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Anti-inflammatory effect of Man-Pen-Fang, a Chinese herbal compound, on chronic pelvic inflammation in rats

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ABSTRACT

Ethnopharmacological relevance: Traditional Chinese Medicine (TCM) has become the focus of research for the treatment of chronic pelvic inflammatory disease (CPID) based on unique medical theory system. Man-Pen-Fang (MPF), a Chinese herbal compound, which is composed of *Thlaspi arvense* L. (Cruciferae), *Gleditsia sinensis* Lam. (Leguminosae), *Smilax china* L. (Liliaceae), *Euonymus alatus* (Thunb.) Sieb. (Celastraceae) and *Vaccaria segetalis* (Neck.) (Caryophyllaceae) MPF has been used for the treatment of CPID and exerted significant clinical curative effects. However, the corresponding active principles and anti-inflammatory mechanism of MPF are still unknown.

Aim of the study: The objective of present study is to evaluate the effect of MPF on CPID in the chronic pelvic inflammation (CPI) rat model and elucidate its possible anti-inflammatory mechanism.

Materials and Methods: The CPI in rats was induced by administration with E. coli, Staphylococcus aureus and Beta-hemolytic streptococcus. MPF (8.112 g/(kg d) (20 times of adult dosage), 4.056 g/(kg d) (10 times of adult dosage) and 2.028 g/(kg d) (5 times of adult dosage)) and Jingangteng Capsule 2 g/(kg d) (20 times of adult dosage) were administered orally for 20 days. The serum levels of five inflammation-associated cytokines (IL-2, IL-6, IL-10, TNF-α and TGF-β_1) were determined by enzyme-linked immunoassay, and the mRNA expression levels of TGF-β_1, P53, Fas, FasL and MMP-2 in the uterus tissue were measured by quantitative RT-PCR. Furthermore, the expression of NF-κB p65 in uterus and ovary tissues was detected by immunohistochemistry assay and the pathological changes induced in the uterus and ovary tissues were observed by histology.

Results: MPF caused a reduction in serum levels of IL-2, IL-6, IL-10, TNF-α and TGF-β_1. The expression of P53 mRNA, Fas/FasL mRNA and MMP-2 mRNA in the uterus tissue was significantly elevated after treating with MPF, in contrast the expression of TGF-β_1 mRNA was decreased. Furthermore, the expression of NF-κB p65 in uterus and ovary tissue was inhibited after treating with MPF.

Conclusions: These results taken together suggest that MPF has a significant anti-CPID effect, probably due to inhibition of the inflammation reaction by the promotion, and the induction of the apoptosis of inflammatory cells and downregulation of the serum levels of inflammation cytokines.

1. Introduction

Pelvic inflammatory disease (PID) is an infection and inflammation of the uterus, ovaries, and other female reproductive organs and is one of the most common infections in non-pregnant women of reproductive age and remains an important public health problem (Loucks, 1983; Snaith, 1959). It can lead to long-term sequelae, such as infertility, ectopic pregnancy, pelvic pain, abscesses, and other serious problems (Cassell et al., 1997). These problems are called sequelae of pelvic inflammatory disease (SPID) or chronic pelvic inflammatory disease (CPID), which seriously influence the health of women and the quality of life and increases the economic burden of family and society (Barrett and Taylor, 2005; Gradison, 2012). The sexually transmitted organisms, *Neisseria gonorrhoeae* and

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Chlamydia trachomatis are present in many cases and microorganisms comprising the endogenous vaginal and cervical flora are frequently associated with PID. These include anaerobic and facultative bacteria, similar to those associated with bacterial vaginosis (Burnakis and Hildebrandt, 1986; Duarte et al., 2015). Some women have no symptoms while others have pain in the lower abdomen, fever, foul smelling vaginal discharge, irregular bleeding, and pain during intercourse or urination (Haggerty et al., 2005). Early treatment is important as waiting too long increases the risk of infertility.

There are many disadvantages of antibiotics and surgical treatment for CPID, hence Traditional Chinese Medicine (TCM) has become the focus of research for the treatment of CPID. There is no definition of CPID in TCM. And in the theory of TCM, CPID is considered to be caused by cold and dampness damage to the uterus and uterine collateral leading to Qi obstruction, stagnation of blood stasis, recurrent and refractory lingering (Chen, 2012; Liu et al., 2014; Bu et al., 2015). The main pathogenesis of CPID is stagnation of blood stasis. Thus, promoting and dredging the channel circulation and removing stasis are principles of the treatment for CPID (Liu et al., 2014).

A Chinese herbal compound, Man-Pen-Fang (MPF), which is composed of *Thlaspi arvense* L. (Cruciferae), *Gleditsia sinensis* Lam. (Leguminosae), *Smilax china* L. (Liliaceae), *Euonymus alatus* (Thunb.) Sieb. (Celastraceae) and *Vaccaria segetalis* (Neck.) (Caryophyllaceae), as shown in Table 1 is effective in dispersing blood stasis and dredging collaterals. In this Chinese medicine formula, *Thlaspi arvense* L. is recorded in Shen Nong's Herbal Classic and is reported to clear heat, is an antitoxicant, activates blood, and resolves stasis. Compendium of Materia Medica indicates that *Gleditsia sinensis* Lam. has effect on removing toxicity for detumescence (Kim et al., 2015; Li et al., 2016). *Smilax china* L. is good at dispelling wind-damp, reducing swelling and activating blood circulation to dissipate blood stasis (Lee et al., 2001; Shah et al., 1962). *Euonymus alatus* (Thunb.) Sieb. activates blood to promote menstruation and *Vaccaria segetalis* (Neck.) has analgesic and anti-inflammatory effects. All these drugs have a role in activating blood circulation to dissipate blood stasis, clearing heat as an antitoxicant, reducing swelling and analgesia.

MPF has a history of clinical use for the treatment of CPID more than 20 years and has a significant curative effect. Herein, CPI model in rats was established to evaluate the anti-inflammatory effect of MPF on CPID. In order to explore the possible mechanism of MPF, the serum levels of five inflammatory cytokines (IL-2, IL-6, IL-10, TNF-α, and TGF-β_1) were measured by Elisa assay followed by the measurement of mRNA expression levels of TGF-β_1, P53, Fas, FasL, and MMP-2 in uterus tissue by quantitative RT-PCR. Furthermore, the expression of NF-κB p65 in uterus and ovary tissues was measured by immunohistochemistry assay and finally the tissues were examined histologically by staining with haematoxylin and eosin to ascertain the effect of MPF on CPID.

Table 1
The composition of Man-Pen-Fang (MPF).

Scientific name	Chinese name	Weight (g)	%
Thlaspi arvense L. (Cruciferae)	Baijiangcao	30	20
Gleditsia sinensis Lam. (Leguminosae)	Zaojiaoci	30	20
Smilax china L. (Liliaceae)	Baqia	30	20
Euonymus alatus (Thunb.) Sieb. (Celastraceae)	Guijianyu	30	20
Vaccaria segetalis (Neck.) (Caryophyllaceae)	Wangbuliuxing	30	20
Total amount		150	100

2. Materials and methods

2.1. Bacterial strains and animals

E. coli (ATCC25922) and Staphylococcus aureus (ATCC29213) were obtained from bacteriological laboratory of Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine. Beta-hemolytic streptococcus (CMCC32210) was purchased from Beina Chuanglian biotechnology research institute. The three bacterial strains were cultured by the conventional bacterial cultivation method. Female Wistar rats, 6–8 weeks old were obtained from animal laboratory of Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine and were kept at room temperature.

2.2. Preparation of Man-Pen-Fang

The crude drugs of Man-Pen-Fang were purchased from Shanghai Kangqiao Chinese herbal pieces co., LTD. All the herbs were prepared in proportion by soaking in 8 times the weights of pure water and were extracted twice by refluxing for 1 h. The filtrate was merged, concentrated under vacuum and the moisture was removed by placing the samples in an oven at 50 ℃. The extract was then used for the following experiments.

2.3. HPLC/ESI-MS and fingerprint analysis

An Agilent 1100 HPLC system, equipped with a quaternary pump, an autosampler, a degasser, an automatic thermostatic column compartment, a DAD and an LC/MSD Trap XCT ESI mass spectrometer (Agilent Technologies, MA, USA), was used for the analysis of the herb. The separation was performed on a GS-120-5-C18-BIO chromatographic column (5 μm, 250 × 4.6 mm i d.) with the column temperature set at 35 ℃. A linear gradient elution of A (0.1% formic acid water) and B (acetonitrile) was used with the gradient procedure as follows: 0 min, B 5%, to 60 min B 40% (v/v). The flow rate was 1.0 mL/min and the injection volume was 10 μL. DAD was on and the target wavelength was simultaneously set at 210 nm. The split ratio to the mass spectrometer was 1:3. The acquisition parameters for negative ion mode were: collision gas, ultra high-purity helium (He), nebulizer gas (N_2), 35 psi, drying gas (N_2), 10 L/min, drying temperature, 350 °C, HV, 3500 V, mass scan range, *m/z* 100–2200, target mass, 500 *m/z*, compound stability, 100%, trap drive level, 100%. All the data were analyzed by Chemstation software.

2.4. The establishment of CPID model in rats and Animal treatment

The oestrus of female Wistar rats was identified by vaginal smear for continuous 3 cycles. Only those with regular cycles were allowed to continue the next experiments. Suitable female Wistar rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate and the endometrial tissue was damaged with a syringe, followed by administration of a mixture of E. coli, Staphylococcus aureus and Beta-hemolytic streptococcus of 9×10^5 IFUs in 0.15 mL distilled water to the uterus on the right hand side (Tuffrey et al., 1992; Chen et al., 2008;). E. coli, Staphylococcus aureus and Beta-hemolytic streptococcus were in a ratio of 2:1:1.

Seven days after infection, successfully infected rats were randomly divided into 5 groups (n = 10) as following: the model group, positive group, and high-dose, mid-dose and low-dose MPF groups (MPF-H, MPF-M and MPF-L). 10 female Wistar rats did not receive any treatment and served as the control group. The drug was intragastrically administrated to the rats for 20 days, and the distilled water was used as control. The rats in the control group and the model group received distilled water in the same volume; in the positive group, Jingangteng Capsule 2 g/(kg d) (20 times of adult dosage) was