Effect of Xiaochaihu Decoction on Cytokines and MAO in Experimental Autoimmune Prostatitis Rats

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ABSTRACT

Objective To observe the effect of Xiaochaihu Decoction on cytokines and MAO in model rats with experimental autoimmune prostatitis (EAP) to provide an experimental basis for the use of Chinese herbal prescriptions in the treatment of chronic prostatitis.

Methods Thirty male Wistar rats were randomly divided into blank Group, model group and treatment group (Xiaochaihu Decoction). Except the blank group, rats in model and treatment groups were injected subcutaneously in multiple points on days 0 and 30 with prostatic protein extractive solution (60 mg/mL), and intraperitoneally injected with diphtheria-pertussis and tetanus vaccine (DPT vaccine) to establish the EAP model. Model rats were administrated Xiaochaihu Decoction and were sacrificed after 4 weeks. Pathological changes in the prostate gland were observed with HE staining and changes in serum interleukin-6 (IL-6), interleukin-8 (IL-8), and Tumor necrosis factor- α (TNF- α) levels were detected with enzyme-linked immunosorbent assay (ELISA). Quantitative PCR to observe the expression of IL-17, CCL2 and MAOA/B.

Results After treatment with Xiaochaihu Decoction, the serum IL-6, IL-8, and TNF- α levels all significantly lower in the treatment group as compared with the model group ($P < 0.05$). The intervention of Xiaochaihu Decoction can change the state of abnormally elevated IL-17, CCL2 and MAOA expression levels.

Conclusions The therapeutic efficacy remarkable from the theory of heating into the sperm chamber to experimental autoimmune prostatitis rats.

Key words Xiaochaihu Decoction; experimental autoimmune prostatitis; cytokines; MAOA

INTRODUCTION

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a commonly observed distress in male patients which is characterized by chronic pelvic discomfort, with voiding symptoms, sexual dysfunction, and associated mental health disorders (MHD)¹. Though current antibiotic, alpha-blocker, and electro-magnetic therapy seems promising, the efficient treatment of CP/CPPS remains rare². Traditional Chinese Medicine (TCM) has therapeutic effects on the disease, but as there is little experimental research and the therapeutic

mechanisms are unclear, TCM popularization and application is hindered. Increasing evidence support that autoimmunity is a major cause of dysfunction of the organs involved in CP/CPPS in both humans and in rodent models of autoimmune CP/CPPS³. Furthermore, inflammatory cytokines play a critical role in pathogenesis of autoimmune dysfunction and prove to be closely correlated to anxiety and depression⁴. Thus, we conducted this study to evaluate the effect of Xiaochaihu Decoction on cytokines and MAO in EAP rats. These results give evidence for the drug mechanism of action and provide a theoretical basis for the selection of TCM therapeutic.

MATERIALS AND METHODS

Experimental animals

Specific pathogen-free (SPF) Wistar rats were procured from the experimental animal center of Shandong University (Jinan, China). Rats were housed in an SPF environment in a standard housing room under controlled temperature (15–25°C), relative humidity (50–70%), and artificial light (12 h light/dark cycle), and were provided free access to food and water. All animal protocols were approved by the ethics committee of The Qilu Hospital of Shandong University (Jinan, China).

Preparation of rat prostate protein extractive solution

Ten male Wistar rats, weighing 350–300g, were intraperitoneally anesthetized under aseptic conditions and a median incision on the lower abdomen was made. The prostate tissue was removed without the adhesive tissue, weighed, and then equal volume of saline containing 0.5% Triton X-100 (Sigma, Germany) (preliminary high temperature sterilization) was added. The tissue was homogenized with a glass homogenizer in an ice-water bath, and centrifuged at 10000 r/min (3000 ×g) for 30 min at 4 °C. The supernatant was removed for detection of protein with the biuret method, and the protein concentration of the extracted solution was diluted to 60 mg/mL with PBS buffer.

Animal model and study design

Adult male Wistar rats (3 months old, weighing 250–300g) were randomly divided into blank group (n = 10), EAP model group (n = 10), and EAP model with Xiaochaihu Decoction treatment group (treatment group) (n = 10). Except for the rats in the control group, rats in other groups were modeled respectively on days 0 and 30 (60 mg/mL), 1 mL suspension of prostate protein extract and complete Freund's adjuvant (Sigma, Aldrich, United States) was injected subcutaneously, and intraperitoneally injected with diphtheria-pertussis and tetanus vaccine (DPT vaccine). The administration was given by intragastric perfusion (equal concentration method) from the second day (on day 31) after the second injection of modeling, once each day, for 28 days.

Detection of serum cytokines with ELISA

Blood samples were collected before rat sacrifice and centrifuged (3,000 g, 15 min, 4°C) to obtain serum. Serum was stored at –70°C until assayed. IL-6, IL-8, and TNF-α were analyzed by ELISA kits (R&D Systems, Minneapolis, MN, United States) according to manufacturer's protocols. Serum IL-6, IL-8, and TNF-α levels were determined following measurement of absorbance at 450 nm using a Bio-Rad microplate reader (Bio-Rad, Hercules, CA, United States).

HE staining of prostate

The rat's prostate was harvested, washed with 0.15 M saline, fixed in 4% paraformaldehyde, and embedded in paraffin at 4°C overnight. Serial 4 μm thick sections were stained with hematoxylin and eosin (HE). After HE staining, pathological changes of the prostatic tissue in each group were observed under light microscopy (Olympus, Japan).

Quantitative PCR

TRIzol was used for the extraction of mRNA; tissues specimens were incubated with TRIzol for 5–10 min at room temperature and subsequently centrifuged at $8,263 \times g$ at 4°C for 10 min. cDNA was obtained following reverse transcription; the primer was designed according to sequences; target sequences were amplified using a TIANScript RT kit (cat. no. KR104-02; Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocol and the reaction system was constructed for PCR. The housekeeping gene β -actin was used as the internal control in sample results.

Statistical analysis

SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, United States) was used for statistical analyses. The data are presented as mean $\pm$ SD and comparisons between groups were performed using one-way ANOVA. A result with $P < 0.05$ was considered statistically significant.

RESULTS

Pathological changes among the groups

Pathological changes among the groups In the blank group, the prostate tissues of the rats were intact, and fibers between acinuses and the smooth muscle were normally distributed, with no infiltration of inflammatory cells in the lumen of the gland and mesenchyme. There was also no hyperemia or edema. In the model group, the lumen of the gland in the rats' prostate tissues were dilated and enlarged, with secretion visible in the lumen, and dilated blood vessels with hyperemia. The epithelial cells of the acinus had obvious papillary hyperplasia, the mesenchyme had obvious fibrosis, and there were a great number of lymphocytes and plasma cells. Compared with the model group, after administration of the Xiaochaihu Decoction, the proliferation of epithelial cells was improved, the secretion of the cells was reduced, the inflammation disappeared to varying degrees, and the lumen of the gland was enlarged. Epithelial cells mildly proliferated with a regular arrangement, and there was less secretion in the lumen (Figure 1).

Figure 1 Pathological changes in the groups (HE staining, $\times 100$)

A blank group

B model group

C treatment group

Effects of prescriptions on serum cytokines in the EAP rats

Compared with the blank group, the IL-6, IL-8, and TNF- α levels were significantly higher ($P < 0.05$) in the model group. After treatment with Xiaochaihu Decoction, the serum IL-6, IL-8, and TNF- α levels all significantly lower in the treatment group as compared with the model group ($P < 0.05$) (Table 1).

Table 1 Changes in serum IL-6, IL-8, and TNF- α levels

Group	n	IL-6 (mol/l)	IL-8 (pg/ml)	TNF- α (pg/ml)
blank	10	6.54 $\pm$ 0.11	3.80 $\pm$ 0.60	4.23 $\pm$ 0.43
model	10	27.52 $\pm$ 2.69 [△]	17.8 $\pm$ 1.43 [△]	18.40 $\pm$ 2.17 [△]
treatment	10	16.54 $\pm$ 3.24 [▲]	4.64 $\pm$ 0.54 [▲]	9.22 $\pm$ 1.04 [▲]

Compared with the blank group, [△] $P < 0.05$, Compared with the model group, [▲] $P < 0.05$.

Effects of prescriptions on prostate tissues cytokines and MAO in the EAP rats

IL-17, CCL2 and MAOA/B exhibited high expression in the prostate tissues of EAP rats, indicating that IL-17, CCL2 and MAOA/B serve an important role in the occurrence and development of inflammation. Compared with the model group, the expression of IL-17, CCL2 and MAOA were significantly decreased in the treatment group ($P < 0.05$). The intervention of Xiaochaihu Decoction can change the state of abnormally elevated IL-17, CCL2 and MAOA expression levels, and there was no significant change in MAOB expression among the groups (Table 2).

Table 2 Changes in prostate tissues IL-17, CCL2 and MAOA/B expression (n=10)

Group	IL-17	CCL2	MAOA	MAOB
blank	1.04±0.10	1.08±0.11	0.761±0.019	0.562±0.032
model	6.45±0.67 [△]	11.95±1.70 [△]	0.786±0.015 [△]	0.557±0.026
treatment	2.90±0.13 [▲]	6.57±0.49 [▲]	0.766±0.011 [▲]	0.564±0.027

Compared with the blank group, [△] $P < 0.05$, Compared with the model group, [▲] $P < 0.05$.

DISCUSSION

Chronic prostatitis is a disease caused by a number of factors⁵ that may lead to discomfort in the groin area, pelvic pain, irritable urination and sexual dysfunction⁶. Its histological characterization is the infiltration of multinucleate and mononuclear cells into interstitial connective tissues⁷. However, the pathogenesis and diagnostic criteria for chronic prostatitis remains unclear, thus research in this area is hindered⁸. Therefore, appropriate animal models are of great value to understanding and treating this disease⁹. With the development of immunology and molecular biology, the effect of autoimmune factors on morbidity of chronic prostatitis is receiving greater attention¹⁰. The method of the current study was simple and highly effective; the mortality rate of animals was low and the model was stable, reliable and had good pathology specificity; the pathological changes were similar to the clinical manifestation and the pathogenesis was similar. Therefore, the model may be beneficial in the study of pathogenesis and efficiency for novel treatments for human chronic prostatitis.

Traditional Chinese medicine (TCM) has been used in the treatment of andrological conditions for over a thousand years, and an analysis of more than 5000 evidence-based medical studies provides strong evidence that TCM can be effective in the treatment of chronic prostatitis¹¹. In the theory of TCM, diseases are treated according to their symptoms. Thus, chronic prostatitis falls into the category of ‘stranguria’ and ‘turbid semen’, which is associated with heating into the sperm chamber. In TCM, the syndrome is characterised by damp and heat (two of the pathogenic factors in TCM), damp heat in the early pathology, blood stasis in the late pathology¹². The therapeutic regimen therefore focuses on reconciliation Shaoyang, improving symptoms by Xiaochaihu Decoction. The whole prescription clears damp heat to remove external pathogens and improve stagnation, which restores the normal physiologic state of the prostate. In the present study, it was found that after treatment with the prescription for reconciliation Shaoyang, serum IL-6, IL-8, and TNF- α levels were significantly lower than those in the model group. The intervention of Xiaochaihu Decoction can change the state of abnormally elevated IL-17 and CCL2 expression levels in the prostate tissues of EAP rats. This result indicates that the prescription has regulatory action on algogenic and inflammatory factors.

Monoamine oxidase (MAO) is a useful target in the treatment of neurodegenerative diseases and depressive disorders. Both isoforms, MAO-A and MAO-B, are known to play critical roles in disease progression¹³. CP/CPPS frequently show depressive symptoms clinically and increasing evidence indicates a correlation between CP/CPPS and depression. However, the underlying mechanisms of CP/CPPS-related depression remain poorly understood¹⁴. Here, our experiment found that MAOA/B exhibited high expression in the prostate tissues of EAP rats. The intervention of Xiaochaihu Decoction can change the state of

abnormally elevated MAOA expression levels. These findings provide preclinical validation of xiaochaihu Decoction for therapeutic intent in EAP rats with MHD.

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