Effect of orthodontic force on inflammatory periodontal tissue remodeling and expression of IL-6 and IL-8 in rats

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1. Introduction

In recent years, with the development of oral medicine and the improvement of people’s living standards, people’s requirements for beautiful teeth and oral health gradually increase. Orthodontic patients and clinicians are more and more concerned about the health of the periodontal tissue during orthodontic treatment\textsuperscript{1}. The basic principle of orthodontic treatment is to transmitting certain intensity and long enough mechanical force from teeth to the alveolar bone and periodontal membrane, to produce a series of biological signal transduction, which cause the alterations of the periodontal tissue surrounding the tooth root. So that the teeth can move to a new location and a satisfactory result of orthodontic treatment was obtained. Most scholars believe that early orthodontic tooth movement is an acute inflammatory reaction characterized by the dilation of

Objective: To investigate effect of orthodontic force on inflammatory periodontal tissue remodeling and expression of IL–6 and IL–8 in rats. Methods: Eighty SD rats were randomly divided into 4 groups, blank control group (group A) with 5 rats, treatment normal group (group B) with 25 rats, inflammation control group (group C) with 25 rats, inflammation treatment group (group D) with 25 rats. Immunohistochemistry and histomorphometric analysis was performed to measure the expression of IL–6, IL–8 and the first molar to the recent movement in the distance. Results: The expression of IL–8 reached a maximum on day 5 and declined thereafter in group B; the expression of IL–6 reached a maximum on day 5 in group B. The expression of IL–6 and IL–8 was gradually weakened with time in group C. The expression of IL–6 and IL–8 were high, and reached a maximum on day 5 and declined thereafter in group D. AD of positive cells in group D were higher than group B at each time point (P<0.05). The time which 0.49 N orthodontic force was loaded was longer, orthodontic tooth movement distance was greater. Movement distance in group D were longer than group B (P<0.05). Conclusions: Orthodontic force as well as inflammatory stimulus can evoke the expression of IL–6 and IL–8. Under the combined effects of inflammation and orthodontic force, the expression of IL–6, IL–8 will increase.
periodontal blood vessels and migration of white blood cells in vascular, then gradually alleviated and replaced by the chronic inflammatory reaction\cite{2,3}. Interleukin–6 (IL–6) and interleukin–8 (IL–8) are important cytokines which activate in inflammation and the chemoattractant of neutral polymorphonuclear leukocyte. The changes of amount and concentration of them in the periodontal tissues can reflect the state of periodontal inflammation. In this study, we observed the changes of expression of IL–6 and IL–8 of the periodontal tissue in animal periodontitis model, analyzed the effect of orthodontic force and inflammatory stimuli on the periodontal tissue remodeling, so as to improve the basic research and clinical treatment of orthodontic.

2. Materials and methods

2.1. Animal models and grouping

A total of 80 8–week–old male SD rats (Spraque–Dawley rat) were selected, weighting (200±20) g. They were fed with common solid forage, free eating and drinking. The experiment began 1 week after adaptive feeding. All SD rats were divided into 4 groups according to the random number table, blank control group (group A) with 5 rats, treatment control group (group B) with 25 rats, inflammation control group (group C) with 25 rats, inflammation treatment group (group D) with 25 rats. Group B, C and D were randomly divided into 5 groups 1d, 3d, 5d, 7d, 14d afterburning, with 5 rats in each group.

2.2. Agents and instrument

Rabbit anti–mouse IL–8 polyclonal antibody (Beijing Bioss Biotechnology Co., Ltd.), Concentrated DAB color kit, the SP immunohistochemical detection kit (Beijing ZSGB Biotechnology Co., Ltd.), 0.5 mol/L EDTA (Chengdu Kelong Chemical Reagent Factory), trypsin (American Sigma Company), 0.010 inches NiTi orthodontic tension spring (Beijing Nonferrous Metal Company), 0.010 inches wire ligatures (Hangzhou New Asia Dental Co., Ltd.), No. 1 silk (Johnson & Johnson Medical Equipment Co., Ltd.), Optical microscope (Olympus, Japan), Motic BA400 automated microscope (Shanghai Precision Instrument Factory), Motic image analysis system software version 3.2 (MOTIC CHINA GROUP CO., LTD).

2.3. Methods

2.3.1. Establishment of animal model

Rats were anesthetized by intraperitoneal injection 3.3 mL/kg 10% chloral hydrate, fixed on board with belly up. The tongue was pulled out to prevent suffocation. A depth of about 0.5 mm shallow trench was milled at the neck of the tooth close to the gingival margin of the distal surface of the tongue and at the two central incisor of maxillary at the same level of the lip. The molar neck was bypassed with the ligation wire, one end of the NiTi tension spring was ligated and fixed at the neck of the first molar crown, and the other end was ligated and fixed at the neck of the maxillary incisor crown. The maxillary first molar was pulled forward. The orthodontic springs dynamometer was used, and the tension was controlled in the range of 0.49N.

The same method was used to anesthetize the rats. They were fixed on board with belly up, with tongue pulled out. Bilateral maxillary first molar gums was stripped with a probe, the neck of the tooth was ligated with No.1 thread. Normal drinking was changed to 10% sucrose, and rats were feed with soft diet. Thread was checked once a day, and religated promptly if there were abscession. Results were confirmed after 2 weeks.

Silk was removed in the periodontitis model, afterburner in accordance with the method which was used to establish orthodontic tooth movement model, using high–sugar diet as the normal diet.

2.3.2. Preparation of maxillary specimens

Rats in different groups were sacrificed on 1d, 3d, 5d, 7d, 14d afterburner. Three molars were taken out on the the experimental side and the maxillary bone surrounding periodontal tissue, and were placed in 4% paraformaldehyde PBS for 24 h. Then the specimen was rinsed with distilled water for 1 min, placed into 0.5 mol/L EDTA decalcification solution for 6 weeks. They were dehydrated by gradient alcohol solution, paraffin–embedded, and then cut into 5 μm thick slices. They were placed on glass slides for HE staining and immunohistochemical staining.

2.4. HE staining and observation under light microscopic

Intact slices were selected including maxillary first molar crown, root and periodontal tissue by HE staining. They were observed under Motic BA400 automatically microscope at high magnification.

Five horizons were selected with a higher concentration of IL–6, IL–8 expression at low magnification. Motic Image Advanced Analysis Software version 3.2 was adopted. IL–6, IL–8 expression was calculated in periodontal tissues positive cell surface density values (area density, AD) was calculated at high magnification, and the mean value was recorded.

2.5. Measurement of the tooth movement distance

An accurate impression of the maxillary was prepared before and after the afterburner for each rat, to make the
standard plaster model. Vernier calipers were used to measure the distance between the right maxillary first molar maxillofacial mesial lingual groove point and the right maxillary second molar maxillofacial distal tongue groove point, each model was measured three times and then averaged. Tooth movement distance was the difference before and after the afterburner.

2.6. Statistical analysis

All of the data were analyzed by SPSS16.0 statistics software, and the measurement material were expressed as mean ± SD values, t-test and χ² analysis was applied, P<0.05 was considered as statistical significance.

3. Results

IL-8 expression of periodontal ligament fibroblasts was weakly positive after 1 d of afterburner; the IL-8 expression of osteoclasts and periodontal ligament fibroblasts was positive after 3 days of afterburner, while the IL-8 expression of vascular endothelial cells was weakly positive; the IL-8 expression of osteoclasts and periodontal ligament fibroblasts reached the peak and was strongly positive; the IL-8 expression of periodontal tissue was weakened after 7 d of afterburner; the IL-8 expression of periodontal tissue was back to normal after 14 d of afterburner. For group C, the IL-8 expression of the vascular endothelial cells and periodontal ligament fibroblasts were positive after 1 d of the removal of inflammatory stimulation. IL-8 expression of periodontal tissue was gradually weakened with time in group C, and it was back to normal after 7 days of the removal of the inflammatory stimulation. For group D, the IL-6 expression of vascular endothelial cells was positive after 1d of the removal of inflammatory stimulation. IL-6 expression of periodontal tissues reached the peak after 5 days of the afterburner, and the IL-6 expression of the vascular endothelial cells was weakly positive after 7 days of afterburner. IL-6 expression of periodontal tissue was back to normal after 14 days of afterburner. For group C, the IL-6 expressions of the vascular endothelial cells and periodontal ligament fibroblasts were positive after 1d of the removal of inflammatory stimulation. For group D, the IL-6 expression of vascular endothelial cells was positive after 1d of the removal of inflammatory stimulation. IL-6 expression of gingival epithelial cells was weakly positive, and the positive IL-6 expression of vascular endothelial cells, gingival epithelial cells and fibroblasts was enhanced after 3 days of afterburner. IL-6 expression in periodontal tissues reached the peak after 5 days of the afterburner, and the IL-6 expression was weakened after 7 days of afterburner. It continued to decline after 14 days of afterburner, but the strength was still higher than the normal control group (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 d</th>
<th>3 d</th>
<th>5 d</th>
<th>7 d</th>
<th>14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>8.03±0.88</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group B</td>
<td>12.03±0.96</td>
<td>17.93±1.12</td>
<td>23.21±0.87</td>
<td>12.05±0.97</td>
<td>10.31±1.06</td>
</tr>
<tr>
<td>Group C</td>
<td>15.49±0.85</td>
<td>15.03±0.95</td>
<td>12.43±0.93</td>
<td>10.62±0.89</td>
<td>8.26±0.99</td>
</tr>
<tr>
<td>Group D</td>
<td>17.86±1.03</td>
<td>23.14±0.98</td>
<td>33.06±1.16</td>
<td>19.12±0.93</td>
<td>12.55±0.94</td>
</tr>
</tbody>
</table>

Difference of the IL-8 expression positive AD values on 1 d and 5 d of group B and group C had statistically significant difference (P<0.05). The difference of the IL-8 expression positive AD values on 1 d, 3 d, 5 d, 7 d, 14 d of group B and group D had statistically significant difference (P<0.05). The difference of the IL-8 expression positive AD values on 3 d, 5 d, 7 d, 14 d of group C and group D had statistically significant difference (P<0.05).

For group B, the IL-6 expressions of periodontal vascular endothelial cells and periodontal ligament fibroblasts were weakly positive after 1 d of afterburner. IL-6 expressions of vascular endothelial cells was strong positive after 3 days of afterburner, the IL-6 expressions of gums, periodontal ligament fibroblasts and gingival epithelial cells were positive and reach the peak after 3 days of afterburner. IL-6 expression was weakened after 5 days of afterburner, and the IL-6 expression of the vascular endothelial cells was weakly positive after 7 days of afterburner. IL-6 expression of periodontal tissue was back to normal after 14 days of afterburner. For group C, the IL-6 expressions of the vascular endothelial cells and periodontal ligament fibroblasts were positive after 1d of the removal of inflammatory stimulation. IL-6 expression of periodontal tissue was gradually weakened with time in group C, and it was back to normal after 7 days of the removal of the inflammatory stimulation. For group D, the IL-6 expression of vascular endothelial cells was positive after 1d of the removal of inflammatory stimulation. IL-6 expression of gingival epithelial cells was weakly positive, and the positive IL-6 expression of vascular endothelial cells, gingival epithelial cells and fibroblasts was enhanced after 3 days of afterburner. IL-6 expression in periodontal tissues reached the peak after 5 days of the afterburner, and the IL-6 expression was weakened after 7 days of afterburner. It continued to decline after 14 days of afterburner, but the strength was still higher than the normal control group (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 d</th>
<th>3 d</th>
<th>5 d</th>
<th>7 d</th>
<th>14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>8.32±0.85</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group B</td>
<td>13.41±0.95</td>
<td>22.16±0.92</td>
<td>19.58±0.96</td>
<td>13.55±0.93</td>
<td>8.62±0.95</td>
</tr>
<tr>
<td>Group C</td>
<td>16.15±1.03</td>
<td>15.32±0.94</td>
<td>13.64±0.92</td>
<td>8.83±0.88</td>
<td>8.15±0.91</td>
</tr>
<tr>
<td>Group D</td>
<td>18.26±0.88</td>
<td>23.26±0.91</td>
<td>31.62±0.92</td>
<td>19.53±0.88</td>
<td>13.12±1.06</td>
</tr>
</tbody>
</table>

The difference of the IL-6 expression positive AD values on 1 d, 3 d, 5 d, 7 d of group B and group C had statistically significant difference (P<0.05). The difference of the IL-6 expression positive AD values on 1 d, 3 d, 5 d, 7 d, 14 d of group B and group D had statistically significant difference (P<0.05). The difference of the IL-6 expression positive AD values on 3 d, 5 d, 7 d, 14 d of group C and group D had statistically significant difference (P<0.05).

The time which 0.49 N orthodontic force was loaded was
longer, orthodontic tooth movement distance was greater. Movement distance in group D on 5 d, 7 d, 14 d were longer than group B (P<0.05) (Table 3).

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 d</th>
<th>3 d</th>
<th>5 d</th>
<th>7 d</th>
<th>14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>0.28±0.06</td>
<td>0.36±0.05</td>
<td>0.42±0.06</td>
<td>0.52±0.05</td>
<td>0.82±0.06</td>
</tr>
<tr>
<td>Group D</td>
<td>0.33±0.06</td>
<td>0.41±0.07</td>
<td>0.55±0.05</td>
<td>0.66±0.07</td>
<td>1.27±0.08</td>
</tr>
<tr>
<td>t</td>
<td>0.616</td>
<td>0.583</td>
<td>2.365</td>
<td>2.428</td>
<td>2.816</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

4. Discussion

Orthodontic tooth movement is a complex biological process which is a common role of many different cellular, molecular and tissue. Noda et al[4] considered periodontal tissue remodeling is the biological basis of orthodontic tooth movement under the action of mechanical force. The periodontal tissue was pulled by orthodontic force, the blood flow and vascular of the periodontal ligament are changed. It leads to the synthesis and release of growth factors, cytokines, colony stimulating factors, neurotransmitters and metabolites of arachidonic acid and other key molecules, and stimulates the responses of periodontal and many cellular of the dental pulp, to form a suitable microenvironment for the tissue to absorb or deposit. So that the periodontal tissue was remodelled and then it lead to orthodontic tooth movement. Most scholars believe that orthodontic tooth movement is essentially an inflammatory process, while the periodontal tissue remodeling process is actually a process of dynamic physiological balance[5,6]. The tissue osteoclasts and osteoblasts of periodontal were activated under orthodontic force. They produce the interleukin family, cytokines, neurotransmitters, prostaglandin and a series of biological signals, causing the reconstruction of alveolar bone and periodontal ligament, and causing the hyperplasia of the alveolar bone on the tension side and the absorption of alveolar bone on the pressure side, to achieve a new dynamic physiological balance of the periodontal system.

Animal experiments is an important tool to study the effect of inflammatory periodontal tissue remodeling by the effect of orthodontic force. The composition and structure of rat molar periodontal tissue is very similar to human. Rats were with cheap price and easy to control, very suited to be used as periodontitis animal model. Periodontitis is a chronic progressive disease, and the malocclusion of dentition is an important factor to causing periodontitis, so orthodontic patients is often accompanied by inflammation of the periodontal tissue[7,8]. It is important to observe whether the process of orthodontic tooth movement will aggravate the inflammatory response of periodontal tissue inflammation and induced further destruction of periodontal tissue. Holliday et al[9] reported that IL-6 and IL-8 as the important cytokines can be involved in the bone metabolism of orthodontic tooth movement process, which can inhibit the metabolism and repair function of the periodontal ligament and then promote the absorption of alveolar bone. They can also promote and activate the production of vascular endothelial growth factor and the release of inflammatory mediators, and aggravated the local inflammatory response.

IL-6 and IL-8 play a very important role in orthodontic tooth movement and the occurrence and development of periodontal disease. O’Brien et al[10] extracted the human gingival crevicular fluid (GCF) of orthodontic tooth movement, measured IL-8 expression levels of the GCF and found the IL-8 concentrations of GCF were significantly increased after 24 h of the force, and the IL-8 concentrations of GCF has gradually decreased on 7d. In group B of this study, the IL-8 expression of the periodontal ligament fibroblasts was weakly positive 1 d afterburner, the IL-8 expression in periodontal tissues reach the peak after 5 d of afterburner. IL-8 expression in periodontal tissue weakened after 7 d afterburner, which is similar to the changes of IL-8 expression reported in literature. It showed the IL-8 expression during orthodontic tooth movement in human can be well simulated and responded by the IL-8 expression of periodontal tissue in rat experimental model. Yan et al[11] applied orthodontic force for the 36 Wistar rats to creat an obvious inflammatory reaction of the periodontal tissue, the content of IL-8 –mRNA began to increase after 1 d afterburner,and reached the peak after 3 days then began to decreased.

Cattaneo et al[12] considered that IL-1β plays an important role in the regulation of the release process of the inflammatory cytokines such as IL-8. With the increation of the IL-1β , IL-8 is also increased simultaneously as the IL-1β “secondary products” . In this study, the IL-8 expression of rat periodontal tissue is consistent with the literature. Compared the IL-8 expression positive AD values at different time points in each group, we found that the IL-8 expression positive AD values in group C gradually decreased with time, while in group B and group D, the IL-8 expression positive AD values gradually increased after of the afterburner, and reached the peak after 5 days. IL-8 expression positive AD values at different time of group D is much higher than that in group B.

In Pan’s[13] study, the expression of IL-8 of the periodontal tissue for 25 normal rats reached the peak on the 5th days after of the afterburner and began to decline after that, it increased at first and then decreased. The IL-8 expression positive AD values at different time of 25 inflammation afterburner rats are much higher than that in the afterburner rats, which reached the peak on the 5th day, and consistent
with the result of this study. Qian et al[14] reported that the IL–6 expression of periodontal tissue in normal rat was related to the orthodontic time, reached the peak after 3 days of the afterburner, while under the combined action of the orthodontic force and the inflammation. IL–6 expression of rat periodontal tissue was much stronger, which reached the peak on the 5th day of the afterburner. For group B, the IL–6 expressions reach the peak on day 3, the IL–6 expression of periodontal tissue was back to normal after 7 days of the removal of the inflammatory stimulation. For group D, the IL–6 expression in periodontal tissues reached the peak after 5 days of the afterburner, and it continues to decline after 14 days of afterburner, but the strength is still higher than the normal control group. We observed the orthodontic tooth movement distance in each group, and found that The time which 0.49 N orthodontic force was loaded was longer, orthodontic tooth movement distance was greater. Movement distance in group D on 5 d, 7 d, 14 d were longer than group B. Liu[15] divided 55 Wistar rats into normal afterburner group and periodontitis afterburner group, and found that the average orthodontic tooth movement distance of the periodontitis afterburner group is greater than the normal afterburner group, and the speed of the periodontitis afterburner group is much faster than the normal afterburner group, the results are consistent with this study.

By observing the changes of IL–6, IL–8 expression level of rats periodontal tissue and the IL–6, IL–8 expression positive AD values, we can learn that orthodontic force is the mainly cause of periodontal tissue remodeling, which has small effect on periodontal tissue destruction. The local periodontal tissue inflammation will aggravate if afterburner immediately after the removal of local inflammatory stimuli, but orthodontic force can not cause serious damage to periodontal tissues. Orthodontic force combined food impaction and some contributing factor for periodontal disease such as periodontal trauma will aggravate and accelerate the absorption of alveolar bone and the destruction of periodontal tissue.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Li CH, Xu S, Liu YL. Study on the changes of oxygen free radicals in gingival crevicular fluid under the effect of orthodontic force.


