

EFFECTS OF DANCHI DECOCTION ON P450AROM, SURVIVIN OF EUTOPIC ENDOMETRIUM OF PATIENTS WITH ENDOMETRIOSIS AFTER CONSERVATIVE SURGERY

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## Abstract

**Background:** Endometriosis is a common gynecological disease, which can seriously affect the physical and mental health of patients. However, there is not a satisfactory way to treat endometriosis with a low recurrence rate.

**Materials and Methods:** Including twenty patients with endometriosis given conservative surgery, a prospective clinical study was performed with randomly grouping and positive control. The traditional Chinese medicine (TCM) and western medicine (WM) groups were given Danchi decoction and GnRH-a or gestrinone respectively. Treatment was given in 1–5 days during the first menstruation. The eutopic endometriums were collected before and 3 months after the treatment, respectively. Then immunohistochemistry was performed to obtain the expressions of P450arom and survivin. In the normal control (NC) group no drugs were administered.

**Results:** Before the treatment, the expressions of P450arom and survivin in both TCM and WM groups were much higher than the NC group ( $P < 0.05$ ) and no significant differences between the two groups ( $P > 0.05$ ). The expressions of the two groups were remarkably decreased after the treatment ( $P < 0.05$ ) and they had no difference between the two groups ( $P > 0.05$ ).

**Conclusion:** Danchi decoction can control the recurrence of endometriosis by restraining abnormal secretion of aromatizing enzyme and regulating cell apoptosis in eutopic endometrium. Excessive expressions of aromatizing enzyme and decreased apoptosis are a possible mechanism of endometriosis recurrence.

**Key words:** Endometriosis, Recurrence, Danchi Decoction, Eutopic endometrium

## Introduction

Endometriosis with the symptoms of progressive dysmenorrhea, chronic pelvic pain, deep dyspareunia, infertility, menstrual abnormalities, and aggravated pelvic can seriously affect the physical and mental health of patients (Liu and Lang, 2011; Sun et al., 2011; Zhou and Qu, 2009; Zhou et al., 2012). It is a common gynecological disease and recently its occurrence rate is increasing (Sun et al., 2011; Zhou and Qu, 2009). Endometriosis has a few malignant biological behaviors such as wide cultivation, invasion, destruction, unlimited proliferation, and metastasis, which therefore is considered as a kind of “benign tumor”.

The etiology of endometriosis is not clear due to the complex pathogenesis (Liu and Lang, 2011; Zhao et al. 2013a). A theory of Sampson’s blood reflux inducing endometrial implantation can explain part of endometriosis etiology. This theory was improved by Lang (2003) with an idea of “the eutopic endometrium determinism”, which was based on the fact that the biological characteristics of the eutopic endometrium of patients with endometriosis are different from those of normal women. Aromatase cytochrome P450 (P450arom) as well as the apoptosis inhibitory protein and survivin were found to be abnormally expressed in the eutopic endometrium (Lang, 2003).

Currently, conservative surgery is the most popular way to treat endometriosis and however, its overall recurrence rate is as high as 30.4% (Koga et al., 2006). The recurrence rate can be reduced if some drugs, e.g., GnRH-a or gestrinone, are taken for 3–6 months in company with the surgery. But these drugs have serious side effects such as hot flashes, sleeplessness, vaginal dryness, joint pain, headache, breakthrough bleeding, bone loss, and liver and kidney damage (Lang, 2005). In particular, this approach affects the fertility of women. Therefore, it is necessary to find

a safe and effective drug to treat endometriosis and reduce its recurrence.

One could achieve such a drug from Traditional Chinese medicines (TCM). Many existing investigations (Cao et al., 2008; Lian et al., 2007; Qi et al., 2011; Song et al., 2005; Yang et al., 2006; Yang et al. 2012; Zhao et al. 2013a and 2013b) showed that TCM can alleviate endometriosis symptoms or improve signs with slight side effects. This can be explained according to the theory of TCM and the physiological and pathological features of females. The etiology of endometriosis is blood stasis (Zhou and Qu, 2009) and qi stagnation is the leading cause of the blood stasis. Therefore, one can study endometriosis through the qi stagnation and blood stasis. In this paper, we investigated the effect of Danchi decoction on qi stagnation and blood stasis after conservative surgery and explored a possible mechanism of endometriosis recurrence.

## Materials and Methods

### Object of study

Twenty patients accepted laparoscopic conservative surgeries at Guanganmen Hospital, China Academy of Chinese Medical Sciences and Beijing Friendship Hospital, Capital Medical University from January 2010 to February 2012. They were confirmed to have ovarian endometriosis cysts and blood stasis caused by qi stagnation. The choosing standards are as follows:

1) The ones following the laparoscopic diagnosis standard and the pathological criteria for the endometriosis diagnosis were classified to pathological type of ovary endometriosis. 2) Before surgery they had belonged to qi stagnation and blood stasis type (premenstrual abdominal pain, menstrual pain, hard menstruation, blood clots, and pain-reduction after block discharge, anal bulge, premenstrual breast pain, and chest discomfort; as well as dark purple tongue with petechiae, white moss, and string pulse at the tongue edge). 3) They underwent endometriosis laparotomy or laparoscopy surgery for the first time. 4) Any drug treating endometriosis or its recurrence cannot be taken in half a year before surgery. 5) They had no plan of pregnancy in half a year after surgery. 6) They had no autoimmune diseases; cardiovascular, liver and kidney diseases; diseases of hematopoietic system; other serious primary diseases; or mental diseases. 7) They were 18~48 years old and not lactating or pregnant. 8) All of them signed the informed consent.

The excluding standard include the following:

1) They were found to also have estrogen adenomyosis, myoma of uterus, endometrial lesion, polycystic ovary syndrome, genital cancer, breast cancer, estrogen dependent disease, tumor, or acute inflammation. 2) After the operation, they used any drugs to treat of the current disease.

In addition, 5 more patients were chosen as the normal control group:

1) They had accepted laparoscopic ovarian cyst surgery and been diagnosed as ovarian cyst after surgery. They had also been excluded to have endometriosis or malignant tumors; 2) The eutopic endometriums had been collected before surgery and diagnosed to be normal; 3) Any drug treating endometriosis or its recurrence cannot be taken in half a year before surgery; 4) They were 18~48 years old and not lactating or pregnant.

### Treatment Methods

After the conservative surgery, the patients with qi stagnation and blood stasis type of endometriosis were randomly divided into TCM and WM groups. Before the current study, we had been approved by the ethics committee of our hospital. Ethics number is No.57 of Guanganmen hospital, China Academy of traditional Chinese medicine in 2009. The diagnostic criteria of endometriosis of qi stagnation and blood stasis type can be found in references (Cao, 2004; Ministry, 1993).

**Traditional Chinese Medicine Group:** the patients began to take Danchi decoction in the first 1–5 days during menstruation. They took one oral dose per day in the form of a decoction in water. A dose was divided into two bags (200 ml one bag) which were taken in the morning and evening, respectively, within 1 hour after a meal. This treatment course lasted 3 months without stopping even during menstruation. The Danchi decoction was provided by the manufacturing laboratory, Guanganmen Hospital, China Academy of Traditional Chinese Medicine. It was composed of: 25 g Radix salviae miltiorrhizae, 15 g Radix paeoniae rubra, 10 g Radix Bupleuri, 15 g Nutagrass flatsedge rhizome, 10 g zedoary, and 15 g spina Gleditsiae. Note that the Danchi decoction is a safe drug as had been examined in our previous project: the "Eleventh Five" National Technology Support Program project (No.2006BAI04A17).

**Western Medicine Group.** The patients began to take gonadotropin-releasing hormone agonist (GnRH-a) or gestrinone capsules in the first 1–5 days during menstruation. GnRH-a (Goserelin, Zoladex) of 3.6 mg in a dose was hypodermically administered once a month and for 3 months. Gestrinone of 2.5 mg in a dose was orally taken twice a week and for 3 months. GnRH-a were purchased from Beijing Baoyuan Medicines Company, Beijing, China (national medicine accurate: H35020520). Gestrinone capsules were purchased from Beijing Zizhu Pharmaceutical Co., Ltd., Beijing, China (national medicine accurate: H19980020).

**Normal Control Group.** No drugs were administered in the normal control group.

### Sample Collection and Processing

In both TCM and WM groups, endometrial tissues were collected before surgery and 3 months after drug withdrawal. In the NC group endometrial tissues were collected before surgery. First, 1–2 g endometrial tissue was collected by an endometrial collector from the posterior wall of the uterus. Next, the tissue samples were immediately washed triple with a phosphate-buffered solution, fixed in another solution with 10% neutral formaldehyde for 24 hours, and then preserved at normal temperatures after paraffin embedding.

### Immunohistochemistry

Then, immunohistochemistry was used to detect P450arom and survivin expressions in the 25 specimens. Color reaction in the cytoplasm of tissue slices was observed under an optical microscope, where the positive signals of P450arom and survivin show pale yellow, yellow, brown yellow, or brown. In each section, three visual fields were randomly chosen to be photographed. A professional image analysis system, Image-Pro Plus5.1, was employed to analyze the visual fields semi-quantitatively. Integrated optical densities (IOD) are used as evaluation parameters of P450arom and survivin expression intensities, where IOD = average optical density (AOD) × positive area (A). These measures were implemented by the same researcher with the same instrument, light, white balance, and other parameters for each section.

### Statistical Methods

We analyzed all experimental data obtained by the statistical software SPSS17.0. All measurement data were first expressed as  $\bar{x} \pm s$ . When the data obeyed a normal distribution and the variance homogeneity, multiple samples with a single factor analysis of variance would be applied and a paired t-test would be used for the comparison within a group before and after treatment. If the data did not obey a normal distribution or the variance homogeneity, a rank sum test would be used. We used bilateral inspection with the inspection level of  $\alpha=0.05$ . Therefore, the significance was set at  $P<0.05$ .

### Results

There were 29 cases first chosen in this study, among which 25 cases were finally taken: 10 ones in the TCM and WM groups, respectively, and 5 ones in the NC group. We had compared the three groups in the ages and times of pregnancy and birth and had not found a statistical difference ( $P > 0.05$ ), as seen in Table 1.

**Table 1:** Comparison of general data among the three groups ( $\bar{x} \pm s$ )

Group	Case	Age (year)	pregnancy time	birth time
NC	5	34.81±7.30	0.89±0.92	0.48±0.52
TCM	10	33.35±5.52	0.90±1.03	0.50±0.61
WM	10	34.09±7.41	1.01±1.40	0.49±0.66
P		0.861	0.938	0.820

### Expression of P450arom

The cytoplasm aromatase cytochrome P450 exists only in the endometrial glandular epithelium and therefore has no expression in the cell

nucleus or membrane and has weak coloring in mesenchymal cells. In Fig. 1E it showed no or low expressions in normal endometrium cells and in Figs. 1A–1D it illustrated positive expressions in eutopic endometrium cells. One can see in Table 2 that both TCM and WM groups showed positive signals of IOD in immunohistochemistry staining and the expressions of P450arom in preoperative eutopic endometrium were significantly higher than that in the NC group ( $P=0.000$  and  $P=0.000$  for the TCM and WM groups, respectively). Between the TCM and WM groups there was no significant difference ( $P=0.541$ ). Three months after drug withdrawal in both TCM and WM groups, the P450arom expressions were lower than those before surgery ( $P=0.005$  and  $P=0.012$ , respectively), as displayed in Table 3. The expressions among the TCM, WM, and NC groups had no significant difference ( $P=0.146$ ), as shown in Table 4.

**Table 2:** Integrated optical densities of P450arom in preoperative eutopic endometrium in different groups

Group	Case	P450arom
TCM	10	2119.53±170.31 <sup>Δ</sup>
WM	10	2077.98±143.81 <sup>Δ</sup>
NC	5	1671.22±105.82

Note:  $\Delta$  denotes a comparison with the NC group. The significant difference was taken as  $P<0.05$ .

**Table 3:** Integrated optical densities of P450arom in eutopic endometrium before surgery and after drug withdrawal in different groups

Group	Cases	P450arom	
		Preoperation	After drug withdrawal
TCM	10	2119.53±170.31	1823.44±128.18 <sup>Δ</sup>
WM	10	2077.98±143.81	1819.86±201.49 <sup>Δ</sup>

Note:  $\Delta$  denotes a comparison with preoperation. The significant difference was taken as  $P<0.05$ .

**Table 4:** Integrated optical densities of P450arom in eutopic endometrium after drug withdrawal in different groups

Group	Case	P450arom
TCM	10	1823.44±128.18
WM	10	1819.86±201.49
NC	5	1671.22±105.82

### Expression of Survivin

Survivin has expressions mainly in the endometrial glandular epithelial cytoplasm and the nucleus. Low expressions were appeared in normal endometrium in Fig. 2E and positive expressions in Figs. 2A–2D. One can see in Table 5 that both TCM and WM groups showed positive signals of IOD in immunohistochemistry staining and the expressions of survivin in the preoperative endometrium eutopic endometrium were significantly higher than that in the NC group ( $P=0.02$  and  $P=0.00$  for the TCM and WM groups, respectively). Between the TCM and WM groups there was no significant difference ( $P=0.80$ ). Three months after drug withdrawal in both TCM and WM groups, the survivin expressions were lower than that before surgery ( $P=0.014$  and  $P=0.032$ , respectively), as illustrated in Table 6. The expressions among the TCM, WM, and NC groups showed no significant differences ( $P=0.104$ ), as observed in Table 7.

**Table 5:** Integrated optical densities of survivin in preoperative eutopic endometrium in different groups

Group	Case	Survivin
TCM	10	1731.71±146.14 <sup>Δ</sup>
WM	10	1781.31±182.37 <sup>Δ</sup>
NC	5	1387.08±77.08

Note: Δ denotes a comparison with the NC group. The significant difference was taken as P<0.05.

**Table 6:** Integrated optical densities of survivin in eutopic endometrium before surgery and after drug withdrawal in different groups

Group	Case	Survivin	
		Preoperation	After drug withdrawal
TCM	10	1731.71±146.14	1535.43±88.78 <sup>Δ</sup>
WM	10	1781.31±182.37	1539.74±288.36 <sup>Δ</sup>

Note: Δ denotes a comparison with preoperation. The significant difference was taken as P<0.05.

**Table 7:** Integrated optical densities of survivin in eutopic endometrium after drug withdrawal in different groups

Group	Case	Survivin
TCM	10	1535.43±88.78
WM	10	1539.74±288.36
NC	5	1387.08±77.08

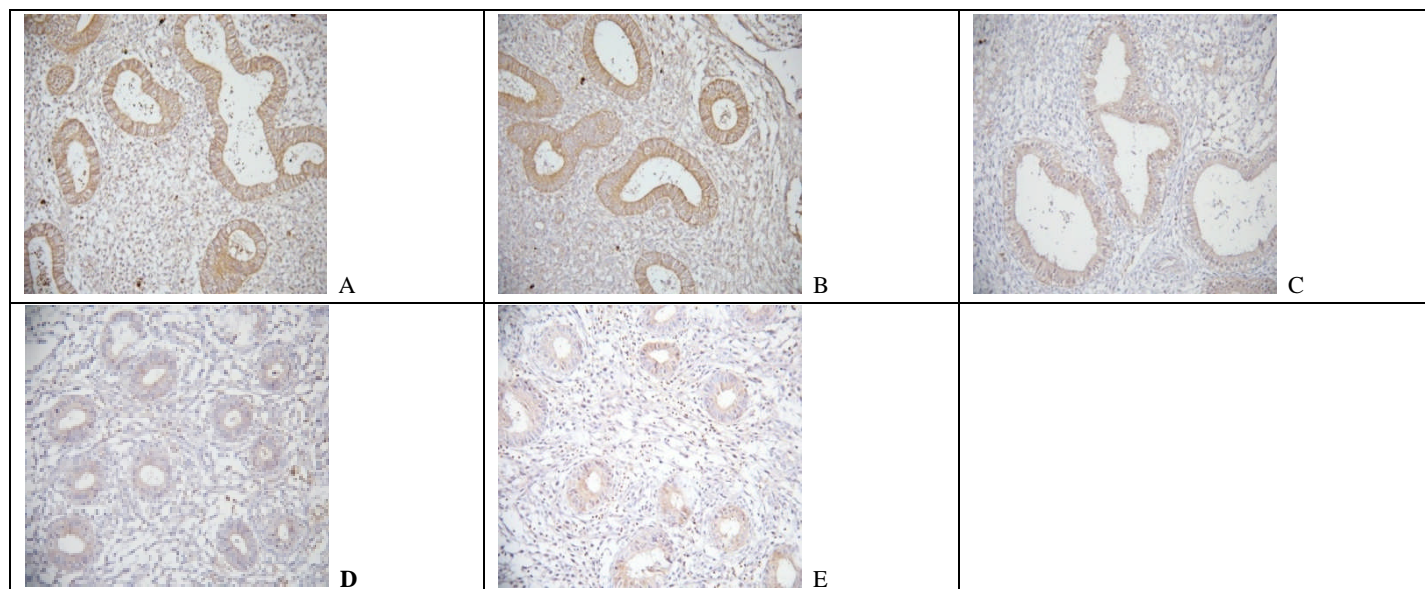


Figure 1: Immunohistochemical staining of P450arom under the microscope (×200). A: TCM group before surgery; B: WM group before surgery; C: TCM group after drug withdrawal; D: WM group after drug withdrawal; E: NC group.

## Discussion

Endometriosis is a disease in which abnormal expression of aromatase causes the growth of local estrogen concentrations (Qiu and Zhang, 2008). Aromatase cytochrome P450 is the rate limiting enzyme in the final step in estrogen synthesis. With it the androstenedione and testosterone are converted into the estrone and estradiol. Smuc et al. (2007) found that the aromatase cytochrome levels are quite low in the normal endometrial ovarian tissues and the levels are significantly enhanced in the ectopic tissues. In the regions with high expressions of P450arom, estrogen receptor contents are also increased.



In our study the P450arom expressions in the TCM and WM groups before surgery were higher than that in the NC group ( $P < 0.05$ ), i.e., the P450arom expression in normal endometrium was low and it was enhanced in ectopic one. This is because local estrogen production is grown in endometriosis. Therefore, endometriosis depends strongly on estrogen. Three months after treatment and drug withdrawal, the expressions of P450arom were significantly decreased in both TCM and WM groups ( $P < 0.05$ ). There also were no differences ( $P > 0.05$ ) in the expressions of P450arom among the TCM, WM, and NC groups, suggesting that the Danchi decoction has a long-term effect on the P450arom by weakening the eutopic endometrium estrogen synthesis of local lesions. It was also indicated that Danchi decoction has roughly the same effect as the western medicines used.

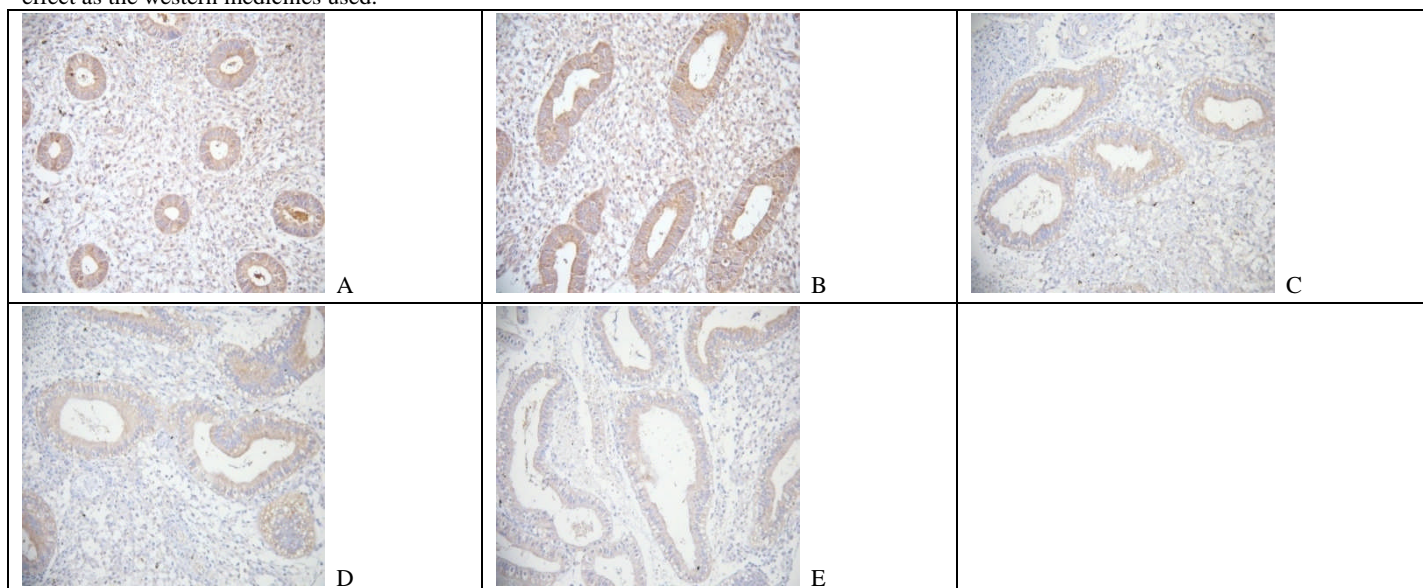


Figure 2: Immunohistochemical staining of survivin under the microscope ( $\times 200$ ). A: TCM group before surgery; B: WM group before surgery; C: TCM group after drug withdrawal; D: WM group after drug withdrawal; E: NC group.

Abnormal apoptosis may be related to occurrence of endometriosis. Apoptosis is a controllable process of cell death in multicellular organisms that can remove excess cells without causing inflammation. Periodic endometrial hyperplasia and shedding proceeds via cell proliferation and apoptosis. Exfoliated endometrial fragments, due to regulation by cell apoptosis, lose the proliferative ability and stop growing, which maintains the normal structure and function of tissues and organs. Therefore, the spontaneous apoptosis of endometrial cells is important to maintaining a normal structure of the endometrium. However, if the apoptosis is inhibited, ectopic endometrium may survive in the pelvic cavity causing the development of endometriosis. Compared with the normal endometrium, cell apoptosis in the eutopic endometrium is significantly reduced. Survivin is an apoptosis inhibitory protein and may be the strongest one. It can inhibit cell apoptosis and therefore promote cell proliferation, regulate cell mitosis, and indirectly promote angiogenesis by inhibiting the apoptosis of vascular endothelial cells. Thanks to these characteristics different from other proteins, it has attracted wide interest. Investigations of Ueda et al. (2002) showed that the expression of survivin in ectopic lesions of the endometrium was higher than that in the normal one. They also found that the expression of survivin in pigmented lesions was higher than the non-pigmented lesions. This suggests that up-regulation of survivin has a positive correlation with endometriosis infiltration and growth.

In our study, the survivin expressions in the TCM and WM groups before surgery were higher than that in the NC group ( $P < 0.05$ ), i.e., the apoptosis rate in ectopic endometrium was lower than that in normal one. Therefore, cells can grow and survive in the pelvic cavity, explaining that endometriosis has the same proliferation properties as tumors. This result was consistent with the reference (Zhang and Shen, 2006). Three months after treatment and drug withdrawal, the expression of survivin were significantly decreased in both TCM and WM groups ( $P < 0.05$ ). There also were no significant differences ( $P > 0.05$ ) in the expressions of survivin among the TCM, WM, and NC groups, suggesting the Danchi decoction can strengthen cell apoptosis.

The previous results on P450arom and survivin indicated that excessive expressions of aromatase and reduction of cell apoptosis in eutopic endometrium may be one of the mechanisms of endometriosis recurrence. Therefore, one could take drugs to inhibit aromatase and promote cell apoptosis to decrease endometriosis recurrence.

## Conclusions

Our investigations showed that high expressions of P450arom and survivin in the eutopic endometriums were closely associated with both occurrence and recurrence of endometriosis. Therefore, the P450arom and survivin could be considered as two important indicators in the treatment of endometriosis. It was also shown that the Danchi decoction can inhibit endometriosis recurrence by regulating the abnormal expressions of P450arom and survivin.

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**Conflicts of Interest:** The authors declare that there is no conflict of interests regarding the publication of this article.

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