

Original Research Article

Effect of Taoren-Quyú decoction on endometriosis in rats

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Abstract

Purpose: To study the effect of traditional Chinese Medicine formula Taoren-Quyú decoction (TQD) on endometriosis.

Method: Fifty female Wistar rats were randomly separated into five groups (10 rats/group): normal control, model (untreated) group, positive control (danazol), 200 mg/kg/day (low dose) or 400 mg/kg/day (high dose). All rats were prepared into endometriosis except for normal control rats. TQD groups rats were orally administered of TQD for 5 weeks. After treatment, the rats were sacrificed by cervical dislocation. The number of total endometriotic lesions were counted. Serum levels of cancer antigen 125 (CA-125), interleukin 13 (IL-13), interleukin 18 (IL-18) and peritoneal fluid tumor necrosis factor- α (TNF- α) were measured by ELISA kits.

Result: Compared with control rats, TQD reduced the number of total endometriotic lesions significantly (12.7 ± 1.2 , $p < 0.01$), as well as serum levels of CA-125 (6.4 ± 1.2 U/mL), IL-18 (118.6 ± 7.4 pg/mL), IL-13 (6.3 ± 0.8 pg/mL) and peritoneal fluid TNF- α (231.5 ± 11.7 pg/mL) ($p < 0.01$).

Conclusion: The results reveal that TQD exerts anti-endometriotic effect in rats by inhibiting inflammatory factors. Therefore, TQD has potentials for use in the treatment of endometriosis.

Keywords: Taoren-Quyú decoction, Endometriosis, Cancer antigen, Endometriotic lesions, Matrix metalloproteinase

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INTRODUCTION

As a common gynaecological disease, endometriosis usually induces dysmenorrhoea, chronic pelvic pain and infertility. The morbidity of endometriosis is 14 % in normal population and 40 % in infertile women [1]. The typical symptoms of endometriosis is ectopic implantation and growth of endometrial tissue and local sterile inflammation of peritoneal cavity. Proliferation, adhesion, and migration of ectopic endometrial tissue are required to establish

endometriotic lesions in the peritoneal cavity [2,3].

The endometriosis-associated inflammatory response, tissue repair and neo-vascularization are dependent on the peritoneal fluids macrophages and their secretory products/cytokines [4]. These cytokines may play major roles in regulating cell proliferation, activation, motility, adhesion, chemotaxis and morphogenesis in the pathogenesis of endometriosis [5]. Increased peritoneal fluids

concentration of cytokines that lead to the migration, proliferation, and activation of macrophages have been reported in patients with endometriosis. The biomarkers such as cancer antigen 125 (CA-125) and IL-13, IL-18 and TNF- α levels in the peritoneal fluids were found linked with endometriosis [6]. Although surgical and hormonal treatments are applied as the common interventions, the unpleasant side effects limited the use of these interventions. Some medicinal plants were shown to be potential treatment for the disease [7]. *Taoren-Quyu* Decoction (TQD), which is composed of *Prunus persica* (L.) Batsch 10 gram, *Salvia miltiorrhiza* Bge. Ten grams *Angelica sinensis* 5 gram and *Leonurus artemisia* (Laur.) S. Y. Hu F 5 gram, is a famous traditional Chinese Medicine formula in China. TQD has been used to treat endometriosis for many years and has achieved good curative action. In this study, the anti-endometriotic effect of *Taoren-Quyu* Decoction (TQD) was investigated in human endometriotic cells and rats.

EXPERIMENTAL

Preparation of TQD

The medicinal herbs of TQD were collected from Zhunyi City, Guizhou Province in China in May 2017. Taxonomic identification of the plants was performed by Prof Lin Hu of Harbin Medical University China. A voucher specimen (no. TQD 20170505) was deposited in the herbarium of College of Pharmacy, Harbin Medical University, China for future reference. *Prunus persica* (L.) Batsch 10 gram, *Salvia miltiorrhiza* Bge. 10 gram, *Angelica sinensis* 5 gram and *Leonurus artemisia* (Laur.) S. Y. Hu F 5 gram were mixed and decocted with ten times of volume of water for 40 minutes. It was repeated for twice. Then the decoction was filtrated and concentrated into the volume of 100 mL to obtain TQD [8].

Animals and grouping

Female Wistar rats weighing 200 - 220 g were obtained from Experimental animal center of Heilongjiang Province, Heilongjiang, China. The animals had free access to feed and water, and were allowed to acclimatize for at least one week before use. All animal experiments were approved by the Animal Care and Use Committee of Harbin Medical University (approval ref no. 20131007) and were carried out in compliance with the Animal Welfare Act and NIH guidelines (NIH publication no. 80-23, revised 1996) [9]. Endometriosis was induced in rats using a previously established method with modifications [10]. The uterine horns of the donor

mice were removed and put into a dish containing PBS. The endometrium-rich fragments (1 cm) from the middle-third of the uterine horn were finely and uniformly chopped. The fragments (~20 pieces) suspended in PBS were injected into the peritoneal cavity of recipient mice with a micropipette to induce the formation of endometriosis-like lesions. Forty (40) rats with induced endometriosis were randomly divided into four groups (10 rats/group): control, Danazol[®], high-dose TQD and low-dose TQD. Ten normal rats were used as normal control. Rats were orally administered either vehicle (normal control, 200 μ L of phosphate buffered saline) alone or TQD 200 mg/kg/day (low dose) or 400 mg/kg/day (high dose) for 5 weeks. Danazol was used as positive control (reference). Danazol produces an environment with high androgen and low estrogen levels, which leads to the atrophy of endometriotic implants [11]. After induction of endometriosis for 4 weeks, the rats were sacrificed by cervical dislocation and the peritoneum and visceral organs were examined visually to measure the number of endometriotic lesions (1 > mm).

Enzyme linked immunosorbent assay

After all the treatment was finished, the rats were sacrificed and the samples of peritoneal fluids and serum were taken. The peritoneal fluid samples of the rats were centrifuged at 12,000 rpm for 10 min at 4 °C. Then, the supernatants were collected, aliquoted, and stored frozen at -80 °C until used for further evaluation. The serum CA-125 levels, and the levels of IL-13, IL-18 and TNF- α of the peritoneal fluids were detected using ELISA as directed by the manufacturer (RUIQI Bio Co. Ltd, Shanghai, China).

Statistical analysis

Data was expressed by means \pm SD, GraphPad Prism version 5 (GraphPad, San Diego, CA, USA) was used. Statistical comparison between two groups was made using Student's *t*-test. For comparing more than two groups, one-way analysis of variance (ANOVA) was used followed by Tukey test with a significance of $p < 0.05$. All errors are shown as standard deviation (SD).

RESULTS

TQD inhibited the formation of endometriosis-like lesions in rats

As shown in Table 1, TQD-treated rats had a reduced number of total endometriotic lesions as

compared with vehicle-treated controls ($p < 0.01$). Thus, TQD inhibit the formation of endometriosis-like lesions in rats.

Table 1: Effect of TQD on the formation of endometriosis-like lesions in rats (n = 10)

Group	No. of lesion/mouse
Normal	11.3±1.1
Control	21.8±1.5
Danazol	16.4±1.3 ^{**}
TQD-400	12.7±1.2 ^{**}
TQD-200	14.5±1.4 ^{**}

^{*} $P < 0.05$, ^{**} $p < 0.01$ compared with control group

Effect of TQD on rat biochemical profile

As shown in Table 2, the serum CA-125 level, and the IL-18 and TNF- α levels of peritoneal fluids in control group rats were higher than those of sham group ($p < 0.01$). The serum CA-125 level, and the IL-18 and TNF- α level of peritoneal fluids of high dose of TQD were significantly lower than that of the control group ($p < 0.01$). The IL-13 level of peritoneal fluids in control group was significantly lower than that of sham group ($p < 0.01$). After treated with high dose of TQD, the IL-13 level of peritoneal fluids was significantly higher than that of the control group ($p < 0.01$).

Table 2: Effect of TQD on biochemical profile of rats

Group	CA-125 (U/mL)	IL-13 (pg/mL)	IL-18 (pg/mL)	TNF- α (pg/mL)
Normal	5.9±0.4	7.6±1.4	112.4±9.8	213.4±15.4
Control	12.3±1.1	2.4±0.6	321.8±21.5	874.2±13.5
Danazol	6.2±1.3 ^{**}	6.5±0.9 ^{**}	124.5±8.7 ^{**}	241.6±14.9 ^{**}
TQD-H	6.4±1.2 ^{**}	6.3±0.8 ^{**}	118.6±7.4 ^{**}	231.5±11.7 ^{**}
TQD-L	7.8±2.4 [*]	5.1±0.7 [*]	154.2±11.4	264.1±12.9 [*]

^{*} $P < 0.05$, ^{**} $p < 0.01$ compared with control group

DISCUSSION

Endometriosis is usually associated with inflammation of the pelvic area and peritoneum. This hallmark has led to searches of inflammatory markers in the circulation which could potentially predict the presence of endometriosis, and the possibility of a clinically silent systemic inflammatory state in women with endometriosis. These results, which covered three classes of molecules associated with systemic inflammation, namely oxylipins, immunomodulatory proteins and CRP, were largely similar to minimal differences at a level which precludes their use as diagnostic biomarkers for endometriosis. This may explain why there has been no unequivocal consensus on the levels of circulating cytokine in endometriosis [12,13].

In this study, CA125, IL-13, IL-18 and TNF- α were chosen to reflect the effects of TQD on the model rats with endometriosis. Serum CA-125 measurement is now a consolidated method for diagnosing endometriosis, and the serum CA-125 values were found significantly elevated in patients with ovarian and mixed endometriosis lesions [14]. The levels of IL-18 in peritoneal fluids were markedly higher in women with peritoneal, minimal-to mild-stage endometriosis than in controls [15]. The level of TNF- α in peritoneal fluid has been demonstrated as a biomarker to discriminate between patients with endometriosis and those without [16]. It was found in the present study that TQD markedly decreased the serum levels of CA-125 and the levels of IL-18 and TNF- α in the peritoneal fluids and significantly increased the levels of IL-13 in the peritoneal fluids.

CONCLUSION

The findings of this study show that TQD exhibits anti-endometriotic effect in rats by inhibiting inflammatory factors, and is thus a promising therapeutic agent for the treatment of endometriosis.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jian-hua Zheng designed all the experiment and revised the paper. Ye Jin performed the experiment and wrote the paper.

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