Assessment of the inflammatory factor as well as invasion and apoptosis gene expression in endometriosis model rats after mifepristone intervention
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ABSTRACT

Objective: To study the effect of mifepristone intervention on the inflammatory factor as well as invasion and apoptosis gene expression in endometriosis lesions of endometriosis model rats. Method: SD female rats were selected as experimental animals, divided into model group (EMs group) and mifepristone group (RU486 group) and made into endometriosis models, then the EMs group received saline intervention and RU486 group received 2.6 mg/kg/d RU486 intervention. 4 weeks after intervention, endometriosis lesions were anatomized to determine the expression of inflammatory factors (COX-2, PGE2, TNF-α, IL-1β and IL-6), invasion genes (OPN, MMP2, MMP9, uPA and S100A6) as well as apoptosis genes (Bcl-2, Livin, Smac and PTEN). Results: COX-2, PGE2, TNF-α, IL-1β, IL-6, OPN, MMP2, MMP9, uPA, S100A6, Bcl-2 and Livin protein expression in endometriosis lesions of Ru486 group were significantly lower than those of EMs group while Smac and PTEN protein expression were higher than those of EMs group. Conclusion: Mifepristone for endometriosis model rats can inhibit the expression of inflammatory factors, invasion genes and anti-apoptosis genes, and increase the expression of pro-apoptosis genes.

1. Introduction

Endometriosis (EMs) is a common disease in women at childbearing age, the pathological characteristic of endometriosis is that the endometrial tissue appears outside the uterine lining mucosa and abnormally grows, and it can cause chronic pelvic pain, menstrual disorders, infertility and other clinical symptoms[1,2]. Mifepristone is a competitive progesterone antagonist, its affinity to progesterone receptor is more than 10 times of that to progesterone, and it can antagonize the progesterone effects, inhibit the secretion of estrogen and block the endometrial reactivity to progesterone when it is used for the treatment of endometriosis[3,4]. At present, mifepristone is widely used in the treatment of endometriosis, but the specific molecular mechanism for mifepristone to treat endometriosis is still not clear. Inflammation is the outstanding pathological change of the ectopic endometrial lesions, which not only has chemotaxis effect on ectopic endometrial cells, but can also adjust the expression of apoptosis and invasion-related molecules in lesions, and eventually participate in invasive growth of ectopic endometrial lesions. In the following study, the endometriosis rat models were established, and the effect of mifepristone on the inflammatory factor as well as invasion and apoptosis gene expression in endometriosis lesions was specifically analyzed.

2. Materials and methods

2.1 Experimental materials

The experimental animals were a total of 24 SD female rats with body weight of 200-250 g, and animal license was SYXK (Shanghai) 2012-0002. Mifepristone (RU486) and estradiol benzoate were bought from Sigma Company, Elisa kits for gene expression detection were purchased from Shanghai Univ-bio Company, PBS buffer was bought from Shanghai Beyotime Company, and chloral hydrate was purchased from Sinopharm Chemical Reagent Co., LTD.
2.2 Experimental methods

2.2.1 Ems model establishment methods and drug intervention
Female SD rats were randomly divided into model group (EMS group) and mifepristone group (RU486 group), and the EMS models were established according to the following method: rats were given 0.2 mg/kg/d estradiol benzoate 1 d before model establishing and received intraperitoneal chloral hydrate anesthesia the next day, a longitudinal incision of 3 cm was made 1.0 cm above the urethral orifice to enter into the abdominal cavity, isolate uterus, do proximal ligation 1.0 from the cornua uteri and do distal ligation 2.0 cm from the ovaries, then the uterus was removed and put in sterile PBS buffer, the endometrial tissue was separated and then cut into tissue blocks of 0.5 cm × 0.5 cm, the endometrium was inoculated in the tissue clearance between abdominal muscle and subcutaneous fascia, and then the abdomen was closed. The abdomen was opened again after 3 weeks to confirm the successful establishment of EMSs, then the models received intervention, RU486 group were given intraperitoneal injection of mifepristone 2.6 mg/kg/d, EMSs group were given intraperitoneal injection of same dose of saline, and the medication lasted for 4 weeks in a row.

2.2.2 Detection methods of gene expression in ectopic lesions
After mifepristone intervention for 4 weeks, the EMSs group and RU486 group of rats were put to death and anatomized, the obtained endometriosis lesion tissue was cleaned with the normal saline, then added in proper amount of PBS buffer, fully grinded and then centrifuged for 20 min at 4 ℃ and 12 000 r/min, the supernatant was separated, and Elisa kits were used to determine COX-2, PGE2, TNF-α, IL-1β, IL-6, OPN, MMP2, MMP9, uPA, S100A6, Bcl-2, Livin, Smac and PTEN contents.

2.3 Statistical methods
SPSS 16.0 software was used to input the gene expression data in ectopic lesions, gene expression analysis between EMSs group and RU486 group was by t test, and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Inflammatory factor contents in ectopic lesions
Analysis of inflammatory factors COX-2 (μg/L), PGE2 (μg/L), TNF-α (ng/L), IL-1β (ng/L) and IL-6 (ng/L) expression in endometriosis lesions between EMSs group and RU486 group was as follows: COX-2, PGE2, TNF-α, IL-1β and IL-6 protein expression in endometriosis lesions of Ru486 group were significantly lower than those of EMSs group. Differences in COX-2, PGE2, TNF-α, IL-1β and IL-6 protein expression in endometriosis lesions were statistically significant between two groups of rats (P<0.05).

3.2 Invasion gene expression in ectopic lesions
Analysis of invasion genes OPN, MMP2, MMP9, uPA and S100A6 expression in endometriosis lesions between EMSs group and RU486 group was as follows: OPN, MMP2, MMP9, uPA and S100A6 protein expression in endometriosis lesions of Ru486 group were significantly lower than those of EMSs group. Differences in OPN, MMP2, MMP9, uPA and S100A6 protein expression in endometriosis lesions were statistically significant between two groups of rats (P<0.05).

3.3 Apoptosis gene expression in ectopic lesions
Analysis of apoptosis genes Bcl-2, Livin, Smac and PTEN expression in endometriosis lesions between EMSs group and RU486 group was as follows: Bcl-2 and Livin protein expression in endometriosis lesions of Ru486 group were significantly lower than those of EMSs group while Smac and PTEN protein expression were higher than those of EMSs group. Differences in Bcl-2, Livin, Smac and PTEN protein expression in endometriosis lesions were statistically significant between two groups of rats (P<0.05).

Table 1.
Comparison of inflammatory factor contents in endometriosis lesions between two groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>COX-2</th>
<th>PGE2</th>
<th>TNF-α</th>
<th>IL-1β</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>RU486 group</td>
<td>12</td>
<td>0.87±0.11</td>
<td>1.42±0.18</td>
<td>11.28±1.63</td>
<td>25.39±4.52</td>
<td>8.92±1.05</td>
</tr>
<tr>
<td>EMS group</td>
<td>12</td>
<td>1.85±0.24</td>
<td>4.28±0.63</td>
<td>29.52±5.58</td>
<td>73.92±9.32</td>
<td>23.27±3.68</td>
</tr>
<tr>
<td>T</td>
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<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.
Comparison of invasion gene expression in endometriosis lesions between two groups of rats (ng/L).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>OPN</th>
<th>MMP2</th>
<th>MMP9</th>
<th>uPA</th>
<th>S100A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>RU486 group</td>
<td>12</td>
<td>5.22±0.89</td>
<td>2.57±0.36</td>
<td>1.89±0.24</td>
<td>1.03±0.16</td>
<td>1.56±0.25</td>
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<td>EMS group</td>
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<td>13.28±2.25</td>
<td>8.49±1.14</td>
<td>6.52±0.94</td>
<td>2.86±0.42</td>
<td>4.57±0.79</td>
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</tr>
</tbody>
</table>

Table 3.
Comparison of apoptosis gene expression in endometriosis lesions between two groups of rats (ng/L).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Bcl-2</th>
<th>Livin</th>
<th>Smac</th>
<th>PTEN</th>
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</thead>
<tbody>
<tr>
<td>RU486 group</td>
<td>12</td>
<td>5.82±0.89</td>
<td>2.47±0.42</td>
<td>3.21±0.52</td>
<td>7.69±0.92</td>
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<tr>
<td>EMS group</td>
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<td>9.32±1.24</td>
<td>7.54±0.93</td>
<td>1.02±0.16</td>
<td>4.21±0.84</td>
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<tr>
<td>T</td>
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<td>8.398</td>
<td>18.585</td>
<td>22.175</td>
<td>8.175</td>
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<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
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</tr>
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</table>
4. Discussion

Mifepristone is a commonly used drug for clinical treatment of endometriosis, it mainly exerts the pharmacological effect through combining with progesterone receptors[5], but the biological effect is not clear after the drug is combined with progesterone receptors. Chronic inflammation is one of the pathological features of endometriosis, and the contents of a variety of inflammatory cytokines significantly increase in serum and peritoneal fluid of patients with endometriosis. Endometriosis lesion is an important source of inflammatory cytokines, and inflammatory factors can induce the endometrial cell adhesion, seeding and growth as well as ectopic lesion formation. COX-2 is an important inducible enzyme of inflammation reaction process, the COX-2 in endometriosis lesions can catalyze arachidonic acid metabolism and generates PGE2[6,7], and the accumulation of PGE2 can cause the expression of TNF-α, IL-6 and other inflammatory factors and mediate the activation of inflammatory response[8,9]. In order to define the effect of mifepristone on the inflammatory response in the development and change of endometriosis, the above inflammatory factor expression in endometriosis lesions were analyzed in the study, and the result showed that COX-2, PGE2, TNF-α, IL-1β and IL-6 protein expression in endometriosis lesions of Ru486 group were significantly lower than those of EMs group. This means that mifepristone can inhibit the inflammatory reaction within the endometriosis lesions, and can reduce the expression and secretion of a variety of inflammatory factors.

Inflammatory factors can promote the adhesion, migration and invasive growth of cells when acting on the ectopic endometrial lesions, and the expression of OPN, MMP2, MMP9, uPA, S100A6 and other invasion genes within the lesions are regulated and affected by the inflammatory response. OPN is an acid glycoprotein, and it can promote cell adhesion and invasion after combination with cell surface receptors αvβ3 and CD44 through RGD domain[10]; MMP2 and MMP9 have direct hydrolysis effect on collagen type IV, collagen type V, gelatin and other compositions in the extracellular matrix, and promote the cells to cross through the extracellular matrix and invasively grow[11-13]; uPA is a kind of serine protease that can directly hydrolyze cell basement membrane and promote cell invasion, and can also activate the plasminoenzyme and promote cell invasion; S100A6 is the calcyclin of S100 family, and it can realize the biological effect of promoting cell invasion through the Wnt/β-catenin pathway[14]. In order to define the effect of mifepristone on cell invasion in the development and change of endometriosis, the above invasion gene expression in endometriosis lesions were analyzed in the study, and the result showed that OPN, MMP2, MMP9, uPA and S100A6 protein expression in endometriosis lesions of Ru486 group were significantly lower than those of EMs group. This means that mifepristone can inhibit the cell invasion within the endometriosis lesions, and it can reduce the expression of a variety of pro-invasion genes.

In the development of endometriosis, the invasive growth of cells within the ectopic lesions is not only regulated by invasion genes, but also regulated by the Bcl-2, Livin, Smac, PTEN and other apoptosis genes. Bcl-2 is the anti-apoptosis molecule of mitochondrial apoptosis that can inhibit cytochrome C release from the mitochondria into the cytoplasm, and thus antagonize the cell apoptosis mediated by cytochrome C[15,16]; Livin is a new member of anti-apoptosis protein family, the BIR domain in protein structure has decisive effect on the anti-apoptotic effect of Livin, and through the structural domain, Livin can inhibit the pro-apoptotic effect of a variety of Caspase molecules and block the pro-apoptotic effect of c-Jun pathway[17]; Smac is a kind of mitochondrial pro-apoptosis protein that can directly act on caspase-9 and caspase-3, and enhance their activity so as to promote cell apoptosis[18]; PTEN is a tumor suppressor gene, and the protein encoded by it can inhibit the activity of PI3K/Akt and the cell proliferation effect mediated by the pathway[19]. In order to define the effect of mifepristone on cell apoptosis and proliferation in the development and change of endometriosis, the above apoptosis gene expression in endometriosis lesions were analyzed in the study, and the results showed that Bcl-2 and Livin protein expression in endometriosis lesions of Ru486 group were significantly lower than those of EMs group while Smac and PTEN protein expression were higher than those of EMs group. It means that mifepristone has promoting effect on the cell apoptosis within endometriosis lesions, and it can reduce the expression of a variety of anti-apoptosis genes and increase the expression of a variety of pro-apoptosis genes.

To sum up, it is believed that mifepristone has regulating effect on the expression of inflammatory factors, invasion genes and apoptosis genes in ectopic lesions of rats with endometriosis, and the specific effects are inhibiting the expression of inflammatory factors, invasion genes and anti-apoptosis genes while increasing the expression of pro-apoptosis genes. In future study, cellular experiment in vitro can be further adopted to explain the specific signaling pathways for mifepristone to exert the above effect.

References


