Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Szechwan Lovage Rhizome Extract Improves Renal Function and Alleviates Inflammatory Responses in Pyelonephritis Rats Infected with *Escherichia Coli* via IL-6/STAT3 Axis

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ARTICLE INFO

Original paper

Article history:
Received: February 4, 2023
Accepted: March 14, 2023
Published: March 31, 2023

Keywords:

Szechwan Lovage Rhizome, Escherichia coli, Pyelonephritis, Renal function, Inflammatory response, IL-6/STAT3 axis

ABSTRACT

The objective of this study was to probe the effect and mechanism of Szechwan Lovage Rhizome (Chuanxiong, CX) extract on renal function (RF) and inflammatory responses (IRs) in acute pyelonephritis (APN) rats infected with *Escherichia coli (E. coli)*. Fifteen SD rats were randomized to intervention, model and control groups. Rats in the control were fed normally without treatment, rats in the APN model were infected with E. coli, and rats in the intervention group were intragastrically administered CX extract after infection with E. coli. HE staining detected pathological changes in the kidney tissues in rats. Levels of renal function indexes and inflammatory factors (IFs) were measured by ELISA and an automatic biochemical analyzer. Besides, levels of IL-6/signal transducer and activator of transcription 3 (STAT3) pathway-related genes in rat kidney tissue were detected by qRT-PCR and western blot. the experimental results showed that IL-1β, IL-8, TNF-α and RF levels were the highest in the model group and the lowest in the control group, with those of the intervention group in between ($P<0.05$). Besides, the IL-6/STAT3 axis was markedly activated in the model group but inhibited in the intervention group ($P<0.05$). Subsequently, activated IL-6/STAT3 signal promoted IFs (IL-1β, IL-8 and TNF-α) and RF (BUN, Scr, β2-MG and UA), but this effect was offset after CX treatment ($P<0.05$). In conclusion, CX extract could improve RF and inhibit IRs of APN rats infected with E. coli by inhibiting the IL-6/STAT3 axis, which may be a new choice for APN treatment in the future.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.3.20

Introduction

Pyelonephritis (PN), either acute or chronic, is a substantial inflammation of the kidney caused by microbial infection, often accompanied by lower urinary tract inflammation. Among them, acute pyelonephritis (APN) accounts for about 50 to 60 percent of all PN and can occur in all ages, especially in women of childbearing age (1). More than 300, 000 new cases of APN are reported globally each year, and the incidence is on the rise (2). APN is mainly attributed to bacterial infection, among which Escherichia coli (E. coli) is the most common. APN has no special clinical symptoms at the initial stage of infection. Once the patient has obvious pain, urinary tract inflammation and systemic reaction, APN usually has progressed to the middle and late stage, significantly increasing the difficulty of cure (3). It is also due to the occultation of early APN that patients do not pay attention to it or deal with it improperly, which increases the possibility of worsening the disease or developing into chronic PN, and eventually leads to renal failure (4). At present, antibiotics are still the main treatment for APN in the clinic, whose purpose is to kill pathogenic bacteria in patients and improve renal function (RF) (5). However, such drugs generally have serious toxic and side effects, including nausea, vomiting, and nervous system reaction in mild cases, and hemolysis and liver and kidney toxicity in severe cases, which may aggravate the patient's condition (6). Therefore, finding a new treatment for APN is the hotspot and difficulty in modern clinical research.

In recent years, increasing clinical attention has been drawn to traditional Chinese medicine (TCM) extracts. Extracting effective components from TCM monomer can not only retain the safety of the original TCM, but also enhance the clinical application effect of the drug, and make remarkable achievements in the treatment of various diseases (7, 8). Among them, Szechwan Lovage Rhizome (Chuanxiong, CX), as a natural Chinese medicine component, has remarkable achievements in the Figureht against sclerosis, oxidation and apoptosis, and can significantly inhibit the occurrence of inflammatory responses (IRs) and bacterial infections (9). At present, CX extract has shown a relatively good and safe improvement effect in leukemia, myocardial ischemia-reperfusion injury and other diseases (10, 11), but its employment in APN remains poorly reported.

Studies have suggested interleukin 6 (IL-6) secretion carries functional significance during APN, and IL-6 expression reflects the severity of clinical APN (12). In mechanism, after IL-6 binds to its receptor, the signal

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transducer and transcription activator 3 (STAT3) undergo JAK-dependent tyrosine phosphorylation, leading to homo-dimer and translocation to the nucleus, where phosphorylation of (p)-Stat3 regulates transcription (13). The IL-6/STAT3 signaling pathway has been documented to modulate several biological processes, such as cell survival, cell proliferation, and the cell cycle. Besides, it plays crucial roles in apoptosis and inflammation (14). Additionally, in previous studies, we have found that inflammasome NLRP3 is involved in the occurrence and development of APN. The downstream pro-inflammatory axis IL-6/STAT3 of NLRP3 is also of great significance to APN, while CX extract blocks the development of asthma by inhibiting the IL-6/STAT3 axis (15).

Hence, we speculated that CX extract may also have a certain improvement effect on APN through the IL-6/STAT3 pathway. Herein, experiments were carried out based on the above conjecture to confirm the application value of CX extract in the treatment of APN, so as to provide a more reliable safety guarantee for patients and lay a foundation for the subsequent research on CX extract and APN.

Materials and Methods

Animal data

Supplied by Beijing Amersey Biotech (laboratory animal certificate number: SYXK (Beijing) 2020-0028), grade-specific pathogen-free (SPF) Sprague Dawley (SD) rats (n=30) weighing (180-240) g were kept at 26℃ and allowed to eat and drink freely.

Grouping and modeling

Fifteen rats were randomized into control, model, and intervention groups, each with 5 rats. Control rats were fed normally without treatment. Those in the other two groups were anesthetized with 2% pentobarbital sodium intraperitoneally after 24 hours of fasting and water deprivation and fixed on the anatomical table in the supine position. Rat genitals were then tied with thin threads, and the abdomen was cut along the midline of the lower abdomen. In the model group, 0.5 mL (108 CFU/mL) of E. coli was slowly injected into the bladder from the ureter. The incision was then sutured and sterilized conventionally, and the genitals were opened 10 days later.

Intervention

Animals in the intervention group were intragastrically treated with CX extract (1200 mg/kg), and those in the other two groups were given the same amount of normal saline, all for 14 days. The body temperature and weight of rats in each group were recorded after the above treatment was finished, and their life activities were observed. All animals were then killed under anesthesia by cervical dislocation.

Blood sample collection and detection

Enzyme-linked immunosorbent assay (ELISA) measurements of inflammatory factors (IFs) including interleukin (IL)-1β, IL-8 and tumor necrosis factor (TNF)-α were performed after collecting rat carotid blood. The relevant kits were all supplied by TransGen Biotech. An automatic biochemical analyzer examined serum urea (BUN), serum creatinine (Scr), serum β2-microglobulin (β2-MG) and uric acid (UA).

Tissue sample collection and testing

Bilateral kidney tissues were taken out through a midline incision in the rat's lower abdomen, the fixed kidney tissue was embedded in paraffin, and then cut into 4 μm thickness. The samples were deparaffinized using xylene and hydrated and stained using a hematoxylin-eosin (HE) reagent (Sigma, USA). Hematoxylin was treated in the sample and incubated for 5 min. After washing, eosin was treated in the sample and incubated for 2 min. The pathological changes were observed under a microscope.

IL-6 and STAT3 mRNA levels in kidney tissues were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA, extracted by Trizol (Invitrogen), was reverse transcribed into cDNA according to the manuals of the kit for PCR amplification (Takara Bio Inc., Japan). qPCR was performed with an ABI Prism 7500 instrument (Applied Biosystems, Japan). Primer sequences could be found in Table 1. Amplification conditions: 94℃ for 30 s, 94℃ for 5 s, and 60℃ for 30 s. The calculation of relative expression adopted $2^{-\Delta\Delta ct}$.

In addition, Western blots were carried out to quantify IL-6/STAT3 pathway protein expression: After cell, lysis using radioimmunoprecipitation assay (RIPA, Elabscience, Wuhan, China), the isolated total protein was treated with 12% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane, which was then closed with 5% skim milk powder for 2 h and immersed in primary antibodies comprising IL-6 (Abcam, ab233706, 1/1000), STAT3 (Abcam, ab68153, 1/1000) and GAPDH (Abcam, ab9485, 1/2500) to be tested, for overnight sealing at 4℃. The membrane was washed the next day to remove the primary antibodies, and incubated with a secondary antibody (Abcam, ab109489, 1/1000) for 1 h, followed by three PBS rinses and development with ECL.

Impacts of IL-6/STAT3 on APN

Another 15 SD rats were randomly divided into 3 groups (groups A, B, and C), and APN models were established as above. After modeling, rats in groups B and C were injected with exogenous IL-6 (2000 U/100 g) through the tail vein to activate IL-6/STAT3 pathway expression, while rats in group A were injected with the same amount of normal saline as a control. Then, rats in group C were treated with CX extract by intragastric administration (same as above). The above tests were repeated to confirm

Table 1. Primer sequences.

	F (3'-5')	R (3'-5')
IL-6	GTTGCCTTCTTGGGACTGATG	TACTGGTCTGTTGTGGGTGGT
STAT3	TGGGCATCAATCCTGTGGTAT	TAGTTCACACCAGGCCCTAAG
β-actin	CACGATGGAGGGGCCGGACTCATC	TAAAGACCTCTATGCCAACACAGT

the impacts of IL-6/STAT3 on APN and the association of CX extract with the IL-6/STAT3 axis.

Statistics and methods

SPSS224.0 analyzed and processed the data. All experiments were performed thrice. All the results were expressed in the form of ($\bar{\chi}\pm s$), and multi-group comparisons were made via repeated measures ANOVA and Bonferroni's post hoc test, with $P<0.05$ as the significance threshold.

Results

Impacts of CX extract on vital signs of APN rats

The control rats responded quickly and acted actively, with obvious resistance to capture. The intervention group animals suffered from listlessness, reduced diet, dull hair, and slightly weak resistance during capture. The mobility of model group rats decreased markedly, most of which had no appetite, hair loss, and no resistance to capture. Besides, model group rats had the highest body temperature among the three groups, followed by the intervention group, while control rats had the lowest body temperature ($P<0.05$, Figure 1A). However, the weight of the model group (171.77±3.62 g) was the lowest, while that of the intervention group (186.97±3.19 g) was even lower when compared to the control group ($P<0.05$, Figure 1B). HE staining showed that the kidney tissues of the control group were basically normal, while the kidney interstitium and pelvic mucosa of the model rats showed severe inflammatory cell infiltration and fibrosis, and the kidney tissues of the intervention group showed significant improvement compared with the model group, and the degree of inflammatory infiltration was reduced (Figure 1C).

Impacts of CX extract on RF of APN rats

BUN, Scr, β2-MG and UA concentrations in the model group were (15.68±0.54 mmol/L), (170.36±6.04 μmol/L), (68.38±1.67 μg/mL) and (389.04±7.29 μmol/L), respectively, which were significantly higher compared with the control group ($P<0.05$), indicating the presence of significant RF injury in the model group. However, BUN, Scr, β2-MG, and UA in the intervention group were (12.72±0.50 mmol/L), (146.24±5.40 μmol/L), (56.83±2.22 μg/mL) and (307.47±14.69 μmol/L), respectively, which were still higher versus the control group, and were significantly lower when compared to the model group ($P<0.05$, Figure 2A-D). The results suggested that CX extract had an excellent improvement effect on the RF of APN model rats.

Impacts of CX extract on IRs in APN rats

The test results of IFs showed that IL-1β concentration in the model group was (82.80±6.53 pg/mL), higher than that in the other two groups, while IL-1β in the control group was lower when compared to the intervention group ($P<0.05$, Figure 3A). Similarly, IL-8 in the model group was (120.55±10.10 pg/mL), also the highest among the three groups, and was lower in the control group versus the intervention group ($P<0.05$, Figure 3B). The concentration of TNF-α in rats in the three groups from low to high was control group, intervention group and model group ($P<0.05$, Figure 3C). It could be seen that there were obvious IRs in the model group rats, and the process of inflammatory reaction could be effectively inhibited by using CX extract.

Impacts of CX extract on IL-6/STAT3 axis in APN rats

PCR results showed that model group rats had the highest IL-6 and STAT3 mRNA levels (0.64±0.05) and (0.65±0.04) in kidney tissues among the three groups. While the levels of IL-6 and STAT3 mRNA in the intervention group were (0.43±0.04) and (0.45±0.03), respectively, higher than those in the control group but lower than the model group ($P<0.05$, Figure 4A). The Western blot analysis showed that IL-6, STAT3 and p-STAT3 protein levels in the intervention group were higher compared with the control group ($P<0.05$), but lower than the model group ($P<0.05$, Figure 4B). Therefore, CX extract could inhibit IL-6/STAT3 pathway expression in APN rats.

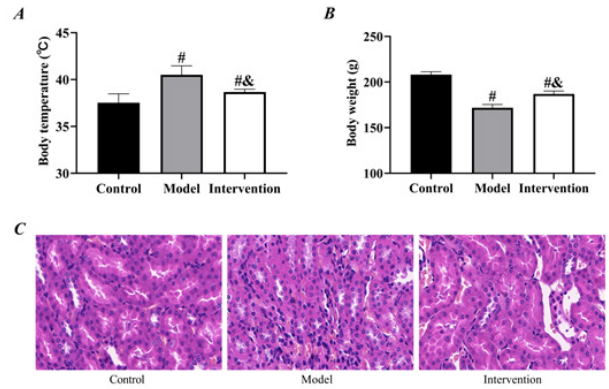

Figure 1. Impacts of CX extract on vital signs of APN rats. A: Comparison of body temperature of rats. B: Comparison of body weight of rats. C: HE staining of kidney tissues from rats. Note: compared with the control group, #P<0.05, compared with the model group, &P<0.05.

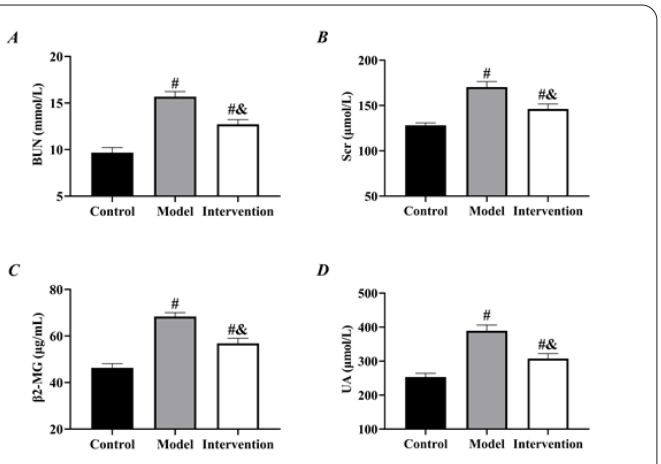

Figure 2. Impacts of CX extract on RF of APN rats. A: Comparison of BUN in rats. B: Comparison of Scr in rats. C: Comparison of β2-MG in rats. D: Comparison of UA in rats. Note: compared with the control group, #P<0.05, compared with the model group, &P<0.05.

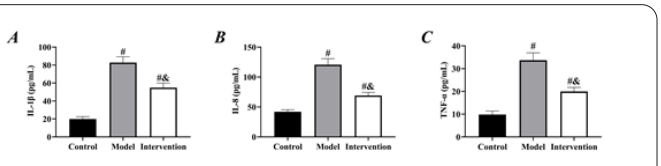

Figure 3. Impacts of CX extract on IRs in APN rats. A: Comparison of IL-1β in rats. B: Comparison of IL-8 in rats. C: Comparison of TNF-α in rats. Note: compared with the control group, #P<0.05, compared with the model group, &P<0.05.

Detection of intervention effect of exogenous IL-6

Firstly, the intervention results of exogenous IL-6 were confirmed by detecting IL-6/STAT3 pathway expression. IL-6, STAT3 and p-STAT3 protein levels showed no difference between group A and C (P>0.05), lower than those in group B (P<0.05, Figure 5), which indicated that the IL-6/STAT3 axis was activated after exogenous IL-6 injection, indicating success intervention.

Impacts of IL-6/STAT3 on RF in APN rats

Subsequently, rat RF in each group was detected. Groups A and C were found with similar BUN, Scr, β2-MG and UA concentrations (P>0.05); While BUN, Scr, β2-MG and UA concentrations in group B were (26.18±1.350 mmol/L), (227.51±9.55 μmol/L), (82.80±3.00 μg/mL) and (467.22±15.07 μmol/L), respectively, the highest among the three groups (P<0.05, Figure 6A-D). It could be seen that activation of the IL-6/STAT3 pathway could lead to more severe RF injury in APN rats, and the use of CX extract could reverse this process.

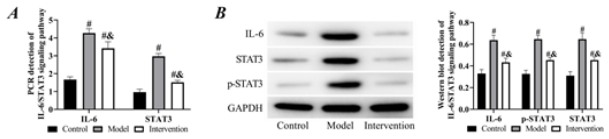

Figure 4. Impacts of CX extract on IL-6/STAT3 axis in APN rats. A: PCR was performed to detect the expression of the IL-6/STAT3 signaling pathway. B: Western blot was performed to detect the expression of the IL-6/STAT3 signaling pathway. Note: compared with the control group, #P<0.05, compared with the model group, &P<0.05.

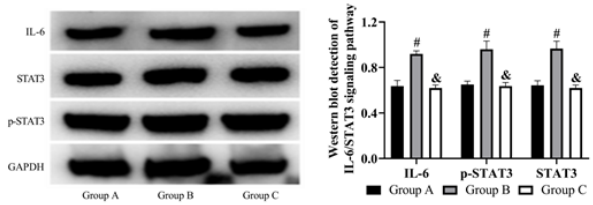

Figure 5. Detection of intervention effect of exogenous IL-6. Note: compared with Group A, #P<0.05, compared with Group B, &P<0.05.

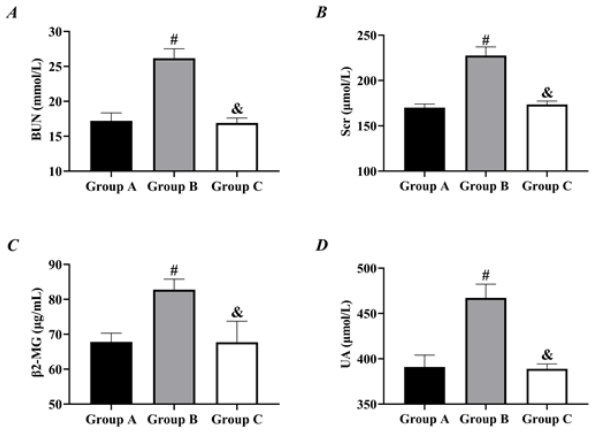

Figure 6. Impacts of IL-6/STAT3 on RF in APN rats. A: Comparison of BUN in rats. B: Comparison of Scr in rats. C: Comparison of β2-MG in rats. D: Comparison of UA in rats. Note: compared with Group A, #P<0.05, compared with Group B, &P<0.05.

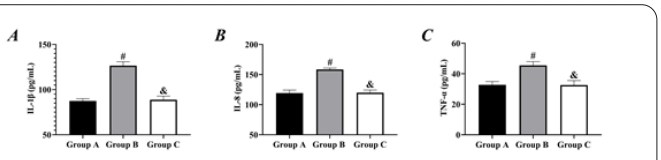

Figure 7. Impacts of IL-6/STAT3 on IRs in APN rats. A: Comparison of IL-1β in rats. B: Comparison of IL-8 in rats. C: Comparison of TNF-α in rats. Note: compared with Group A, #P<0.05, compared with Group B, &P<0.05.

Impacts of IL-6/STAT3 on IRs in APN rats

The test results of IFs showed that IL-1β, IL-8 and TNF-α differed insignificantly between groups A and C (P>0.05), which were lower when compared to group B (P<0.05, Figure 7A-C). This indicated that IRs in APN rats were significantly intensified after activation of the IL-6/STAT3 pathway, and the use of CX extract also reversed the aggravated IRs in APN rats.

Discussion

Clinically, the occurrence of APN is considered to be a complex process after pathogen infection, in which inflammation plays a crucial role (16). CX, an excellent anti-inflammatory drug, has been found to have significant pathological IR inhibition effects, antioxidant capacity and antibacterial ability in previous studies (17). Today, CX has shown excellent application results in neuroinflammation and antibacterial treatment (18, 19), but research on its application in APN is still rare. Although Yang et al. have found that APN can alleviate diabetic nephropathy to some extent (20), its specific mechanism is still not completely clear. Therefore, by analyzing the impacts and mechanism of CX extract on APN, this study could not only provide a new choice for future APN treatment but also lay a reliable foundation for subsequent researches.

In this experiment, we first established the APN rat model and found that the body temperature of the modeled rats increased significantly, while the body weight decreased, and the vital sign activity weakened significantly, confirming the success of the modeling. Under CX extract intervention, the rats' vital signs were obviously improved, which indicated that CX extract had a certain therapeutic effect on APN. Subsequently, it was showed obvious RF damage and IRs in model group rats, which was also in line with the pathological manifestations of APN (21). In the intervention group, the levels of IFs and IRs were all reduced, which further suggested that CX extract could repair RF injury to a certain extent and inhibit inflammatory processes. We also found through previous literature that CX extract had significant effects on repairing acute kidney injury and inhibiting inflammatory reactions in chondrocytes and vascular endothelial cells (22-24), which can also testify to the results of our experiment.

As we all know, the IL-6/STAT3 axis is one of the classic pro-inflammatory pathways, which has been proven to be critical in regulating IRs in many diseases (14, 25). In APN, significant abnormal activation of IL-6/STAT3 has also been confirmed (26). In this study, the IL-6, STAT3 and their phosphorylated proteins were increased in the model group, confirming the activation of this axis in APN. However, CX extract intervention led to obviously inhibited the IL-6/STAT3, demonstrating that the effect of CX extract on APN might be realized through the inhibi-

tion of the IL-6/STAT3 axis. To verify this conjecture, we further used exogenous IL-6 to intervene in APN rats. The results also showed that an activating IL-6/STAT3 axis could promote the pathological process of APN, which was consistent with former studies (27). However, the accelerated pathological process of APN caused by the IL-6/STAT3 axis was completely reversed by CX extract, confirming the regulatory relationship between them.

However, because there are some differences between animal models and human bodies, we need to carry out clinical trials as soon as possible to confirm the use of CX extract. Moreover, it is still worth carrying out more comprehensive basic experiments to analyze the effect mechanism of CX extract on APN. In the follow-up study, we will conduct a more complete study on the effect of CX extract on APN, so as to provide more reliable experimental results for clinical reference.

Conclusion

CX extract could effectively improve the renal function and inflammatory response in APN rats infected with E. coli by inhibiting the IL-6/STAT3 signaling pathway, which indicated that CX extract may become a new treatment option for APN in the future to protect the health and safety of patients.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors Contributions

Song Li designed the study, Juan Ji and Chaoyu Bi wrote the manuscript. Ling Tian and Shufeng Hou collected and analyzed data. Qian Ji supervised the research, Juan Ji and Chaoyu Bi have the same contribution. All authors read and approved the final submitted manuscript.

Acknowledgments

Not applicable.

Funding

This work was supported by the Baoding Science and Technology Plan Project (Approval No.2241ZF348).

Ethics approval and consent to participate

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

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