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EXPERIMENTAL STUDY

Effect of warming *Yang* and removing blood stasis method on matrix metalloproteinases / tissue inhibitor metalloproteinases levels secreted by cultured endometrial cells from patients with endometriosis

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Abstract

OBjECTIVE: To investigate the effect of Chinese medicines using the warming *Yang* and removing blood stasis method on levels of matrix metalloproteinases (MMPs)/tissue inhibitor metalloproteinases (TIMPs) secreted by cultured endometrial cells from patients with endometriosis.

METHODS: Ectopic and eutopic endometrial cells obtaind from 15 endometriosis patients were cultured *in vitro* , and divided randomly into five groups: high dose; moderate dose; low dose; nemestran; blank control. The three dose groups were treated with a decoction prepared according to the principle of warming *Yang* and removing blood stasis; nemestran and 0.9% NaCl were administered to the nemestran group and balnk control group, respectively. Eutopic endometrial cells obtaind from 10 hysteromyoma patients were cultured *in vitro* , as the normal control group , 0.9% NaCl were administered to the normal control group. Cell culture supernatants were collected and levels of matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), tissue inhibitor metalloproteinase-1 (TIMP-1) and tissue inhibitor metalloproteinase -2 (TIMP-2) detected by enzyme-linked immuno sorbent assay (ELISA).

RESULTS: Compared with the normal control group, levels of MMP-1, MMP-2, and MMP-9 in eutopic and ectopic endometrium cell supernatants in the blank control group were increased, whereas levels of TIMP-1 and TIMP-2 were decreased ($P < 0.05$). Compared with the blank control group, levels of MMP-1 and MMP-2 in ectopic and eutopic endometrium cell supernatants cultured in low-dose, middle-dose, and high-dose groups were decreased, whereas levels of TIMP-1 and TIMP-2 were increased significantly ($P < 0.05$).

CONCLUSION: The warming *Yang* and removing blood stasis method affects expression of MMPs and TIMPs.

Key words: Endometriosis; Matrix metalloproteinases; Tissue inhibitor of metalloproteinases; Warming *Yang*; Removing blood stasis

INTRODUCTION

The incidence of endometriosis (EMS) in women of childbearing age is about 5%-10%. EMS is a relatively common gynecologic disease characterized by the proliferation, invasion and metastasis of cells, as well as recurrence, and malignancy.[1] Studies have shown that matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) are associated with EMS onset.[2,3]

MMPs are a group of proteases that can degrade almost all components of the extracellular matrix (ECM; e.g., collagen, protein-fiber connections, proteins, polysaccharides). MMPs are a class of highly conserved enzymes in nature. TIMPs are a family of natural, specific inhibitory factors of MMPs that can combine with the corresponding MMP preferment or active enzyme to inhibit MMP activity. These two factors have important roles in maintenance of the stability and structural integrity of the internal environment of the ECM.[4,5] Studies have shown that disorder of MMPs/TIMPs can promote destruction and degradation of the peritoneal ECM and prompt the development of EMS.[6,7] However, the occurrence, development, and prognosis of EMS warrants further study because these factors are incompletely understood.

EMS treatment with Traditional Chinese Medicine (TCM) has gained increasing attention in recent years. Main pathologic change of EMS is periodic bleeding of the ectopic endometrium (known as "blood circulating out of vessels" in TCM). Numerous scholars believe that the basic pathogenesis of EMS (in terms of TCM theory) is blood stasis; treatment is based on promotion of blood circulation and removal of blood stasis. However, the clinical outcome for some EMS patients is not significant, which suggests that the pathogenesis and therapeutic principle of TCM in EMS merits further investigation.

Clinical observations as well as ancient and modern literature suggest that the pathogenesis of EMS in terms of TCM is *Yang* deficiency and blood stasis. Thus, EMS treatment should consider both of these aspects. In clinics, warming *Yang* and removing stasis (by consumption of Dangguisini soup) has been applied, replacing the convention of activating blood and removing stasis for EMS treatment. Results have shown that the curative effect has been obviously improved (especially in terms of relieving dysmenorrheal and chronic pelvic pain) for EMS patients.

In the present study, the effect of warming *Yang* and removing blood stasis on cultured endometrial cells in EMS patients was observed. Pathogenesis of EMS, as well as the therapeutic effect of warming *Yang* and removing blood stasis on EMS, was investigated further.

METHODS

Subjects

Twenty-one cases of EMS patients and 12 cases of Hysteromyoma patients were recruited from the Department of Gynecology in Guangdong Provincial Traditional Chinese Medicine Hospital (Guangdong, China) from October 2010 to August 2012. ALL patients were underwent surgery, the ectopic endometrium and eutopic endometrium in patient were gain and cultureed *in vitro*. The cultivation of 15 cases of EMS and 10 cases of Hysteromyoma patients were successful, The average age of the recruited patients was (32 ± 4) years. Hormonal drugs had not been used within 3 months in all cases.

Preparation of Chinese medicines for warming Yang and removing blood stasis

Chinese medicines used for warming *Yang* and removing blood stasis were purchased from Kangmei Pharmaceutical Company (Puning, China) and were: Danggui (*Radix Angelicae Sinensis*), 1.5×10^4 mg; Guizhi (*Ramulus Cinnamomi*), 1.0×10^4 mg; Baishao (*Radix Paeoniae Alba*), 1.5×10^4 mg; Xixin (*Herba Asari Mandshurici*), 3.0×10^3 mg; honey-fried Gancao (*Radix Glycyrrhizae*), 1.0×10^4 mg; Tongcao (*Medulla Tetrapanaicis*), 1.0×10^4 mg; Dazao (*Fructus Jujubae*), 1.0×10^4 mg; Wuyao (*Radix Linderae Aggregatae*), 1.0×10^4 mg; Fuzi (*Radix Aconiti Lateralis Preparata*), 6.0×10^3 mg; Ganjiang (*Rhizoma Zingiberis*), 9.0×10^3 mg. Total weight of these ingredients was 9.6×10^4 mg. Ingredients were boiled in a ceramic frying pan and centrifuged (1699 × *g* for 10 min, Again, 3999 × *g* for 10 min) to obtain a crude drug concentration of 700 mg/mL in liquid; and then stored in a sterile vessel. Phosphate-buffered saline was used to dilute nemestran to 8 mmol/L.

Culture and identification of cultured endometrial cells

Culture and identification of cultured endometrial cells were undertaken according to a method reported previously,[8] with slight modification. Obtained tissue was shredded and washed with Dulbecco's modified Eagle's media: Nutrient Mixture F-12 ("FD medium"; Sigma-Aldrich, Saint Louis, MO, USA). After centrifugation, a double-enzyme digestion liquid was added in an incubator. After washing, centrifugation and discarding supernatants, the mixture was filtered. The filtrate contained mainly interstitial cells, with clusters of glandular cells on the filter. Cells in the filtrate were in-