Effect of mifepristone on invasion gene and apoptosis gene expression in ectopic endometrial tissue of patients with endometriosis

Wei Xie

Department of Obstetrics and Gynecology, People’s Hospital of Dongxihu District Wuhan City Hubei Province, Wuhan City, Hubei Province, 430000

ARTICLE INFO

Article history:
Received 8 Sep 2017
Received in revised form 12 Sep 2017
Accepted 19 Sep 2017
Available online 28 Sep 2017

Keywords:
Essential hypertension
Endometriosis
Mifepristone
Invasion gene
Apoptosis gene

ABSTRACT

Objective: To study the effect of mifepristone on invasion gene and apoptosis gene expression in ectopic endometrial tissue of patients with endometriosis. Methods: Patients with endometriosis who were treated in People’s Hospital of Dongxihu District Wuhan City between March 2015 and June 2017 were selected as the research subjects and randomly divided into two groups, mifepristone group received mifepristone therapy 3 months before surgery, and control group received no special treatment. The endometriosis lesions were collected after surgical resection to determine the expression of invasion and apoptosis genes. Results: β-catenin, GSK3 β, uPA, NK-kB p65, OPN, Ki-67, c-IAP1, Bcl-2, Livin and Id-1 protein expression in endometriosis lesions of mifepristone group were significantly lower than those of control group while PTEN, Smac, Bax and Fas protein expression were significantly higher than those of control group. Conclusion: Preoperative mifepristone therapy can inhibit cell invasion and promote cell apoptosis in endometriosis lesion.

1. Introduction

Endometriosis is a common gynecological benign disease, which specifically means that the endometrial tissue appears in the area outside the uterine cavity lining mucosa and abnormally grows, and can cause symptoms such as chronic pelvic pain and dysmenorrhea, and affect patients’ daily life[1,2]. Although ectopic endometrial tissue belongs to benign lesions, it has the characteristics of malignant tumors such as abnormal proliferation, invasion and seeding, and the abnormal expression of a variety of invasion and apoptosis genes can cause change the biological behavior of endometrial cells and cause the occurrence of endometriosis growth[3-4]. The biological characteristics of ectopic endometrial lesions can affect the surgical removal of the lesion, and the incomplete removal of the lesion can increase the risk of disease recurrence. Progesterin receptor antagonist, gonadotropin-releasing hormone agonist and other drugs are the common means for treatment of endometriosis[5], preoperative drug therapy is able to adjust the biological behaviour of ectopic cells in lesions, but the specific molecular mechanism of drug treatment is still not clear. In the following studies, we analyzed the effect of mifepristone on invasion gene and apoptosis gene expression in ectopic endometrial tissue of patients with endometriosis.

2. Research subjects and methods

2.1 General information of research subjects

A total of 78 patients with endometriosis who were treated in People’s Hospital of Dongxihu District Wuhan City between March 2015 and June 2017 were selected as the research subjects, all patients conformed to the diagnosis of endometriosis and the indications of surgical resection, and the patients with a history of hormone therapy before inclusion were ruled out. The enrolled patients were divided into mifepristone group and control group by random number table, each with 39 cases. Mifepristone group were 22-45 years old, and the course of disease was 9 months to 6 years; control group were 21-48 years old, and the course of disease was 11 months to 7 years. There was no statistically significant difference in general information between the two groups (P>0.05).
2.2 Therapy

Mifepristone group received mifepristone therapy three months before operation, and the method was as follows: mifepristone 10 mg/d, taken orally, for 3 months in a row; control group received no drug therapy before operation. Two groups of patients received endometriosis lesion removal or affected-side adnexectomy performed by the same group of doctors.

2.3 Research methods

2.3.1 Sample collecting

After surgical resection, the endometriosis lesion specimens were collected, washed with saline to remove the residual blood, then quickly frozen in liquid nitrogen tank for 20 min, taken out and placed in -80℃ refrigerator.

2.3.2 Gene expression detecting

Endometriosis lesion specimens were taken, added in RIPA lysate and then homogenized to get the homogenate, and enzyme-linked immunosorbent assay kit was used to detect β-catenin, GSK3β, uPA, NK-kB p65, OPN, PTEN, Smac, Bax, Fas, Ki-67, c-IAP1, Bcl-2, Livin and Id-1 protein expression.

2.4 Statistical methods

SPSS 20.0 software was used to input data, measurement data between two groups was by t test and \( P < 0.05 \) indicated statistical significance in differences in test results.

3. Results

3.1 Invasion gene expression

Analysis of invasion genes β-catenin (ng/mL), GSK3β (pg/mL), uPA (ng/mL), NK-kB p65 (ng/mL) and OPN (pg/mL) protein expression in surgically removed endometriosis lesions between two groups of patients was as follows: β-catenin, GSK3β, uPA, NK-kB p65 and OPN protein expression in endometriosis lesions of mifepristone group were significantly lower than those of control group. Differences in β-catenin, GSK3β, uPA, NK-kB p65 and OPN protein expression in endometriosis lesions were statistically significant between two groups of patients (\( P < 0.05 \)).

3.2 Apoptosis gene expression

Analysis of pro-apoptosis genes PTEN (ng/mL), Smac (pg/mL), Bax (ng/mL) and Fas (ng/mL) protein expression in surgically removed endometriosis lesions between two groups of patients was as follows: PTEN, Smac, Bax and Fas protein expression in endometriosis lesions of mifepristone group were significantly higher than those of control group. Differences in PTEN, Smac, Bax and Fas protein expression in endometriosis lesions were statistically significant between two groups of patients (\( P < 0.05 \)).

Analysis of anti-apoptosis genes Ki-67 (ng/mL), c-IAP1 (ng/mL), Bcl-2 (ng/mL), Livin (pg/mL) and Id-1 (pg/mL) protein expression in surgically removed endometriosis lesions between two groups of patients was as follows: Ki-67, c-IAP1, Bcl-2, Livin and Id-1 protein expression in endometriosis lesions of mifepristone group were significantly lower than those of control group. Differences in Ki-67, c-IAP1, Bcl-2, Livin and Id-1 protein expression in endometriosis lesions were statistically significant between two groups of patients (\( P < 0.05 \)).

Table 1. Invasion gene protein expression in endometriosis lesions

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>β-catenin (ng/mL)</th>
<th>GSK3β (pg/mL)</th>
<th>uPA (ng/mL)</th>
<th>NF-kB p65 (pg/mL)</th>
<th>OPN (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mifepristone group</td>
<td>39</td>
<td>1.95±0.22</td>
<td>93.52±10.26</td>
<td>2.55±0.36</td>
<td>0.82±0.11</td>
<td>142.32±16.96</td>
</tr>
<tr>
<td>Control group</td>
<td>39</td>
<td>4.52±0.58</td>
<td>268.42±35.25</td>
<td>7.94±0.93</td>
<td>3.29±0.52</td>
<td>364.86±42.39</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2. Pro-apoptosis gene protein expression in endometriosis lesions

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>PTEN (ng/mL)</th>
<th>Smac (pg/mL)</th>
<th>Bax (ng/mL)</th>
<th>Fas (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mifepristone group</td>
<td>39</td>
<td>3.22±0.46</td>
<td>175.68±20.35</td>
<td>1.58±0.20</td>
<td>4.51±0.62</td>
</tr>
<tr>
<td>Control group</td>
<td>39</td>
<td>1.29±0.17</td>
<td>74.52±9.41</td>
<td>0.68±0.09</td>
<td>1.89±0.22</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>17.689</td>
<td>12.478</td>
<td>13.028</td>
<td>14.427</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3. Anti-apoptosis gene protein expression in endometriosis lesions

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Ki-67 (ng/mL)</th>
<th>c-IAP1 (ng/mL)</th>
<th>Bcl-2 (ng/mL)</th>
<th>Livin (pg/mL)</th>
<th>Id-1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mifepristone group</td>
<td>39</td>
<td>3.26±0.56</td>
<td>1.03±0.14</td>
<td>2.27±0.35</td>
<td>162.32±18.94</td>
<td>125.29±14.86</td>
</tr>
<tr>
<td>Control group</td>
<td>39</td>
<td>7.94±0.92</td>
<td>3.35±0.47</td>
<td>6.72±0.92</td>
<td>354.28±42.94</td>
<td>228.68±31.37</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
4. Discussion

Surgical resection is an important means of clinical treatment of endometriosis, but the endometriosis lesions are with similar features as those of malignant tumors, and the ectopic endometrial cell proliferation and invasion will affect the effect of surgical resection, so surgical resection can’t completely remove ectopic endometrial lesions. In the development of endometriosis, the abnormal expression of multiple invasion genes and apoptosis genes can result in the invasive growth of endometrial cells. Mifepristone is the common drug for clinical treatment of endometriosis, which can competitively antagonize the estrogen to influence the estrogen stimulation to ectopic endometrium growth, thereby inhibiting the growth of ectopic endometrial lesions[6,7]. Although several studies have confirmed that mifepristone can inhibit the growth of endometriosis lesions, it is not yet clear whether mifepristone will influence the invasion gene and apoptosis gene expression in endometriosis lesions.

Invasive growth is the most prominent biological feature of endometrial lesions, and β-catenin and NK-kB p65 are the key signaling molecules that regulate the invasion of endometrial cells. β-catenin is the signal transduction molecule in downstream of Wnt signaling pathways, β-catenin degradation is restrained when Wnt signaling pathways are activated, it gathers inside the cells, transfers into the nucleus and increases the expression of GSK-3β and uPA, thus promoting the extracellular matrix degradation and the cell invasion[8,9]. NK-kB p65 is the signaling molecule in OPN downstream, and OPN can identify cell surface receptor integrins and CD44 to make NF-kB p65 dissociated from NK-kB—IκBs complexes, which can transfer into the nucleus and then regulate the expression of downstream MMPs and promote cell invasion[10,11].

In the study, analysis of above invasion gene expression in endometriosis lesions showed that β-catenin, GSK3β, uPA, NK-kB p65 and OPN protein expression in endometriosis lesions of mifepristone group were significantly lower than those of control group. This means that mifepristone has inhibitory effect on the expression of various invasion genes within the endometriosis lesion, and inhibiting the invasion gene expression can restrain the invasion of the ectopic endometrial cells and help the complete resection of lesions.

The invasive growth of the cells in ectopic endometrial lesion is related to the enhancement of cell proliferation ability, and the expression deletion of pro-apoptosis genes and the increased expression of anti-apoptosis genes can promote cell proliferation. PTEN, Smac, Bax, and Fas are genes closely associated with apoptosis of endometrial cells. PTEN is a tumor suppressor gene closely related to tumor, which can catalyze dephosphorylation to inhibit the activation of PI3K/Akt pathway, inhibit cell proliferation and induce apoptosis[12]; Smac is a mitochondria-derived cysteine protease activator, which can promote the conversion from pro-caspase-3 to caspase-3 and enhance the activity of caspase-9 to promote apoptosis[13]; Bax and Fas are upstream molecules that regulate apoptosis of mitochondrial pathway and death receptor pathway, which can promote cells to enter into apoptosis process[14]. In the study, analysis of the above pro-apoptosis gene expression in endometriosis lesions indicated that PTEN, Smac, Bax and Fas protein expression in endometriosis lesions of mifepristone group were significantly higher than those of control group. This means that mifepristone has up-regulating effect on the expression of various pro-apoptosis genes within the endometriosis lesion, and increasing the pro-apoptosis gene expression can induce ectopic endometrial cell apoptosis and help the radical resection of lesions.

The apoptosis of endometrial cells is not only associated with the change in pro-apoptosis gene expression, but is also closely related to the changes in the expression of Ki-67, c-IAP1, Bcl-2, Livin, Id-1 and various other anti-apoptosis genes. Ki-67 is a type of proliferating cell nuclear antigen, which plays an important role in DNA replication and can promote cell proliferation and inhibit apoptosis[15]; both cIAP-1 and Livin are the IAPs family members that can antagonize the activation of various caspase molecules and inhibit apoptosis through the BIR domain; Bcl-2 is the regulator of mitochondrial pathway apoptosis, which can inhibit the activation of apoptosis by reducing the release of cytochrome C in mitochondria[16]; Id-1 belongs to the HLH transacting factor family, which suppresses the apoptosis through negatively regulating the activity of various transcription factors. In the study, analysis of the above anti-apoptosis gene expression in endometriosis lesions indicated that Ki-67, c-IAP1, Bcl-2, Livin and Id-1 protein expression in endometriosis lesions of mifepristone group were significantly lower than those of control group. This means that mifepristone has inhibitory effect on the expression of various anti-apoptosis genes within the endometriosis lesion, and reducing the expression of anti-apoptosis genes can inhibit the ectopic endometrial cells proliferation and help the complete resection of lesions.

In the study, the analysis of the invasion and apoptosis gene expression in endometriosis lesions can be concluded as follows: mifepristone therapy before endometriosis surgery can inhibit cell invasion and promote cell apoptosis.
References


