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Research Article

Effect of Zhuang Yi Liu Fang Teng Fang on the Expression of TNF-α and NF-κB in Chronic Pelvic Inflammatory Disease (CPID) Model Rats

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Abstract: ***Objective:*** To investigate the expression of TNF-α and NF-κB in the uterus of chronic pelvic inflammatory disease (CPID) model rats. ***Methods:*** 40 female rats that adaptive fed for 5 days were randomly divided into normal group, model group, high dose group, medium dose group and low dose group. In addition to the normal group, the rats in each group were made chronic pelvic inflammatory model by mechanical injury combined with implantation of bacteria. The rats in each group were administrated by gavage for 20 days. After the last administration, the level of TNF-α and NF-κB in serum was measured by ELASA method. ***Results:*** (1) after the establishment of the model, the uterus of the chronic pelvic inflammatory model rats showed the pathological damage of chronic inflammation; the levels of TNF - α and NF-κB in serum were higher than those in the normal group, the difference was statistically significant (P<0.05). (2) After the drug intervention, the uterine tissue morphology of the rats in the Zhuang Yi Liu Fang Teng Fang Group was basically restored to normal, with only a small amount of inflammatory cell infiltration and other pathological changes; compared with the rats in the model group, the TNF-α in serum of the rats in each treatment group was lower, the difference was statistically significant (P<0.05). The expression of NF-κB in serum of each treatment group was lower than that of the model group (P<0.05). ***Conclusion:*** Zhuang Yi Liu Teng Fang can effectively improve the endometrial histomorphology of CPID model rats, and regulate the levels of TNF - α and NF - κ B in the uterus of chronic pelvic inflammatory model rats.

Keywords: Zhuang Yi Liu Fang Teng Fang; Chronic pelvic inflammatory disease; TNF - α; NF-κB.

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1 Introduction

Chronic pelvic inflammatory disease is a common gynecological disease, showing an increasingly high incidence in recent years. TNF-α is an important pro-inflammatory factor with more biological effects, which plays a role in bidirectional regulation in the process of the inflammation, in the early stage, it can play an immunomodulatory role and improve anti-infection ability; but its sustained release will stimulate endothelial cells to produce adhesion factors to induce producing inflammatory mediators, causing pathological damage which is positively correlated

with the expression of TNF-α[1]. NF-kB is a nuclear transcription factor widely present in human tissues and cells, it can participate in human inflammation, injury and repair, and has been confirmed that it plays a critical role in the regulation of inflammation[2]. The Zhuang Yi Liu Teng Fang is a special prescription formed under the guidance of Zhuang Yi theory with good clinical effect. However, it remains to be discovered that what mechanism of this side acting on chronic pelvic inflammatory disease. It is expected to explore the mechanism of the treatment on chronic pelvic inflammatory disease by the Zhuang Yi Liu Teng Fang in this experiment so as to be used in clinical better.

1 Materials

1.1 Animals

40 SD female rats, 7-9 weeks old, weighing (220 ± 20)g, and were purchased from Changsha Tianqin Biotechnology Co., Ltd., and the certificate number is SCXK (Xiang) 2014-001. They were all fed at the same temperature and same environment.

1.2 Drugs

The treatment group was given Zhuang Yi Liu Fang Teng Fang: Liu Fang Teng 30 g, Jiu Long Teng 20 g, Hematoxylin 20 g, wu zhi mao tao 20 g, maodongqing 25 g, sealwort 20 g, Bidens 10 g, Chuanxiong 10 g, and liquoric root 10g. (Made by the preparation room of Ruikang Affiliated Hospital of Guangxi University of Traditional Chinese Medicine) All fried into 200ml of decoction, which is used in the high-dose group; the middle-dose group is half of the high-dose; the low-dose is half the medium-dose concentration.

1.3 Equipment

TNF-αtest kit and NF-κB test kit; strains (E. coli, Staphylococcus aureus); 10% chloral hydrate, scalpel, tissue scissors, hemostatic forceps, stitch scissors, tissue forceps, non-absorbent surgical silk thread, sterilized medical suture needle and iodophor disinfectant are all provided by Guangxi Key Laboratory of Traditional Chinese Medicine.

2 Experiment methods

2.1 Experiment procedure

2.1.1 Experiment grouping

Forty SPF female SD rats were randomly divided into normal group, model group, high, middle and low dose group of Zhuang Yi Liu Teng Fang. Eight rats of each group.

2.1.2 Experiment molding

Take methods of mixed bacteria plus mechanical injury modeling on rats which have been fed for 7 days.

2.1.3 Experiment administration

After the molding is done. All rats of model group, except blank group, were intragastric filled by high, middle and low dose of Zhuang Yi Liu Teng Fang for 20 days.

2.1.4 Experiment materials

After modeling is succeeded, blood was taken from the eye sockets and the serum was prepared for later use. After the last administration, pathological observation of the uterine tissue was performed. Animals were anesthetized with 10% chloral hydrate, blood was collected in the abdominal aorta, left at room temperature for 2 hours, and centrifuged at a low-temperature high-speed centrifuge (about 3000r / min) for 5 minutes. Then the supernatant was collected and placed in a refrigerator below -20 ℃ save for future use, and TNF-α level and NF-κB level in the serum was determined by ELASA method.

2.3 Statistical analysis

SPSS 21.0 statistical software was used for statistics. The measurement data was expressed in the form of mean ± standard deviation ($\bar{x} \pm s$), using t-test, and groups were analyzed by variance. $P<0.05$ was considered to show significant difference, and $P<0.01$ was extremely significant.

3 Experiment results

3.1 After molding

The uterine morphology of rats with chronic pelvic inflammatory disease showed pathological damage of chronic inflammation. The expression levels of serum TNF-α and NF-κB were higher than that of blank group, and the difference was statistically significant ($P<0.01$). See table 1.

Table 1. Contents of TNF-α、NF-κB in serum of chronic pelvic inflammatory model rats($\bar{x} \pm s$)

Group	Number	TNF-α(pg/mL)	NF-κB(ng/mL)
Blank group	8	245.87±12.34**	9.05±2.01**
Model group	32	418.08±11.31	25.15±0.63

Note: compared with blank group **P<0.01.

3.2 After drug intervention

The uterine morphology of the rats in the Zhuang Yi Liu Fang Teng Fang group showed returning normal of the uterine cavity and glands. The expressions of serum TNF-α and NF-κB in the high and middle dose groups of Zhuang Yi Liu Teng Fang were dramatically lower than those in the model group. (P<0.01); The expression of serum TNF-α and NF-κB in the low-dose group of Zhuang Yi Liu Teng Fang was obviously lower than that in the model group (P<0.05). See Table 1.

Table 2. Effect of Zhuang Yi Liu Teng Fang on TNF-α、NF-κB of chronic pelvic inflammatory model rats ($\bar{x} \pm s$)

Group	Quality	TNF-α(pg/mL)	NF-κB(ng/mL)
Blank group	8	233.67±13.12##	8.94±2.13##
Model group	8	421.87±8.72	24.56±3.51
High dose of Zhuang Yi Liu Teng Fang group	8	252.93±12.48##	11.67±3.23##
Middle dose of Zhuang Yi Liu Teng Fang group	8	287.52±11.99##	13.78±4.19##
Low dose of Zhuang Yi Liu Teng Fang group	8	331.12±8.31#	16.15±2.34#

Note: compared with model group, #P<0.05, ##P<0.01.

4 Discussion

Chronic pelvic inflammatory disease is a common gynecological disease in clinic, TCM treatment have a variety of methods according to the different types of evidence. However, there are methods of external and internal treatment in general. The treatment of 60 cases of the disease was divided into two groups, which were clinically treated with Pelvic Inflammatory Disease Clearing Recipe and Kanggongyan capsule belonging to decoction of traditional Chinese medicine by Zhou Xiaoying et al[4]. Luo Suqin[5] also achieved good results in the treatment of blood stasis chronic pelvic inflammatory disease with Guizhi Fuling pill and Xuefu Zhuyu decoction; Zhang Yufeng et al[6] used Heat-Clearing and Blood-Activating Decoction to treat damp-heat type of chronic pelvic inflammatory disease. The clinical effect of Herbal decoction is the most significant. There are plenty of external treatment methods in TCM, such as fumigation, application, traditional medicine clyster, acupuncture and vaginal administration. With the increasing development of modern medical technology, the changes of tissue and molecule caused by pathological changes of organism are revealed gradually. Chronic pelvic inflammatory disease (CPID) is exactly an inflammatory reaction, and the related inflammatory molecules such as IL-6, TLR4, TNF-, IL-2 and NF-b signal pathway have been found to be closely related to CPID.

Zhuang Yi Liu Fang Teng Fang is the wisdom crystallization of Zhuang Doctors for thousands of years, and has been used to treat chronic pelvic inflammatory disease clinically with remarkable effect[8]. In this study, the expression of TNF-α and NF-κB in Liu Teng Fang decoction was investigated, and the mechanism of it was to be further elucidated. The Liu Teng Fang was composed of Liu Fang Teng 30g, Jiu Long Teng 20g, Hematoxylin 20g, wu zhi mao tao 20g, maodongqing 25g, sealwort 20g, Bidens 10g, Chuanxiong 10g, and liquoric root 10g. All was jointly used to dredge the channels and collaterals, dissipate blood stasis and relieve pain, regulating spirit blood and nourish yin. The experiment showed that Zhuang Yi Liu Teng Fang could effectively alleviate the inflammation and inhibit the expression of TNF-and NF-κB. To explore the mechanism of Liu Teng Fang on decreasing the expression of TNF-αand NF-b. It is easy to find that Liu Teng Fang has its unique advantages in treating chronic pelvic inflammatory disease in modern pharmacology research. Liu Fang Teng is also named Tripterygium hypoglaucum Hutch, Jiang Xiao et al[9] found that Tripterygium hypoglaucum Hutch total alkaloids (THHTA) play an anti-inflammatory role by inhibiting the production of inflammatory mediators TNF-and NO. Guo Chunfeng[10] experiments found that extracts

of Sappanwood Ethyl Acetate can reduce blood levels of TNF-α. Conlin[11] found that Sappanone A(SA) can inhibit the activation of the NF-b channel and improved inflammation. Xue Xuebin et al[12] found that polygonatum polysaccharide (PSP) inhibit the expression of NF-κB, control the proliferation and development of inflammatory factors by inhibiting the expression of NF-κB. There were also experiments[13] found that PSP reduce the serum levels of IL-6, TNF-α, CKMB and Lactate dehydrogenase (LDH) in rats by inhibiting the NF-b channel. The Ligusticum Chuanxiong Hort can relieve pain and activate blood circulation, whose extract can cause NF-κB nuclear factor shift, shortening half-life period of TNF-αand increasing TNF-αdegradation. Besides, it was also found that Ligustilide can inhibit the activation of NF-κB and inhibit inflammation[14]. Wang Maojun et al[15] did experiments finding that compound glycyrrhizin inhibited TNF-α. The strict prescription can effectively inhibit the expression of TNF-αand NF-κB, and cure chronic pelvic inflammatory disease with each medicine in place.

The experiment results showed that high-dose group had the best effect and could reduce the expression of TNF-and NF-b in serum of rats. It needs enough dose of Zhuang Yi Liu Teng Fang to play a role in the treatment of chronic pelvic inflammatory disease.

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