Effects of minocycline combined with periocline on the levels of inflammatory factors, ALP and NO in gingival crevicular fluid of patients with periodontitis

Xiao-Li Yuan¹, Zai-Chun You², Li Wen¹, Kai-Lan Peng¹, Xin Zheng¹

¹. Traditional Chinese Medicine Hospital of Shijingshan District, Beijing 100043, China
². Emergency Department, Second Affiliated Hospital of Military Medical University of the Army, Chongqing 400037, China

ABSTRACT

Objective: To investigate the effect of minocycline combined with periocline on patients with periodontitis, and analyze the effect of inflammatory factors, ALP and NO levels in the gingival sulcus fluid. Methods: A total of 100 cases of patients with periodontitis admitted in our hospital from December 2016 to May 2017, with a total of 128 teeth. They were divided into control group and observation group by the random number method, 64 teeth in each group. Patients in both groups were given conventional supragingival scaling and subgingival curettage therapy. The control group patients were given minocycline ointment in periodontal pockets until overflow, once a week; the observation group was given periocline treatment on the basis of control group. Oral administration 2 capsules each time, 2 times each day. The two groups were both treated for 4 weeks. The general indicators of teeth in both groups were compared. The levels of inflammatory factors, ALP and NO in gingival crevicular fluid of the two groups were detected before and after the treatment. Results: Before treatment, there was no significant difference in the levels of PLI, PD, SBI, AL and TNF-α, CRP, ALP and NO in the gingival crevicular fluid of the both groups. After treatment, the levels of PLI, PD, SBI, AL and TNF-α, CRP, ALP and NO in the gingival crevicular fluid of the two groups were significantly decreased than those before the treatment, and the levels of PLI, PD, SBI, AL and TNF-α, CRP, ALP and NO in gingival crevicular fluid after treatment in the observation group were all significantly lower than that in the control group after treatment, the differences were statistically significant. Conclusion: Minocycline combined with periocline can improve the periodontal condition and gingival crevicular fluid inflammatory state in patients with periodontitis, reduce the activity of ALP and NO. It has the effect of antiseptic and induced osteogenesis and improving the clinical effect.

1. Introduction

Periodontitis is a type of periodontal supporting tissue chronic inflammation in which inflammation spreads from the gums deep into the periodontal ligament, the alveolar bone, and the cementum. Characteristics as following: periodontal pocket formation, periodontal pocket inflammation, alveolar bone resorption, loosening of teeth, and even shedding, severely affect the quality of patients' life[1-3]. The treatment of periodontitis is mainly topical treatment. It can be treated with supracondylar scaling, subgingival scraping, or direct administration to the lesion. Minocycline is a broad-spectrum antibiotic with a strong antibacterial effect[4-6]. Buprofen is a non-steroidal anti-inflammatory drug that can effectively improve the clinical symptoms of periodontitis and has a significant therapeutic effect on periodontitis[7]. This study analyzed the effects of minocycline combined with ibuprofen on periodontal status, inflammatory factors, ALP and NO levels in gingival crevicular fluid in patients with periodontitis. The report is as following.
2. Materials and methods

2.1 Clinical data

A total of 100 patients with periodontitis admitted into our hospital from December 2016 to May 2017 were selected, with a total of 128 teeth. The random number table method was used to divide them into the control group and observation group, with 64 teeth in each group. All patients met the diagnostic criteria of the "Clinical Diagnosis and Treatment Plan of Dental Disease"[8]. 50 patients in the observation group, 32 males and 18 females, aged from 23 to 56 years old; 50 patients in the control group, 26 males and 24 females, aged from 22 to 57 years old. The difference in general data between the two groups was not statistically significant and they were comparable. Inclusion criteria: (1) No periodontal systemic therapy was performed within one year before the consultation; (2) No antibiotics and anti-inflammatory drugs were administered within one month; (3) No other chronic or systemic inflammation; (4) No history of tetracycline and ibuprofen drug allergy; (5) The number of functional teeth>20 teeth, the number of odontoprisis>4. Exclusion criteria: (1) pregnant or lactating women; (2) combined with oral and maxillofacial tumors. All selected patients signed informed consent.

2.2 Treatment method

All patients were given basic treatment such as routine supracondylar scaling and subgingival scraping. On this basis, the control group was treated with minocycline ointment, the minocycline ointment (Sunstar INC, Japan) was placed in periodontal pockets of the affected teeth until overflow, once/week; the observation group was given ibuprofen capsules (Shenyang Dongxin Pharmaceutical Co., Ltd.) on the basis of control group, orally 2 times/time, 2 times/d. Both groups were treated for 4 weeks.

2.3 Detection methods

Collected the gingival crevicular fluid of each group by the bag bottom method before and after treatment respectively[9], Stored in the EP tube at -70 °C liquid nitrogen.

2.3.1 General indexes monitoring

Before and after treatment, periodontal probes were used to systematically examine the periodontal condition of teeth in both groups, including plaque index (PLI) and periodontal pocket probing depth (PD), sulcular bleeding index (SBI) and adhesion level (AL).

2.3.2 Detection of Inflammatory Factor

The patient's gingival crevicular fluid was separated and stored at -70 °C. The level of C-reactive protein (CRP) in gingival crevicular fluid was measured using an AU5800 automatic biochemical analyzer (Beckman Coulter, Inc., USA). Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure TNF-α levels in gingival crevicular fluid. Refer to the instruction manual for the procedure.

2.3.3 ALP activity, NO level detection

ALP activity level in gingival crevicular fluid was measured using AU5800 automatic biochemical analyzer (Beckman Coulter, Inc., USA); NO level in gingival crevicular fluid was measured by nitrate reductase method[10].

The kits used for the above tests were all provided by the Nanjing Jiancheng Bioengineering Institute. All operations were strictly conducted in accordance with the instrument and kit instructions.

2.4 Statistical methods

SPSS 17.0 software was used to analyze the experimental data. The measurement data were tested by normality, and the data in accordance with the normal distribution, measured data were expressed as mean±standard deviation. The t-test of independent samples was used to compare the difference in two groups. The paired t-test was used for comparison before and after treatment. 

3. Results

3.1 General indicators

Before treatment: There was no significant difference in PLI, PD, SBI and AL levels between the two groups (P>0.05). After treatment, the levels of PLI, PD, SBI, and AL were significantly decreased than before treatment in both groups, and the above indexes in the observation group were significantly lower than those in the control group after treatment. The differences were statistically significant (P<0.05). See Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of teeth</th>
<th>Time</th>
<th>PLI</th>
<th>PD (mm)</th>
<th>SBI (Point)</th>
<th>AL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>64</td>
<td>Before</td>
<td>2.41±0.52</td>
<td>5.93±0.67</td>
<td>2.96±0.71</td>
<td>3.87±0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>1.39±0.35</td>
<td>5.07±0.55</td>
<td>1.93±0.42</td>
<td>2.27±0.65</td>
</tr>
<tr>
<td>Observation group</td>
<td>64</td>
<td>Before</td>
<td>2.51±0.56</td>
<td>6.02±0.61</td>
<td>2.93±0.68</td>
<td>3.81±0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>0.68±0.30</td>
<td>4.23±0.39</td>
<td>1.15±0.33</td>
<td>0.98±0.24</td>
</tr>
</tbody>
</table>

*P<0.05 compared with before treatment; *P<0.05 compared with the control group after treatment.
Comparison of inflammatory factor levels in gingival crevicular fluid of two groups before and after treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time</th>
<th>TNF-α (ng/mL)</th>
<th>CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>64</td>
<td>Before treatment</td>
<td>5.31±1.48</td>
<td>3.02±0.79</td>
</tr>
<tr>
<td>Observation group</td>
<td>64</td>
<td>Before treatment</td>
<td>5.27±1.42</td>
<td>3.05±0.82</td>
</tr>
</tbody>
</table>

\[ P<0.05 \text{ compared with before treatment; } ^* P<0.05 \text{ compared with the control group after treatment.} \]

Comparison of ALP activity and NO level in gingival crevicular fluid of two groups before and after treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Time</th>
<th>ALP (U/L)</th>
<th>NO (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>64</td>
<td>Before treatment</td>
<td>496.19±106.55</td>
<td>22.3±2.1</td>
</tr>
<tr>
<td>Observation group</td>
<td>64</td>
<td>Before treatment</td>
<td>494.09±102.25</td>
<td>22.4±2.6</td>
</tr>
</tbody>
</table>

\[ P<0.05 \text{ compared with before treatment; } ^* P<0.05 \text{ compared with the control group after treatment.} \]

3.2 Analysis of inflammatory factors

Before treatment: There was no significant difference in TNF-α and CRP levels in gingival crevicular fluid between the two groups (\( P>0.05 \)). After treatment, the levels of TNF-α and CRP were significantly decreased in the two groups than before treatment, the difference was significant (\( P<0.05 \)); and the levels of TNF-α and CRP in the gingival crevicular fluid of the observation group were significantly lower than control group after treatment. The difference was statistically significant (\( P<0.05 \)). See Table 2.

3.3 ALP activity, NO level analysis

Before treatment: There was no significant difference in ALP activity and NO level in gingival crevicular fluid between the two groups (\( P>0.05 \)). After treatment: ALP activity and NO levels were significantly decreased than before treatment in the two groups (\( P<0.05 \)); and the ALP activity and NO level in the gingival crevicular fluid in the observation group after treatment were significantly lower than the control group after treatment, the difference was statistically significant (\( P<0.05 \)). See Table 3.

4. Discussion

Periodontitis is a chronic infectious disease caused by microorganisms in dental plaque. It develops from long-standing chronic gingivitis to deep periodontal tissue, leading to inflammation and destruction of the periodontal supporting tissues[11]. Its incidence increases with age. At present, clinical routine periodontitis treatment methods include supracondylar scaling and subgingival scaling, which can effectively remove plaque, calculus and diseased cementum, but the removal effect is not complete, and with no definite therapeutic effect on the infection caused by anaerobic bacteria in the periodontal packets. Therefore, the drug-assisted therapy has become a hot topic[12-15]. Minocycline can significantly improve various symptoms of chronic periodontitis caused by P. gingivalis, F. nucleatum, Actinobacillus, etc., and has a certain control effect on anaerobic infection[16]. Studies have shown that ibuprofen can reduce the level of inflammatory factors in patients with periodontitis, relieve clinical symptoms, and with specific clinical curative effects on chronic periodontitis[17].

The study found that the severity of periodontitis disease was related to inflammatory factors level in gingival crevicular fluid of patients[18-23]. TNF-α can promote T cells to generate a variety of inflammatory factors, promote the occurrence of inflammatory reactions, participate in the occurrence and development of chronic periodontitis[24]. CRP is a part of the body's non-specific immune system, CRP can be elevated to 2000 times than normal level within 48 hours after inflammation[25]. The results of this study showed that after treatment, the levels of PLI, PD, SBI, AL, TNF-α, and CRP were significantly decreased in the two groups than before treatment, and the PLI, PD, SBI, AL, TNF-α, and CRP in the gingival crevicular fluid of the patients in the observation group after treatment were obviously lower than the control group after treatment, the difference was significant (\( P<0.05 \)). It indicated that: minocycline combined with ibuprofen treatment, can significantly improve the periodontal status, reduce and improve the periodontal supporting tissue inflammatory response by reducing TNF-α and CRP levels in gingival crevicular fluidin of patients, and compared with simply use of minocycline, the combination therapy had more significant anti-inflammatory effects on periodontitis.

The study found that ALP is closely related to the calcification of bone. ALP in osteoblasts produces phosphate by hydrolyzing phosphatase, and combined with calcium to generate calcium phosphate deposits in the bone and promotes mineralization. Therefore, in the view of molecular biology, ALP activity can reflect the destruction degree of periodontitis[26]. Excessive production of NO in the inflammatory state of oral inflammatory diseases can cause damage to the corresponding tissue or organ[27]. In this study, after treatment, the ALP activity and NO levels in the two groups were significantly decreased than before treatment, and the ALP activity and NO level in the gingival crevicular fluid of the
observation group were significantly lower than those in the control group after treatment. All had statistical significance (P<0.05). The results showed that minocycline combined with ibuprofen can reduce the ALP activity and NO level in gingival crevicular fluid of patients, and the effect of combination therapy on ALP activity and NO level was more significant than that of minocycline alone. The mechanism may be inferred that ibuprofen is a non-steroidal anti-inflammatory drug that can effectively inhibit the bioactivity of cyclooxygenase (COX) in the body[7]. COX is a key enzyme in the synthesis of prostaglandins. Prostaglandin is a bone resorption factor. The use of drugs that inhibit the synthesis of prostaglandins in the treatment of periodontal disease is currently a new research direction.

In summary: minocycline combined with ibuprofen can improve periodontal status and gingival crevicular fluid inflammation in patients with periodontitis, reduce ALP activity and NO levels, play a role in antibacterial and induced osteogenic, and improve clinical efficacy.

References

[4] Zhang Dafeng, Huang Shengbin, Zhang Youting. Clinical efficacy and the effect of combination therapy on ALP activity and NO level in gingival crevicular fluid of patients, results showed that minocycline combined with ibuprofen can reduce the ALP activity and NO level in gingival crevicular fluid of patients, play a role in antibacterial and induced osteogenic, and improve clinical efficacy.