Expression and significance of IL-1β and COX-2 in gingiva tissues in rat periodontitis model with different estrogen levels

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ABSTRACT

Objective: To explore the expressions of IL-1β and COX-2 in the gingiva tissues in rat periodontitis model with different estrogen levels, and the effect of estrogen level on periodontitis. Methods: A total of 40 female Wistar rats were randomized into 4 groups, i.e. normal control group (n=10), periodontitis group (n=10), castration periodontitis group (n=10), and estrogen therapy group (n=10). RT-PCR was used to detect the expressions of IL-1β and COX-2 in the gingiva tissues in each group. Results: The expression intensity of IL-1β and COX-2 in the estrogen therapy group was significantly lower than that in the castration periodontitis group (P<0.05). Conclusions: Estrogen can significantly down regulate the expressions of IL-1β and COX-2 in order to alleviate the symptoms of periodontitis.

1. Introduction

Periodontitis is a common chronic infectious periodontal disease in the clinic, with various pathogenic factors, in which local stimulation is involved. In recent years, it has been found that the general condition is also closely associated with the occurrence and development of periodontitis. Some researches demonstrate that estrogen deficiency will aggravate the inflammatory reaction of periodontitis and bone loss. Estrogen replacement therapy can regulate the endocrine disorder caused by estrogen deficiency through exogenous supplementation and replacement of estrogen or antagonizing in vivo estrogen deficiency. The study is aimed to explore the effect of estrogen level on the occurrence and development of periodontitis, and the effect of estrogen replacement therapy in improving periodontitis.

2. Materials and methods

2.1. Experimental animals

A total of 40 female Wistar rats, 3 months old, body weight of (220-260 g), were purchased from North China University of Science and Technology Animal Center. The experiment was performed in Wuhan University Animal Experiment Center. The experiment animal disposition was approved by the Ethical Committee of our college, and strictly abide by the Experiment Animal Administration Regulation.

2.2. Reagents

Trizol (Sigma), DEPC (Sigma), chloroform (Sinopharm Chemical Reagent Beijing Co., Ltd), FastQuant RT Super Mix (TIANGEN BIOTECH), and SuperReal PreMix Plus (SYBR Green) (TIANGEN BIOTECH) were purchased.

2.3. Experimental grouping and methods

Wistar rats were randomized into 4 groups with 10 in each group. Normal control group: normal feeding with no processing.
Periodontitis group: the cotton silk thread was used to embrace the tooth cervix of second molar of upper jaw in rats for ligation, and was placed under the gingiva. Castration periodontitis group: the tooth cervix of second molar of upper jaw in rats was ligated, and ovariectomy was performed. Estrogen therapy group: the tooth cervix of second molar of upper jaw in rats was ligated, ovariectomy was performed, and estradiol benzoate (produced by Tianjin Jinyao Pharmaceutical Co., Ltd, Approval No. H12020529, 1 mL: 1 mg) was subcutaneously injected 2 d after operation, 20 µg/kg, 1 time/3 d. The rats were sacrificed 5 d after estrogen treatment. The gingiva tissues of second molar of upper jaw in rats were obtained, immediately frozen, and preserved in the refrigerator at -80 °C for RT-PCR detection.

2.4. RT–PCR procedure steps

2.4.1. RNA extraction

After melting, the gingiva tissues (100 mg) were extracted, and ground into homogenate. Trizol (1 mL) was added for blending, and then chloroform (200 µL) was added. The mixture was centrifuged at 12,000 r/min, 4 °C for 15 min. After the supernatant was taken, isovolumetric water phenol was added. Then the mixture was centrifuged at 12,000 r/min, 4 °C for 10 min. After the supernatant was taken, isovolumetric chloroform was added. Then the mixture was centrifuged at 12,000 r/min, 4 °C for 10 min. After the supernatant was taken, isovolumetric isopropyl alcohol was added. The mixture was precipitated at -20 °C for more than 1 h, and centrifuged at 12,000 r/min, 4 °C for 20 min. After the supernatant was taken, 75% ethanol with appropriate volume was added. The mixture was blended, and centrifuged at 7,500 r/min, 4 °C for 5 min. After the supernatant was taken, RNA sediment was dried by air or vacuum, and DEPC with appropriate volume was added. The sediment was dissolved. A small amount of RNA was taken to detect OD value. OD260/280 value between 1.6-2.0 was regarded as normal. The rest RNA was preserved in the refrigerator at -80 °C for reservation.

2.4.2. Synthesis of cDNA by reverse transcription

RNA concentration was adjusted to 200 ng/µL. The template RNA/primer mixed liquor was formulated in Microtube. The template RNA (10 µL) was taken, 4-FQ-RT Super Mix 5 µL and RNase-Free ddH2O 10 µL were added, with a total amount of 20 µL, and preserved at 42 °C for 15 min, and at 95 °C for 3 min.

2.4.3. RT–PCT detection

IL-1 β primer: upstream primer: 5'-TCC ATG AGC TTT GTA CAA GG-3'; downstream primer: 5'-GTT GCT GAT GTA CCA GTT GG-3'.

COX-2 primer: upstream primer: 5'-TGA TGA CTG CCC AAC GCC CAT G-3'; downstream primer: 5'-AAT GTT GAA GGT GTC GGG CAG C-3'.

GAPDH primer: upstream primer: 5'-ACC ACA GTC CAT GCC ATC AC-3'; downstream primer: 5'-TCC ACC ACC CTG TTG CTG TA-3'.

RT-PCR reaction procedure: degeneration at 95 °C for 2 min, 95 °C for 20 s, 58 °C for 30 s, circulation for 40 times, and finally at 72 °C for 5 min.

2.5. Statistical analysis

SPSS 17.0 software was used for the statistical analysis. The measurement data were expressed as mean±SD. ANOVA was used for the comparison among groups. \( p<0.05 \) was regarded as statistically significant.

3. Results

When compared with the control group, IL-1 β expression quantity in the periodontitis group, castration periodontitis group, and estrogen therapy group was significantly up regulated \( (p<0.05) \). When compared with the castration periodontitis group, IL-1 β expression quantity in the periodontitis group and estrogen therapy group was significantly reduced \( (p<0.05) \). The difference of IL-1 β expression quantity between periodontitis group and estrogen therapy group was not statistically significant \( (p>0.05) \). When compared with the normal control group, COX-2 expression quantity in the periodontitis group, castration periodontitis group, and estrogen therapy group was significantly up regulated \( (p<0.05) \). When compared with the castration periodontitis group, COX-2 expression quantity in the periodontitis group and estrogen therapy group was down regulated, but the difference was not statistically significant \( (p>0.05) \). The difference of COX-2 expression quantity between periodontitis group and estrogen therapy group was not statistically significant \( (p>0.05) \) (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-1 ( \beta )</th>
<th>COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>10</td>
<td>0.167±0.024</td>
<td>0.023±0.009</td>
</tr>
<tr>
<td>Periodontitis group</td>
<td>10</td>
<td>1.03±0.088*</td>
<td>0.286±0.059*</td>
</tr>
<tr>
<td>Ovariectomy periodontitis group</td>
<td>10</td>
<td>1.40±0.058*</td>
<td>0.633±0.088*</td>
</tr>
<tr>
<td>Estrogen therapy group</td>
<td>10</td>
<td>1.10±0.058*</td>
<td>0.336±0.055*</td>
</tr>
</tbody>
</table>

\* \( p<0.05 \), when compared with the normal control group; \# \( p<0.05 \), when compared with the castration periodontitis group.
4. Discussion

IL-1 β is an important prophase inflammatory cytokine. It is found by previous studies[2-5] that IL-1 β can mediate matrix degradation, bone absorption, and connective tissue adhesion loss through stimulating human fibroblasts to secrete mesenchyme lysozyme, collagenase, and gelatin degrading enzyme, and can also promote human fibroblasts to secrete plasminogen activator and PGE to strengthen bone absorption. The results in the study showed that IL-1 β expression in the castration periodontitis group was significantly up regulated (P<0.05), and was significantly higher than that in the periodontitis group and estrogen therapy group (P<0.05), indicating that the reduction of estrogen level will aggravate the inflammation of periodontitis, while the estrogen replacement therapy can maintain the estrogen level, and alleviate the inflammatory reaction. It is found from the experiment results that IL-1 β expression in the estrogen therapy group was significantly up regulated when compared with the castration periodontitis group (P<0.05), but was not significantly different from that in the periodontitis group (P>0.05).

COX-2 is a cyclooxygenase with high stimulability, can catalyze arachidonic acid to convert to PG, and is a HMG-CoA for the synthesis of PG. The higher COX-2 expression is, the higher PG content is, the higher inflammation degree is, and the more severe tissue damage is[6,7]. The results in the study showed that COX-2 expression in the castration periodontitis group was significantly up regulated when compared with the periodontitis group and estrogen therapy group (P<0.05), indicating that COX-2 can further up regulate the expression of PG to aggravate the periodontitis and bone absorption, while the estrogen replacement therapy can maintain the estrogen level, and alleviate the inflammatory reaction and bone absorption. It is found from the experiment results that COX-2 expression in the estrogen therapy group was significantly up regulated when compared with the castration periodontitis group (P<0.05), but was not significantly different from that in the periodontitis group (P>0.05).

In conclusion, the reduction of estrogen level will aggravate the periodontitis and bone loss, while estrogen substitution therapy can supplement the estrogen to alleviate the inflammation and bone loss.

References