The influence of mifepristone to caspase 3 expression in adenomyosis

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Summary

Objective: To discuss the influence of mifepristone to caspase 3 expression in adenomyosis tissue. *Materials and Methods:* Sixty patients were equally divided into four groups. Groups 1,2, and 3 were treated with 5, 10, and 15 mg mifepristone, respectively and group 4 was treated with placebo. The expression of caspase 3 was examined by immunohistochemical method in both eutopic and ectopic endometria of the 40 cases. *Results:* Compared with placebo group, the expression of caspase 3 in both eutopic endometrium and ectopic endometrium in the three treatment groups was significantly increased. There was no difference in the expression of caspase 3 in both eutopic and ectopic endometria between the ten and 25 mg treatment groups, while both the ten and 25 mg treatment groups had a higher expression intensity of caspase 3 in both eutopic and ectopic endometria, compared with the five mg treatment group ($p < 0.01$). *Conclusion:* Mifepristone can increase the expression of caspase 3 in both eutopic and ectopic endometria and initiate cell apoptosis in both eutopic and ectopic endometria. Therefore mifepristone can effectively inhibit the emergence and development of adenomyosis.

Key words: Adenomyosis; Apoptosis; Caspase3; Mifepristone.

Introduction

Adenomyosis (AM) is a disease caused by the endometrum penetrating into the myometrium and growing into ectopic glandular tissue. It is often dispersed in the myometrium and is a common gynecological disease prevalent in women aged 40-50 years [1]. It is a medical condition characterized by volume increase, prolonged menstrual period, and dysmenorrhea which is sexually aggravated [2]. The incidence rate shows an increasing trend, its cause of dysmenorrhea, menorrhagia, and anemia, leading to infertility causes a great deal of pain and mental burden to patients and families. Severe dysmenorrhea especially affects the patient's life and work quality. The incidence of AM has gradually increased over many years and there is a trend of younger women reported at home and abroad.

There is still no ideal treatment for AM. Clinical treatment options range from use of drug therapy, surgery, and uterine artery embolization (UAE). Treatment of AM uterine artery embolization is a novel approach that not only can ease the symptoms of dysmenorrhea, be able to retain the uterus, and have very clear short-term effects, but it still requires further in-depth evaluation regarding the recurrence rate, lesion vascular recanalization, and its influence on ovarian and reproductive function with longer-term studies [3, 4].

Clinically, surgery is still the main treatment, and generally includes hysterectomy, but patients that become infertile can also often lead to endocrine disorders, and even reduce the quality of life after hysterectomy. Therefore, many of the younger patients often cannot accept these side-effects. Drug treatment in AM can relieve the symptoms of dysmenorrhea, but cannot completely cure, the side-effects of the drug, and AM is still very easy to relapse after discontinuation [5]. There is a certain lack of treatment options and this is due to the pathogenesis of AM which remains unclear.

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms. Biochemical events lead to programmed cell degeneration and necrosis of the process [6]. Spontaneous apoptosis of endometrial cells is a key factor for metrial tissue to maintain its normal structure and function, and ectopic endometrial cells to grow and continue to survive outside the uterine cavity are particularity related to the changes in cell apoptosis and proliferation. Recent studies have found that there were significant differences between AM endometrial cells and normal endometrial apoptosis rates. Abnormal apoptosis is an important reason for the ectopic implantation and the growth of endometrial cells. Abnormal apoptosis may be a key cause for the occurrence and development of uterine gland muscle disease [7, 8].

Caspase family is widely present in the cells mainly in the form of the zymogen. As an important process of the cell death, once the cutting of caspase is activated, cell death will inevitably occur [9]. Fourteen or more types of the caspase family have been identified; caspase 3 is the most important member of this family. Most of the factors that trigger apoptosis eventually require signal transduction pathway mediated by caspase 3 leading to apoptosis [10]. It is not yet clear about the expression level and its clinical significance of caspase in endometriosis (EM) and AM endometrial carcinoma. In the study of the treatment of en-

Revised manuscript accepted for publication February 25, 2013

dometriosis using BAY 11-7085 inhibitor of nuclear factor called, Nasu *et al.* [11] found that BAY 11-7085 can activate apoptosis caspase common pathway of caspase 8, 9, and finally activates factor caspase 3, to accelerate the eutopic of apoptosis and ectopic endometrial cells, achieving the purpose of the treatment of endometriosis. This shows that caspase 8, 9 and caspase-3 activity decreased in AM eutopic and ectopic endometrial cells. Kim *et al.* [12] found that the expression level of caspase 3 in endometriosis group was significantly lower than the normal control group and the ectopic endometrial activity than the corresponding lower eutopic endometrium. All these may indicate that in utero film endometriosis endometrium low expression of caspase 3 results in the significantly reduced apoptosis rate in the EMs ectopic endometrial cell apoptosis in the process of EM occurred. The reduced apoptosis rate undermines the balance of cell proliferation and apoptosis. Cell death is less than the proliferation, cell accumulation, and prolonged survival, and leads to the implantation and growth of these cells, which promoted EMs. Therefore, caspase 3 plays an important role in the development of in Ems and may be a new target for the treatment of EMs [13].

Mifepristone, synthetic 19-demethyl-testosterone derivative, has anti-progesterone and anti corticosteroids activity. Previous studies confirm that the mifepristone block progesterone through binding to its receptor [14]. It is the anti-progesterone drugs played on the level of the receptor, can suppress ovarian function, and induce amenorrhea, and make ectopic endometrial atrophy [15]. Reinsch *et al.* [16] reported that after mifepristone (25 mg/d) three months, uterine artery blood flow of patients is reduced by up to 40%, significantly reduced uterine volume, and elevated resistance index. Similar to these results Zhou *et al.* [17] reported through animal tests, that mifepristone is not only able to suppress the occurrence of mice AM, while narrows mice AM lesion, but also relieves symptoms. These results in humans are consistent in the treatment of AM by using mifepristone [18]. Clinical trials confirmed that mifepristone can maintain the patient's blood E2 level in early or mid follicular phase. Furthermore, mifepristone, is more economic, easier to accept, and does not cause bone loss. Therefore, it opens up a new field for the treatment of AM. In recent years, scholars studied the relationship between AM and apotosis. Some studies have shown that mifepristone may promote apoptosis by acting directly on endometrial cells [19]. Mifepristone used in the clinical treatment of AM has a certain effect, [20] but its mechanism of action and therapeutic dose has yet to be further understood.

The study by detecting the expression of cysteine-aspartate-specific protease (caspase 3) in the AM, make the role of caspase 3 clear in the regulation of apoptosis in AM development, and explore influences of the caspase 3 expression on eutopic and ectopic endometrial cells by using different doses of mifepristone in patients with AM and determine the mechanism of mifepristone treatment on AM.

Table 1. — *The ectopic Caspase3 expression level.*

Groups	n	Caspase 3 expression level –	+	++	+++	Positive rate
Control	15	13	1	1	0	13.3%
5 mg Group A	15	4	6	3	2	73.3%
10 mg Group B	15	1	3	5	6	93.3%
25 mg Group C	15	1	2	6	6	93.3%

A, B, and C with the control group compared to the expression intensity of $p < 0.05$; positive rate of $p < 0.01$.
Group B compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C and group B ratio of expression intensity $p > 0.05$; positive rate $p > 0.05$.

Table 2. — *The ectopic endometrial Caspase3 expression level.*

Groups	n	Caspase 3 expression level –	+	++	+++	Positive rate
Control	15	12	2	1	0	20%
5 mg Group A	15	3	6	4	2	80%
10 mg Group B	15	1	4	5	5	93.3%
25 mg Group C	15	1	2	5	7	93.3%

A, B, and C with the control group compared to the expression intensity of $p < 0.05$; positive rate of $p < 0.05$.
Group B compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C compared with group A, the expression of the intensity $p < 0.05$; positive rate of $p < 0.05$.
Group C and group B ratio of expression intensity $p > 0.05$; positive rate $p > 0.05$.

Material and Methods

The study target

Patients who accepted hysterectomy in the present hospital were selected from October 2008 to October 2010 .The same consented to participate in this research project and signed the consent form and were a total of 60 patients. The patients were randomly divided into four groups. Group A took no medicine for preoperative medication treatment, group B took mifepristone at dose of five mg qd for three months before surgery, group C took mifepristone at dose of ten mg qd for three months before surgery, and group D took mifepristone at a dose of 25 mg qd for three months before surgery. Mifepristone was dispensed to patients by the research group and methods and precautions were explained. The four groups of patients underwent hysterectomy after treatment and observation. Desired eutopic endometrium and myometrium ectopic endometrium were fixed by paraformaldehyde and then embedded with paraffin. The specimens were stained and the expression levels of caspase 3 by immunohistochemistry. Patients had an age of 41 to 49 years, with an average age of 44.54, were women who had given birth, menstrual regulations, with no liver and kidney diseases, no blood diseases, and endocrine history. The age of the study group had no significant difference ($p > 0.05$).

All tissues were paraformaldehyde-fixed, paraffin-embedded, in continuous four-μm thick slices. Immunohistochemistry streptomycin avidin-peroxidase method (SP) was adopted and carried out in the experiments according to the kit instructions.

Observation and judgment of the results.

Caspase 3 expressed in the cytoplasm of epithelial cells and the positive staining cells was the brown granules in the cytoplasm, however, there is no colored nucleus (Figures 1 and 2). Observed

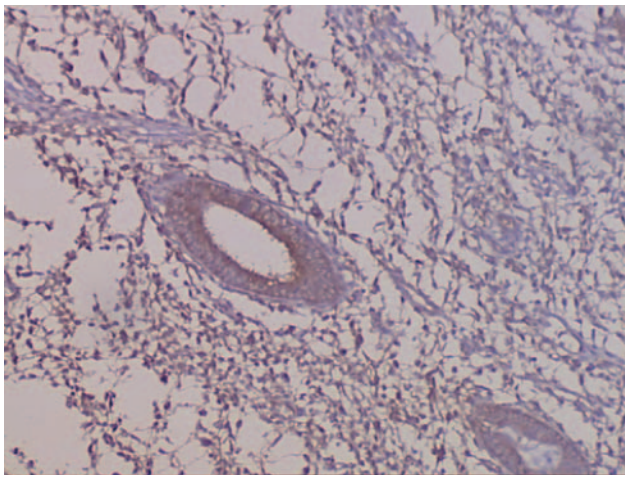

Figure 1. — The ectopic endometrial caspase3 expression, visible brown granules in the cytoplasm of positive cells (×100).

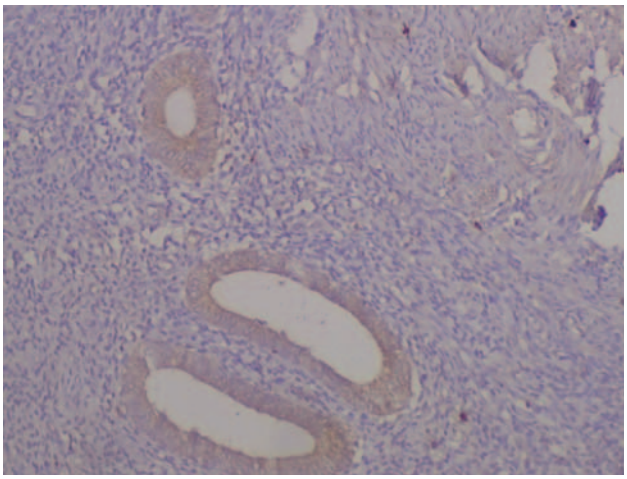

Figure 2. — The ectopic endometrial caspase3 expression, visible brown granules in the cytoplasm of positive cells (×100).

at ten fields of view under a high power microscope and counts were divided into four grades according to the proportion of positive cells: (1) +: positive cells < 25%, (2) ++: positive cells were 25% to 50%; (3) +++: the positive cells > 50% ; (4) cell-brown or light brown is consistent with the background of the negative.

Experimental data were processed using statistical software SPSS 17.0 for Windows. A $p = 0.05$ level of inspection and a $p < 0.05$ of differences were considered significant. Chi-square (X^2) test between the positive rate of statistical methods and expression of strength between the rank-sum test were used for comparison.

Results

The specimens of uterine myometrium eutopic endometria from the four groups of patients were examined immunohistochemically through anti-human caspase 3 antibody. The results showed that there were visible caspase 3 expressions in the two cases samples in the control group, 11 cases in five mg treatment group, and 14 cases in ten mg and 25 mg treatment groups. Compared with unmedicated control group, caspase 3 expression intensity in eutopic endometrium of all the three mifepristone treatment groups were significantly increased. In the two treatment groups of ten mg and 25 mg, the positive expression rate of caspase 3 in ectopic endometrium showed no significant difference. Compared with five mg treatment group, the eutopic positive expression rates of endometrial caspase 3 was significantly different in both. The intensity of expression of each group is shown in Table 1.

The specimens of uterine myometrium ectopic endometria from the four groups of patients were examined immunohistochemically through anti-human caspase 3 antibody. The results showed that there was a visible caspase 3 expression in the three cases samples with the control group, 12 cases in five mg treatment group, 14 cases in ten mg and 25 mg treatment groups. Compared with unmedicated control group, caspase 3 expression intensity in ectopic endometrium of all the three mifepristone treatment groups was significantly increased. In two treatment groups of ten mg and 25 mg, the positive expression rate of caspase 3 in ectopic endometrium showed no significant difference. Compared with five mg treatment group, the eutopic positive expression rates of endometrial caspase 3 was significantly different in both. The intensity of expression of each group is shown in Table 2.

Discussion

This study explores the influence of varying doses of mifepristone in patients with AM eutopic and ectopic endometrial by detecting AM eutopic endometrium and ectopic endometrium cysteine-aspartate-specific proteases (caspase 3) expression level.

Confirmed by this experiment, three mifepristone treatment group compared with the unmedicated control group, eutopic endometrium and ectopic endometrium of caspase 3 expression intensity of both $p < 0.05$, the difference was statistically significant. All these results indicate that long-term use of mifepristone does enhance caspase 3 expression level.

Previous studies have confirmed that the increased expression of caspase 3 can promote apoptosis, and concluded that mifepristone can achieve a therapeutic effect by urging apoptosis of eutopic and ectopic endometrial cells. Ferrero *et al.* showed that mifepristone may directly act on endometrial cells and promote apoptosis [19]. It has been widely confirmed that during mifepristone-treated adenomyosis patients, one mechanism of dysmenorrhea alleviation is that mRNA expression of caspase-3 was

significantly enhanced to promote apoptosis of endometrial cells, which play a role in the treatment, after the drug intervened on glandular epithelial cell proliferative endometrium of patients with adenomyosis.

The results in this study further identified the treatment effect of mifepristone on adenomyosis and support the studies from the scholars at home and abroad, and may provide a theoretical data for future studying adenomyosis treatment from apoptosis.

Over long periods, a large number of oral mifepristone may affect the patient's liver and kidney function. Severe cases may lead to liver, kidney, and adrenal failure. Therefore it is particularly important to find a minimum effective dose. Most frequently-used clinical application dose of mifepristone is 25 mg qd. This study carried out three different doses of mifepristone treatment groups to detect the caspase 3 expression level. There was no significant difference in the positive expression intensity of caspase 3 between 10 mg and 25 mg treatment groups, but compared to the five mg treatment, positive expression rate of caspase 3 in the eutopic and ectopic endometria was significantly increased in both 10 mg and 25 mg treatment groups. This provides a new way of thinking for exploring the optimal dose of mifepristone treatment for AM.

Due to the small samples in this study and the fact that only caspase 3 expression was studied in the factors affecting apoptosis, may have influenced the accuracy of the final results. In the next study, the number of sample cases and expressions of other apoptotic factors detected in adenomyosis endometrium should be increased, and a better method of treatment of adenomyosis and drug dose will be found to relieve the suffering of patients.

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