effect on CUTI rats, but the mechanism is still unclear[5]. When microorganisms infect the body, NLRP3 inflammasome and other related proteins and related cytokines in the body tissues will be significantly increased, resulting in a series of body damage[6-8]. This experiment by observing the histopathological changes of rats, constituting the NLRP3 inflammatory corpuscle pathways of each protein expression and the expression of serum inflammatory cytokines, to investigate whether the "Fuzheng Qingretonglin decoction" by regulating inflammatory corpuscle NLRP3 activation and the production of inflammatory cytokines, reduce the complicated urinary tract infections caused by *E. coli* damage in the body. It provides theoretical basis for clinical application.

2. Materials and methods

2.1 Materials

2.1.1 Laboratory reagents

Traditional Chinese medicine formula: Phellodendri Chinensis Cortex, Astragalus membranaceus, Rhizoma anemarrhenae, Semen Plantaginis, Polygonum aviculare, Dianthi Herba, Gardenia jasminoides Ellis, Poria cocos, Bitter cardamon, Cuscuta chinensis Lam, Atractylodes macrocephala Koidz, Scutellariae Radix and Radix Achyranthis Bidentatae. The above herbs are provided by the pharmacy of The Affiliated Hospital of Traditional Chinese Medicine of Southwest Medical University, and levofloxacin hydrochloride was purchased from Heilongjiang Norjet Pharmaceutical Co., LTD. Hematoxylin eosin staining Kit (C0105S), Primary resistance diluent and Secondary resistance diluent (P0023A, P0023D) all purchased from Biyuntian Biotechnology Company; NLRP3 antibodies(512694), ASC antibodies(340097), Caspase-1 antibodies(381016) purchased from Sichuan Zhengneng Biological; HRP antibodies(bs-0295G-HRP) purchased from Beijing Bioss Biotechnology Co., LTD; Interleukin IL-1β and IL-18ELISA kit (SEKR-0002, SEKR-0054), GADPH antibodies (bs-2188R) and other consumables were purchased from Beijing Solarbio Technology Co. , LTD.

2.1.2 Experimental animals and groups

Fifty SPF female SD rats, aged 8 to 9 weeks and weighing 240 to 250 g, were purchased from Southwest Medical University, which license: SYXK (Chuan) 2018-065. After 1 w of adaptive feeding, modeling was carried out. After successful modeling, SD rats were divided into five groups: sham group, model group, levofloxacin group (Lev group), levofloxacin+Fuzheng Qingre Tonglin decoction group (FZ+lev group), and Fuzheng Qingre Tonglin decoction group (FZQRTL group), with 10 rats in each group. The animal experiment program was approved by the ethics Committee of animal Experiment Center of Southwest Medical University.

2.1.3 Experimental instruments and equipment

Gel electrophoresis apparatus(liuyi Beijing); Bio-rad gel imager; Automatic microplate reader (Gene); Low temperature high speed centrifuge (eppendorf); Paraffin slicer (Leica); Ultrasonic tissue homogenizer (BRANSON).

2.2 Methods

2.2.1 Building methods

According to the model experiment of urinary tract infection in the early stage of our research group and relevant literature reports[9], the model of complex urinary tract infection caused by drug-resistant bacteria was created. In the sham group, 0.5 mL normal saline was injected into the bladder after the foreign body was inserted during modeling. Rats were fed a normal diet and drinking water was restricted daily during modeling (drink water once in the morning and evening for 1 h each time).

2.2.2 Dosing method

Five days later, after complete modeling success, 36 SD rats were randomly divided into four groups:model group, Lev group, FZ+lev group, and FZQRTL group. There were 9 in each group. It was obtained by the conversion formula of human-rat body surface area ratio: The Lev group was given levofloxacin 0. 025 $g \cdot kg^{-1}d^{-1}$;The FZ+lev group was given levofloxacin 0.025 $g \cdot kg^{-1}d^{-1}$ and Fuzheng Qingretongling Soup 16.1 $g \cdot kg^{-1}d^{-1}$; The FZQRTL group was given Fuzheng Qingretongling Soup 16.1 $g \cdot kg^{-1}d^{-1}$; sham group and model group were given the same amount of normal saline intragastric treatment. Continuous administration for 2 w.

2.2.3 Hematoxylin isocyanin staining

The kidney and bladder tissues of rats were fixed with 4% paraformaldehyde, dehydrated with gradient alcohol, transparent with xylene, embedded in paraffin, sliced, and stained with HE. See kit instruction for detailed operation steps.

2.2.4 Immunohistochemical

The prepared pathological paraffin sections were placed in a thermostatic drying oven, baked at 65 ℃ for 3 h, dewaxed with xylene, hydrated with 100%, 95%, 80% and 70% ethanol respectively, and then washed with PBS. After antigen repair and cooling, 3% hydrogen peroxide was added and incubated at room temperature for 10 min to inactivate endogenous enzymes. Then, after rinsing with PBS again, each section was sealed with goat serum. After incubation at room temperature for 10 min, NLRP3 antibody diluent was added (antibody:diluent=1:200); Place in 4 ℃ refrigerator overnight. The next day, DAB was used for color rendering, and then hematoxylin was used for redyeing. The slices were dehydrated and dried with gradient alcohol, transparent

with xylene and sealed with neutral gum. Finally, the prepared immunohistochemical sections were used to observe the staining of each experimental group under light microscope.

2.2.5 Enzyme-linked immunosorbent assay

The serum levels of IL-1 β and IL-18 in SD rats were determined by Elisa, according to kit instructions.

2.2.6 Western blotting

BCA protein extraction kit was used to extract protein from kidney tissue samples of each group, and then automatic microplate analyzer was used to measure the concentration and loading of sample protein. 20 μg samples were taken from each group and transferred to PVDF membrane by SDS-PAGE of 8%, 10% and 12%, respectively. Then, biyuntian sealing solution was used for room temperature sealing for 2 h, and biyuntian washing solution was used for film washing after the sealing was completed. NLRP3 (1:1 000), GAPDH (1:1 000), Caspase-1 (1:1 500) and ASC (1:1 500) primary antibody incubating solution were used for overnight incubation at 4 ℃. Goat anti-rabbit antibody (1:6 000) was incubated at room temperature for 1 h. Finally, ECL developer was used for development, bio-Red instrument was used for exposure development, and Image J software was used to analyze gray value for data analysis.

2.2.7 Statistical analysis

SPSS 26.0 software was used for data analysis, and quantitative data were expressed as mean ± standard deviation ($\bar{x}\pm s$). One-way ANOVA was used for comparison between multiple groups, and *Chi*-square test was used for counting data. $P<0.05$ was considered statistically significant.

3. Results

3.1 General condition of rats in each group

The number of rats in each group should have been 10, but a total of 6 rats died within five days of modeling. Blood culture of the dead rats after dissection confirmed that all 6 rats died of urinary sepsis. In order to randomly divide model rats into four groups with the same sample size, 2 rats were randomly selected from the sham group for infection model construction. However, in the subsequent experiment, 1 rat in the model group died of severe sepsis infection;One died in the FZ+lev groups due to aspiration. Rats in the sham group showed rapid response, good spirit, normal diet, normal activities, thick, smooth and shiny hair, clean and dry dressing, and normal urine odor. The rats in the model group showed listlessness, decreased eating and activity, fluffy and dull hair, slight weight loss or insignificant increase, frequent urination and other symptoms, obvious wetting of dressing, and punchy odor of urine. The reaction, spirit, diet and activity of the treatment groups were improved compared with that of the model group, and the improvement of the FZ+lev groups was the most obvious.

3.2 Comparison of positive rate of urine bacteria culture in each group

The positive rate of urine bacteria culture in the model group was 100%, indicating that the modeling was successful. The positive rate of FZ+lev group was 37.50%, and the difference was statistically significant($\chi^2=6.167$, $P<0.05$) (Table 1).

Tab 1
Comparison of positive rate of urine bacteria culture in each group

Group	Number	Positive result	Positive rate (%)
Sham	8	0	0.00*
Model	8	8	100.00
Lev	9	6	66. 67
FZ+lev	8	3	37. 50*
FZQRTL	9	6	66. 67

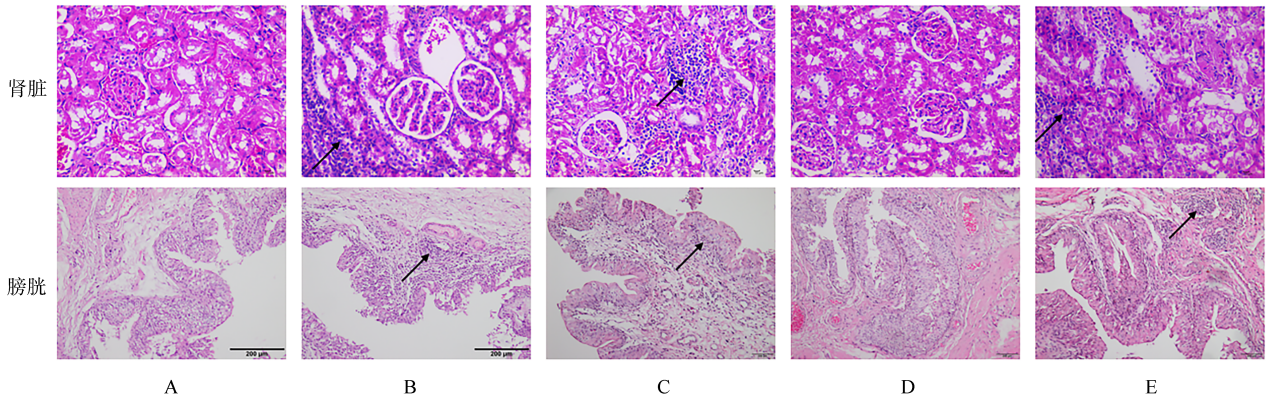
Note: Compared with model group, *$P<0.05$.

3.3 Effect of "Fuzheng Qingretonglin" decoction on renal and bladder histology in rats with urinary tract infection

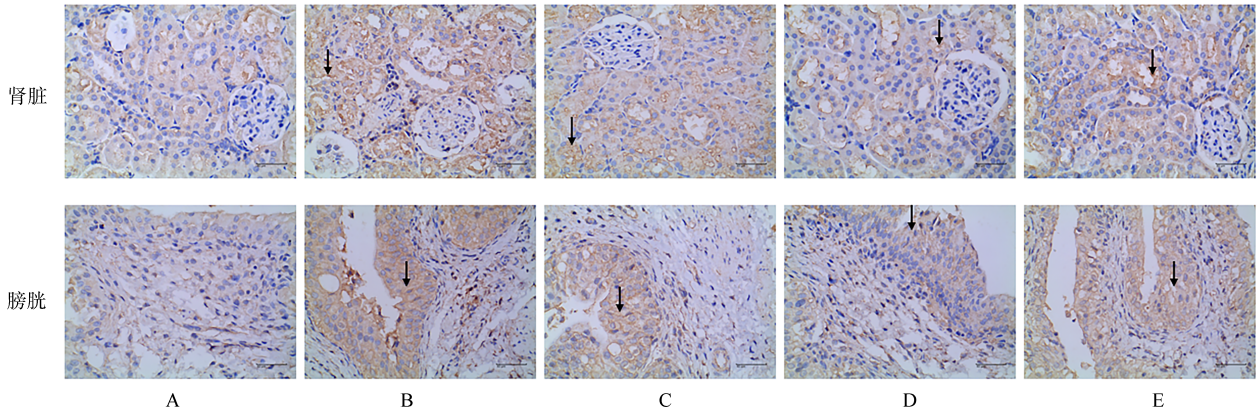
The renal and bladder tissues of the sham group were intact without obvious inflammatory cell infiltration. In the model group, the glomerulus atrophy, tubules dilation and lumen enlargement were observed, and tubule epithelial cells were necrotic and exfoliated. Microscopically, a large number of inflammatory cells were observed. The epithelium of bladder tissue was necrotic and exfoliated, and the submucosa was infiltrated with inflammatory cells. In the Lev group, the FZ+lev medicine group and the FZQRTL group, the glomerulus was slightly atrophy or no obvious atrophy, the renal tubules were slightly dilated, and the epithelial cells of the renal tubules were not necrotic or exfoliated. A little infiltration of inflammatory cells;The bladder epithelium tissue was orderly and the number of layers was relatively intact. There was little or no obvious inflammatory cell infiltration in the submucosa of the bladder. Among them, the HE staining results of kidney and bladder tissues in the FZ+lev groups were most similar to those in the sham group (Figure 1).

3.4 Expression of NLRP3 in kidney and bladder tissues of rats in each group

Mild NLRP3 positive cells were observed in the kidney and bladder tissues of the sham group in microscope. NLRP3 positive cells were found in renal tubule epithelial cells, renal interstitial cells and bladder epithelial cells in the model group. The staining degree of NLRP3 positive cells in Lev group, FZ+lev group and FZQRTL group was lower than that in model group (Figure 2)



A: Sham group; B: Model group; C: Lev group; D: FZ+lev group; E: FZQRTL group. Arrows indicate inflammatory cells.
Fig 1 Histological effects of kidney and bladder in rats with urinary tract infection in each group (HE staining, kidney 400×; 200×)



A: Sham group; B: Model group; C: Lev group; D: FZ+lev group; E: FZQRTL group. Arrow indicates NLRP3 immunohistochemical staining positive cells; The degree of yellow staining indicated the expression level of NLRP3, and the deeper the staining, the higher the protein expression level.
Fig 2 NLRP3 expression in kidney and bladder tissues of rats in each group (immunohistochemistry 400×)

Due to the death of rats during modeling and subsequent experiments, the sample sizes of rats in each group were different. In order to maintain the consistency of sample sizes in each group, 7 rats were randomly selected from each group (8-9) as experimental samples for immunohistochemistry. There were statistically significant differences in kidney and bladder tissues between the model group and the sham group (P<0.001). The kidney and bladder tissues of the Chinese and Lev group and the FZQRTL group are statistically different from the model group (P<0.001); There was statistically significant difference in kidney tissue between the Lev group and the model group, but there was no statistically significant difference in bladder tissue between the Lev group and the model group. In the bladder tissue, there were statistically significant differences between the Lev group and the FZQRTL group compared with the FZ+lev groups (P<0.05). However, in the kidney tissues, there was no statistically significant difference between the Lev group and the FZQRTL group compared with the FZ+lev groups (Table 2).

Tab 2
Semi-quantitative analysis data of NLRP3 in kidney and bladder tissues of rats in each group ($\bar{x}\pm s$, n=7)

Group	Number	Kidney	Bladder
Sham	7	0. 008±0.003 9***	0. 001±0. 000 08***
Model	7	0. 041±0.007 3	0. 041±0. 008 75
Lev	7	0. 015±0.004 7***	0. 033±0. 013 45$^{\#}$
FZ+lev	7	0. 011±0.003 0***	0. 008±0. 001 28***
FZQRTL	7	0. 014±0.002 4***	0. 018±0. 005 23$^{***\#}$
F		55.105	25.421
P		0.000	0.000

Note: Compared with model group, $^{***}P$<0.001; compared with the FZ+lev groups, $^{\#}P$<0.05.

3.5 Expression of IL-1β and IL-18 in serum of rats in each group

The number of rats in the FZQRTL group was 9, but due to operational problems in the blood drawing process, two of them did not receive blood, so the number was 7. Compared with model group, the expressions of IL-1β and IL-18 in serum of rats in Lev group, FZ+lev group and FZQRTL group were significantly decreased, and the differences were statistically significant

(P<0.001). The expressions of IL-1 β and IL-18 in the serum of rats in the Lev group and the FZQRTL group compared with the FZ+lev groups are statistically significant (P<0.05) (Table 3).

Tab 3

Expression of IL-1 β and IL-18 in serum of rats in each group (pg/mL, $\bar{x}\pm s$)

Group	Number	IL-1 β	IL-18
Sham	8	405.0±41.64***	109.7±09.05***
Model	8	705.4±31.71	220.3±12.03
Lev	9	585.2±53.07$^{***\#}$	178.9±12.30$^{***\#}$
FZ+lev	8	453.3±51.07***	127.2±11.07***
FZQRTL	7	527.7±54.21$^{***\#}$	159.3±14.54$^{***\#}$
F		50. 560	111. 073
P		0. 000	0. 000

Note: Compared with model group, $^{***}P$<0.001; Compared with the FZ+lev groups, $^{\#}P$<0.05.

3.6 The relative expressions of NLRP3, Caspase-1 and ASC proteins in kidney of rats with urinary tract infection in each group

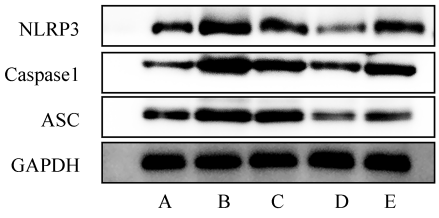
Compared with the sham group, the relative expressions of NLRP3, caspase-1 and ASC proteins in the model group were significantly increased, with statistical significance (P<0.001). Compared with model group, the relative expressions of NLRP3, Caspase-1 and ASC proteins in kidney of Lev group, FZ+lev group and FZQRTL group were significantly decreased, with statistical significance (P<0.001). Compared with the FZ+lev group, the relative expressions of NLRP3, Caspase-1 and ASC proteins were increased in the Lev group and FZQRTL group, and the differences were statistically significant (P<0.05) (Table 4 and Figure 3).

Tab 4

Relative expressions of NLRP3, Caspase-1 and ASC proteins in kidney of rats with urinary tract infection in each group (pg/mL, $\bar{x}\pm s$)

Group	Number	NLRP3	Caspase-1	ASC
Sham	8	0.59±0. 02***	0.42±0.01***	0.77±0.01***
Model	8	1.14±0.04	1.24±0.04	1.31±0.03
Lev	9	0.80±0.04$^{***\#}$	0.88±0.02$^{***\#}$	1.04±0.03$^{***\#}$
FZ+lev	8	0.39±0.02***	0.52±0.02***	0.48±0.01***
FZQRTL	9	0.81±0.03$^{***\#}$	0.86±0.02$^{***\#}$	0.70±0.01$^{***\#}$
F		196.095	626.318	880.217
P		0.000	0.000	0.000

Note: Compared with model group, $^{***}P$<0.001; Compared with the FZ+lev groups, $^{\#}P$<0.05.



A: Sham group; B: Model group; C: Lev group; D: FZ+lev group; E: FZQRTL group.

Fig 3 Relative expressions of NLRP3,Caspase-1 and ASC proteins in kidney of rats with urinary tractinfection in each group

4. Discussion

The CUTI in urinary tract infections is equivalent to the category of "stranguria due to overstrain" in traditional Chinese medicine. Many physicians believe that the disease belongs to asthenia in origin and asthenia in superficiality. Among them, "the deficiency of the kidney is the root, and the heat of the bladder is the mark". Syndrome of lingering pathogen due to deficient vital qi, It not only clears the heat and leaches, but also strengthens the body[10]. According to the above principles, the research group combined with the modern pharmacological research of Traditional Chinese medicine, Self-drafted "Fuzheng Qingretonglin" prescription. In the formula: Phellodendri Chinensis Cortex, Semen Plantaginis, Dianthi Herba have the effect of eliminating damp-heat and promoting urinary discharge. *Astragalus membranaceus*, *Bitter cardamon*, *Cuscuta chinensis* Lam, *Poria cocos*, *Atractylodes macrocephala* Koidz have the effect of strengthening and consolidating body resistance. The Compound have moistening tonification, tonify without causing stagnation;strengthen body resistance and eliminating evil. To play the power of tonifying the kidney, strengthening the body and clearing away heat[9]. The clinical manifestations of rats in this experiment are emotional frustratin, improper diet, spleen-kidney deficiency and Endoretention of damp heat; In line with the traditional Chinese medicine "stranguria due to overstrain" category. Therefore, it is treated with Tonifying and righting, tonify Qi of the kidney, damp invasion of lower energizer and promoting diuresis and relieving stranguria. After the treatment of "Fuzheng Qingretonglin" decoction, the above performance of rats was significantly improved.

Studies have shown that single herbs such as *Phellodendron phellodendri* and *Astragalus membranaceus* can inhibit the growth of multidrug-resistant bacteria *in vitro*[11-13]. The results of urine bacteria culture in rats indicated that the Lev group and the FZQRTL group had no obvious bacteriostatic effect on the urine bacteria in rats. The positive rate of urine bacteria culture in FZ+lev groups was only 37.50% (P<0.05). It is suggested that "Fuzheng Qingretonglin"

combined with levofloxacin has inhibitory effect on urine bacteria of CUTI rats. The mechanism may be related to the decoction changing some drug resistance characteristics of *E. coli*. For example, it can inhibit and eliminate bacterial biofilms[14], so that levofloxacin restores its antibacterial effect. However, the other action mechanism of the decoction on bacteria is not clear and needs further study.

Previous studies have found that NLRP3 inflammasome, as a key protein in NLRP3 signaling pathway, plays a key role in the occurrence and development of a variety of inflammatory diseases. NLRP3 inflammasome can be activated by microorganisms such as *Escherichia coli* through the following pathways: first, the lipolysaccharides located on the cell wall of *Escherichia coli* are recognized by toll-like receptor 4 (TLR4) on the immune membrane, and the signal is transduced into the cell, resulting in downstream nuclear factor- κ B (NF- κ B) protein expression. The increase of NLRP3 mrna expression in the nucleus is sufficient to activate NLRP3 inflammasome, which ultimately leads to caspase-1 cleavage of pro-IL-1 β and pro-IL-18, the formation of mature pro-inflammatory cytokines IL-1 β and IL-18, and the release of extracellular[15,16]. These pro-inflammatory cytokines will cause inflammatory changes in the body, so that more cells repeat the above-mentioned pathway and release more pro-inflammatory cytokines, causing the tissue or body to carry on such a vicious cycle, resulting in a series of damage. The previous experiment of the research group found that "Fuzheng Qingre Tonglin" decoction can reduce the release of pro-inflammatory cytokines by down-regulating the expression of key molecular proteins in TLR4 related pathways, thereby reducing the inflammatory response of kidney tissue[5]. Further research in this experiment showed that the expression of key proteins such as NLRP3, Caspase-1 and ASC in the kidney tissue of the drug intervention group was significantly reduced (P<0.001), and the serum IL-1 β and IL-18 inflammatory factors were also significantly reduced(P<0.001). At the same time, observe and compare the pathological slices of the kidney and bladder tissues of each group. It is found that Lev, FZQRTL, and FZ+lev have a certain protective effect on the kidney and bladder tissue structure of rats. The inflammation of tissues and cells is better than that of the model group. Different degrees of relief, among which the kidney and bladder tissue structure of the FZ+lev group is the closest to those of the sham group, and the inflammation is the lightest. Combined with the previous study on the effect of "Fuzheng Qingre Tonglin" decoction on TLR4 receptors[5], it is suggested that the decoction can reduce the activation of NLRP3 inflammasomes by inhibiting the effect of TLR4, and down-regulate the expression of its related proteins, significantly reducing the release of IL-1 β and IL-18 inflammatory factors, thereby reducing the body's inflammatory response caused by bacterial infection, and protecting target organs such as the kidney and bladder.

Studies have shown that levofloxacin can bind to the hydrophobic region of TLR4-myeloid differentiation protein-2, inhibit LPS-induced intracellular TLR4/NF- κ B signaling pathway, thereby inhibiting the activation of NLRP3 inflammasomes and the release of pro-inflammatory cytokines[17]. In addition, Dupont *et al*.[18] found that autophagy can positively regulate the activation of NLRP3 inflammasomes. Levofloxacin can activate the PI3K/Akt signaling pathway in cells, inhibit the expression of autophagy-related proteins, reduce the level of autophagy in cells, and reduce tissue damage in infection models[19,20]. Therefore, regardless of whether levofloxacin has a killing effect on bacteria, it may down-regulate the related proteins and inflammatory factors of the NLRP3 pathway by inhibiting the binding of LPS and TLR4 and reducing the level of autophagy in cells of the body, thereby reducing inflammation in the kidney. This may explain the following phenomena observed in this experiment: Levofloxacin is not effective against drug-resistant E. coli in conventional *in vitro* drug susceptibility tests, but after administration in rats, NLRP3 related pathway proteins and inflammatory factors in the kidneys decrease, and the kidneys and bladder The tissue inflammation was also lighter than the model group (P<0.001).

This experiment established a stable and reliable CUTI model by implanting foreign bodies and injecting drug-resistant Escherichia coli bacteria. We found that drug-resistant *Escherichia coli* can activate NLRP3 inflammasome in a large amount, and make IL-1 β and IL-18 and other cytokines be released in large quantities. "Fuzheng Qingre Tonglin" decoction reduces the activation of NLRP3 and the expression of related proteins, thereby reducing the release of IL-1 β and IL-18 and other cellular inflammatory factors, and has a certain protective effect on the structure of rat kidney and bladder tissues. In addition, this experiment also confirmed that the combination of levofloxacin and "Fuzheng Qingre Tonglin" decoction has a significant inhibitory effect on drug-resistant *Escherichia coli* in the bladder of rats, but its specific mechanism of action still needs to be further studied. However, this experiment also has the following shortcomings: one is that the number of sample cases is too small; the other is that when the control drug levofloxacin is selected in the experiment, it is only considered as a common drug for past urinary tract infections, and it has not been checked for its own effects on NLRP3 inflammasomes. A certain down-regulation effect leads to certain difficulties in the analysis of experimental results, and there may be deviance, which need to be further improved in future studies.

Author conflict of interest statement

All authors declare that there is no conflict of interest.

Authors′ contribution

Zhong Yuwen carried out experimental operation, data collection and processing and wrote articles; Luo Xiaoquan, Liu Xin, Zhu Yongsheng, Lai Junyu for experimental design, modeling guidance; Su Hongwei was responsible for proposing the experimental scheme and revising the article.

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