The effect of endometritis on level of inflammatory protein and oxidation factor via NF–κB signal pathway

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ABSTRACT

Objective: This study explored effect of endometritis induced by LPS on level of inflammatory protein and oxidation factor via NF–κB signal pathway. Methods: Endometrial cell was treated with LPS (50, 100, 200 ng/mL). The viability of cell was detected by MTT assay. The concentration of IL-6 and PGE_2 was tested by elisa method. The concentration of MDA was tested by thiobarbituric acid method. The concentration of SOD was tested by xantine oxidation method. The concentration of NO was tested by Gries method. The expression of NF–κB p65 was assayed by western blot. Results: MTT assay demonstrated LPS (50, 100, 00 ng/mL) could suppress cell viability, and the inhibitory effect was highest in 48 h. With the increasing dose of LPS, the activity of SOD decreased, the level of MDA, NO, IL-1 and PGE_2 elevated. Finally, LPS stimulated NF–κB p65 phosphorylation. Conclusion: These results suggested endometrial cell treated by LPS make inflammatory factor secreted and anti-oxidant ability decreased, which might be related to NF–κB signal pathway.

1. Introduction

Endometritis refers to inflammation on the uterine surface, but not yet reaching the spongy layer; its symptoms are fever, hypogastralgia, many leucorrhea, odor, etc, which is deemed as a common gynecological disease with occurrence rate of about 10%–32%, and is a major reason for abortion[1,2]. Endometritis results from bacteria inflammation, such as staphylococcus aureus, escherichia coli, streptococcus, etc. Uterine inflammation means the invasion of pathogenic microorganism, which gathers on uterine epithelial cells and relieves disease-induced bacteria and toxin, such as LPS[3].

LPS is the composite of cell walls of gram negative bacteria, to which LPS will lead, hence resulting in a series of inflammatory response to body mechanisms. LPS can stimulate many monocytes, macrophages, endothelial cells, smooth muscle cells, epithelial cells or fibroblasts in the body, and such parenchymal cells cause inflammatory response[4–8]. Once LPS is activated, it will interact with membrane receptors; through LPS-membrane receptor compounds, downstream signal pathway will be further activated; through activating NF–κB signal pathway, relieving such inflammatory factors as NO, IL-1, PGE_2, producing free radical oxide film receptor, eventually leading to diseases, such as systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome, multiple organ failure(MOF), etc[9–12]. A great many research findings prove[4,13,14], that LPS perfusion in vivo can induce rat endometritis model, stimulate in vitro endometrial cells, uterine epithelial cells induce endometritis model cells. The growth and development of endometritis, inflammation, and free radical balance are interrelated[1,2]. Therefore, through discussing LPS stimulation in endometrial cells NF–κB signal pathway related inflammatory protein and contents of oxidant factors change, transference of pathological signal can be attempted to interrupt, hence alleviating organism pathological response and achieving the aim of signal interrupting and treating endometritis.

2. Materials and methods

2.1. Materials

2.1.1. Drugs and reagents

LPS(Sigma Inc., U.S.A); mouse anti NF-κ B p65, pp65, β
actin (Bi Yun Tian Biotechnological Research Institute); I type collagenase, MTT (Gibco Inc.,); fetal bovine serum, DMEM/F-12 medium (Hyclone Inc.,); MDA content examination kit, SOD content examination kit, NO content examination kit (Nanjing Jiancheng Bioengineering Research Institute). IL-6, PGE2 elisa kit (Corning Inc., U.S.A.).

### 2.1.2. Experimental animals
SD big mice, (100±20) g, were purchased from Shanghai Slike Experimental Animals Co., Ltd., certificate number: SCXK(Hu 2012-0005). Indoor temperature was controlled between (23±2) °C, and the big mice freely ate and drank.

### 2.2. Methods

#### 2.2.1. The preparation of endometrial cells from big mice
In the bacteria-free environment, endometrium of 3D big mice was obtained and cut up. 0.25% trypsin was used for digestion for 30 min, eradicating connective tissue; 0.2% collagenase and 0.1% hyaluronidase were in digestion for 3 h. After obtaining single cell suspension, DMEM/F-12 was used for the culture of based heavy suspension cells in incubator under the temperature of 37 °C and in 5% CO₂.

#### 2.2.2. Mice endometrial cells activity testing (MTT method)
Have the second to third generation endometrial cells digested, adjust cell density, inoculate, and culture in the incubator under the temperature of 37 °C and in 5% CO₂ for 24 h. Add DMEM/F-12 medium containing 5% fetal bovine serum (including 50, 100, 200 ng/mL LPS) for continuing to culture 24 h, 48 h, 72 h; in every hole add MTT(5 mg/mL) 20 μL, then the supernatants were removed, and in every hole add DMSO 150 μL; 10 min later, measure OD value using the enzyme mark instrument at 570 nm.

#### 2.2.3. The measurement of endometrial cell supernatants NO, IL-1, MDA, SOD content
Have the second and third generation endometrial cell digested, adjust cell density, inoculate, and culture them in the incubator under the temperature of 37 °C and in 5% CO₂ for 24 h, making the cells fully adherent. Add DMEM/F-12 medium containing 5% fetal bovine serum (including 50, 100, 200 ng/mL LPS) for continuing to culture 48 h, 72 h; in every hole add supernatant 100 μL for measurement, according to kit description respectively to examine each density.

#### 2.2.4. Samples treated by Western blot
Samples treated by Western blot were cracked in the adding, pre-cooling RIPA lysate so as to obtain the protein sample. Use BCA kit to measure the protein density. Take the protein sample as the upper sample, undertake SDS gel electrophoresis, and then have wet label, use 5% skim milk powder to be closed under room temperature for 1h, add first antibody under 4 °C for overnight incubation. In the following day cleanse using TBST for three times, add second antibody, incubate under room temperature for 1h, later cleanse three times and drop plus exposure liquid for exposure.

#### 2.2.5. Statistical analysis
Data are indicated in mean±SD, using analysis of variance, analyzing the statistical significance of the groups’ differences. P value less than 0.05 means two groups’ difference has statistical significance.

### 3. Results

#### 3.1. LPS’s impact on proliferation ability of endometrial cells.
As shown in table 1, with the increased density of inducer, LPS’s inhibition on endometrial cell keeps on strengthening, whose inhibition rate is the highest at 20 ng/mL; with increased administration time at 24 h, 48 h, and 72 h, LPS’s inhibition on endometrial cell gradually strengthens, where the effect density at 48 h and 72 h is identical, therefore the authors choose 48 h as the measurement time in the ensuing experiments.

#### 3.2. Changes of anti-oxidation ability of LPS induced endometrial cell
As shown in table 2, compared with the normal group, LPS induction led to the decrease of SOD activity in cells and became dosage-dependent, therefore having statistical significance (*P<0.05). Compared with the normal group, LPS increased MDA content in

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>LPS on the inhibitive effect of the growth of endometrial cells (mean±SD).</td>
</tr>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Control group</td>
</tr>
<tr>
<td>LPS</td>
</tr>
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<td></td>
</tr>
</tbody>
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Note: Compared with normal group, *p<0.05, **p<0.01.
the cells, in which the LPS effect of 100 ng/mL and 200 ng/mL is comparatively identical.

Table 2
Changes of anti-oxidation ability of LPS induced endometrial cell (Mean±SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>dose/(ng/mL)</th>
<th>SOD/(U/L)</th>
<th>MDA/(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>372.31±39.58</td>
<td>1.73±0.28</td>
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<tr>
<td>Control</td>
<td>50</td>
<td>280.37±25.36</td>
<td>5.78±1.02</td>
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<tr>
<td></td>
<td>100</td>
<td>254.65±38.49</td>
<td>6.12±1.23</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>210.29±32.76</td>
<td>6.14±1.09</td>
</tr>
</tbody>
</table>

Note: Compared with normal group, *P<0.05, **P<0.01.

3.3. LPS’s impact on the inflammatory factor of endometrial cell

As shown in table 3, compared with the normal group, LPS promoted the secretion of inflammatory cell IL-6 and PGE2, improved NO content, with dosage-dependent, hence having statistical significance (P<0.05).

Table 3
LPS’s impact on the inflammatory factor of endometrial cell (Mean±SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>dose/(ng/mL)</th>
<th>NO/(μmol/L)</th>
<th>IL-6/(ng/mL)</th>
<th>PGE2/(ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0.61±0.43</td>
<td>120.43±23.28</td>
<td>0.54±0.05</td>
</tr>
<tr>
<td>LPS</td>
<td>50</td>
<td>10.08±1.05</td>
<td>223.65±33.27</td>
<td>2.31±0.29</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>11.69±2.23</td>
<td>223.73±44.36</td>
<td>3.08±0.54</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>12.13±2.59</td>
<td>235.38±45.67</td>
<td>3.12±0.53</td>
</tr>
</tbody>
</table>

Note: Compared with the normal group, **P<0.01.

3.4. LPS’s impact on NF-κB p65 expression and phosphorylation in endometrial cell

As shown in table 1, compared with the normal group, with the increase of effect density, LPS can significantly promote the secretion of inflammatory cell IL-6 and PGE2, improved NO content, with dosage-dependent, hence having statistical significance (P<0.05), but not having any impact on itself.

Figure 1. LPS’s impact on NF-κB p65 expression and phosphorylation in endometrial cell. *P<0.05, **P<0.01.

4. Discussion

When bacteria inflammation led to the damage on uterine tissue and endometrial structure, for example, inflammation caused in uterine dense layer could lead to the decrease of proliferation ability, resulting in infertility and aphoria. As the major component of the cell wall of gram-negative bacterium, LPS caused “cascade effect” through stimulating mononuclear/macrophages to secrete multiple cytokines and inflammatory mediators, stimulating partial mechanism or the whole body to cause obvious inflammatory response and immunomodulatory effect. In parallel with literature and reports[4,5], a certain dosage of LPS can induce endometrial inflammation, promote the secretion of IL-6, NO and PGE2, inflammatory factors, increase MDA content, decrease SOD content, and further clarify the success of model creation.

When the mechanism had inflammation, the inflammatory response would have many genetic expressions. NF-κB is a nuclear factor generally existed in mammalian cells with multiple transcriptional regulations, and capable of participating in the mechanism’s inflammatory response, immune response, cell differentiation and death and its effect. When cells are in resting state, NF-κB is combined with IκB protein and stuck in cytoplasm; when receiving stimulus from the outside, IκB is phosphorylated and degraded, and dissociated from NF-κB, hence promoting NF-κB to transfer into the nuclear and regulating relevant gene transcription and translation. A great deal of experiments prove that many factors lead to the activation of NF-κB signal pathway, including virus, virus gene products, bacteria toxin, cytokines, etc[15]. The most important downstream pathway in the LPS induced signal pathway is NF-κB signal pathway[16,17]. The finding in this experiment is also indicated that LPS stimulates endometrial cells so as to promote the phosphorylation of NF-κB p65, without impacting its own expression.

Inflammation is the complicated process of pathological response caused by the external stimulus to the mechanism, whereas pathological examination will indicate that there is a great deal of inflammatory cell immersion to the infected site. IL-6 is a promotion inflammation cytokine developed in the early period of inflammation and a key to regulate cytokine network, resulting in inflammatory response and a stronger anti-viral activity. A higher level of IL-6 is related to endometritis[18,19]. This experiment results found that LPS stimulates endometrial cells to develop a great deal of IL-6. LPS can stimulate rats’ endometrial epithelial cells to cause the NF-κB activation and to raise the IL-6 content[6]. Therefore it indicates that a higher level of IL-6 is related to NF-κB activation. Prostaglandin E2(PGE2) is one of the metabolites of arachidonic acid, closely related
to inflammation, and can participate in the growth and development of various diseases, therefore being the synthesized rate-limiting enzyme of COX-2. LPS stimulates NF-κB signal pathway, makes COX-2 accelerate transcriptional translation, and raises the enzyme expression amount. NF-κB deactivation can inhibit the growth of PGE2. Existing reports proved that LPS could induce macrophages and monocytes to produce a great deal of PGE2. Existing reports have proved that LPS stimulates endometrial cells to produce a great deal of PGE2. This experiment results also found that LPS stimulated endometrial cells to produce a great deal of PGE2, which was related to NF-κB activation. NO is closely related to inflammation and can react with superoxide anion that cause inflammation in the uterine to produce peracetic nitrate with strong oxidizability; then further cause lipid peroxidation, inhibit mitochondrial membrane for breath, form multiple toxic materials, and cause injury to tissues. Through administration, it can inhibit the mitochondrial membrane for breath, form multiple toxic materials, and cause injury to tissues. Through administration, it can inhibit the LPS induced macrophages to produce a great deal of NO. The synthetization of NO is closely related to NF-κB activation. This experiment result fully clarified that LPS stimulates endometrial cells to secrete NO, IL-6, and PGE2, and is related to NF-κB signal pathway.

Meantime inflammation is a protective response after the body is invaded by the external bacteria; when the pathogen invades the uterine, the neutrophil nuclear macrophage are summoned up to enter the uterine and swallow the invading microorganisms; then there will occur the phenomenon of “breath explosion”, or the dramatic increase of oxygen consumption. Then active oxygen is increased accordingly, which results in free radical response and participates in eradicating pathogen. But in the meantime this will lead to the lysosome’s massive relief to harm the normal cells, to damage cell structure, and to make the membrane lipid peroxidation, hence aggravating the inflammation. Existing reports proved that LPS stimulates endometrial cells to secrete NO, IL-6, and PGE2, and is related to NF-κB signal pathway.

In conclusion, when LPS induced endometritis occurs, SOD activity significantly decreases, MDA content increases, which indicates that antioxidation ability in the body decreases; NO, IL-6, and PGE2 content abnormally increase, and it shows that the inflammatory response in the body aggravates, and the phosphorylation of NF-κB p65 is strengthened. This fully clarifies that lipid peroxidation and inflammatory response participate in the growth and development of endometritis via NF-κB signal pathway.

References


