Effect of smoking on EA and NOS expression as well as NO and ET-1 content in gingival tissue of patients with chronic periodontitis

Ying Feng1‡, Li Guo1, Wen-Jia Cheng2

1Stomatology Department, Ninth Hospital of Xi’an Shaanxi Province, Xi’an City, Shaanxi Province, 710054
2Pathology Department, Ninth Hospital of Xi’an Shaanxi Province, Xi’an City, Shaanxi Province, 710054

1ARTICLE INFO

Objective: To study the effect of smoking on EA and NOS expression as well as NO and ET-1 content in gingival tissue of patients with chronic periodontitis. Methods: Patients diagnosed with periodontitis in our hospital between May 2013 and March 2016 were selected to screen 72 cases of smokers and 80 cases of non-smokers who were enrolled in smoking group and non-smoking group respectively, periodontal tissue was collected to detect the expression of EA, NOS and NLRP3 inflammasome, and gingival crevicular fluid was collected to detect the content of ET-1, NO, inflammatory factors and MMPs. Results: EA expression and ET-1 content in gingival tissue of smoking group were significantly higher than those of non-smoking group while NOS expression and NO content in gingival tissue were significantly lower than those of non-smoking group; NLRP3, ASC, Caspase-1, IL-1β and IL-18 expression in gingival tissue of smoking group were significantly higher than those of non-smoking group, and IL-1β, IL-18, TNF-α, IFN-γ, MMP1, MMP8 and MMP13 content in gingival crevicular fluid were significantly higher than those of non-smoking group; NLRP3, ASC, Caspase-1, IL-1β and IL-18 expression as well as IL-1β, IL-18, TNF-α, IFN-γ, MMP1, MMP8 and MMP13 content were positively correlated with EA and ET-1, and negatively correlated with NOS and NO. Conclusion: Smoking can cause increased EA and ET-1 as well as decreased NOS and NO in gingival tissue of patients with chronic periodontitis, thus adjusting the expression of NLRP3 inflammasome and MMPs to periodontal tissue inflammation and structure damage.

2. Subjects and methods

2.1 Research subjects

Patients who were diagnosed with periodontitis in our hospital
between May 2013 and March 2016 were selected as the research subject, and 72 cases of smokers and 80 cases of non-smokers were selected as the research subjects. All patients were diagnosed with periodontitis by oral examination, signed informed consent and were then included in the study. Smoking periodontitis patients were the smoking group, and they smoked more than 20 cigarettes every day and smoked for more than 4 years, including 58 male cases and 14 female cases that were 23-75 years old and with the course of disease (3.41±0.44) years; non-smoking periodontitis patients were the non-smoking group, including 62 male cases and 18 female cases that were 24-77 years old and with the course of disease (3.36±0.61) years. The two groups of patients were not significantly different in general information.

2.2 Clinical sample collection methods

The gingival crevicular fluid specimens were collected as follows: clear water was used to rinse, the dental face was blow-dried, then filter paper strip of 2 mm×10 mm was placed in the buccal- and palatal-side periodontal pocket for 60 s, then taken out, put in EP tube and fully immersed with 0.3 mL of deionized water, then the EP tube was centrifuged in centrifugal machine at 3 000 r/min for 10 min, and the supernatant was collected as gingival crevicular fluid specimen. The periodontal tissue specimens were collected as follows: diseased gingival tissue was collected during periodontal surgery or tooth extraction, cleaned with saline for 3-5 times, then placed in cryopreserved tube, quickly frozen in liquid nitrogen for 10-20 min, taken out and stored in a -80 °C refrigerator.

2.3 Gingival crevicular fluid index detection methods

Gingival crevicular fluid specimens were collected and thawed at 4 °C, then enzyme-linked immunosorbent assay kits were used to determine endothelin-1 (ET-1), nitric oxide (NO), interleukin-1 β (IL-1 β), IL-18, interferon γ (IFN-γ) and tumor necrosis factor-α (TNF-α) as well as matrix metalloproteinase 1 (MMP1), MMP8 and MMP13 content, BCA kits were used to determine total protein content, and the target protein content per mg total protein was calculated.

2.4 Periodontal tissue index detection methods

Proper amount of periodontal tissue was collected, added in protein lysis buffer and then fully grinded, the grinded tissue suspension was centrifuged in centrifugal for 20 min at 12 000 r/min, the supernatant was collected, Western-blot experiment steps were followed to incubate the monoclonal antibody of EA, NOS, NLRP3, Caspase-1, IL-1 β, IL-18 and GAPDH, protein bands were obtained, band grey values were scanned, then GAPDH was used as reference to calculate EA, NOS and NLRP3 protein expression, and the pro-Caspase-1, pro-IL-1 β and pro-IL-18 were used as reference to calculate the active-Caspase-1, active-IL-1 β and active IL-18 protein expression respectively.

2.5 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data analysis between two groups was by t test and P<0.05 meant statistical significance in the obtained results.

3. Results

3.1 EA and NOS expression as well as ET-1 and NO content in gingival tissue

Analysis of EA and NOS expression as well as ET-1 and NO content in gingival tissue between two groups of patients was as follows: EA expression and ET-1 content in gingival tissue of smoking group were significantly higher than those of non-smoking group while NOS expression and NO content in gingival tissue were significantly lower than those of non-smoking group. Differences in EA and NOS expression as well as ET-1 and NO content in gingival tissue were statistically significant between smoking group and non-smoking group (P<0.05).

3.2 NLRP3 inflammasome expression in gingival tissue

Analysis of NLRP3 inflammasome NLRP3, ASC, Caspase-1, IL-1 β and IL-18 expression in gingival tissue between two groups of patients was shown in Table 2: NLRP3, ASC, Caspase-1, IL-1 β and IL-18 expression in gingival tissue of smoking group were significantly higher than those of non-smoking group while NOS expression and NO content in gingival tissue were significantly lower than those of non-smoking group. Differences in NLRP3, ASC, Caspase-1, IL-1 β and IL-18 expression in gingival tissue as well
as IL-1β, IL-18, TNF-α, and IFN-γ content in gingival crevicular fluid were statistically significant between the two groups (P<0.05). Pearson test showed that NLRP3, ASC, Caspase-1, IL-1β, and IL-18 expression in gingival tissue as well as IL-1β, IL-18, TNF-α, and IFN-γ content in gingival crevicular fluid were positively correlated with ET-1, and negatively correlated with NOS and NO.

### 3.3 Matrix metalloproteinase content in gingival crevicular fluid

Analysis of matrix metalloproteinase MMP1, MMP8 and MMP13 content in gingival crevicular fluid between two groups of patients was as follows: MMP1, MMP8 and MMP13 content in gingival crevicular fluid of smoking group were significantly higher than those of non-smoking group. Differences in MMP1, MMP8 and MMP13 content in gingival crevicular fluid were statistically significant between smoking group and non-smoking group (P<0.05). Pearson test showed that MMP1, MMP8 and MMP13 content in gingival crevicular fluid were positively correlated with EA expression in gingival tissue.

### 4. Discussion

Smoking is an independent risk factor for periodontal disease, and study has shown that the smoking patients are with deeper periodontal pocket depth and more severe alveolar bone resorption and tooth defect. At present, the close relationship between smoking and periodontal disease has received more and more attention, but the key molecules regulated by smoking in the process of periodontal injury are not clear. Chronic periodontitis is one of the most common clinical periodontal diseases, and chronic periodontal tissue inflammation and structure damage are its basic pathological characteristics. EA is one of the important proteases causing periodontal tissue damage in patients with chronic periodontitis, and it not only has direct degrading effect on type I-IV collagen, fibrin, elastin and various other periodontal tissue compositions, but can also activate many members in the MMPs family and indirectly degrade extracellular matrix[6,7]. MMPs family is a zinc ion-dependent protease family, and MMP1, MMP8 and MMP13 in the family are associated with the periodontal tissue damage in patients with periodontitis[8,9]. In the study, analysis of EA expression in periodontal tissue and MMPs content in gingival crevicular fluid between smoking and non-smoking patients with chronic periodontitis showed that EA expression in periodontal tissue as well as MMP1, MMP8 and MMP13 content in gingival crevicular fluid of smoking group was significantly higher than those of non-smoking group and EA expression was positively correlated with MMP1, MMP8 and MMP13 content. It indicates that smoking will increase the EA expression in periodontal tissue of patients with chronic periodontitis, and then cause periodontal tissue damage through the effect of EA on degrading extracellular matrix.

Persistent periodontal tissue inflammation is the basic characteristic of patients with chronic periodontitis, and the balance between ET-1 and NO is an important mechanism that regulates local tissue inflammation. ET-1 is a active molecule with strong vasoconstrictive effect, a variety of inflammatory cells in local tissue can express and release ET-1, and it plays a significant role in promoting the cascade amplification of the inflammatory response[10,11]; NO is an important gas signal molecule in local tissue, it is produced after NOS catalyzes L-arginine, and the compensatory increase of NO from NOS catalysis in periodontitis group can mitigate the degree of periodontal tissue inflammation. In the study, analysis of the content of ET-1 and the NO from NOS catalysis in the periodontal tissue showed that the NOS expression in periodontal tissue and NO content in gingival crevicular fluid of smoking group were significantly lower than those of non-smoking group while ET-1 content in gingival crevicular fluid was significantly higher than that of non-smoking group. This means that smoking can lead to the
increased ET-1 generation in periodontal tissue and the inhibited NO generation from NOS catalysis in patients with chronic periodontitis, and then cause periodontal tissue inflammation through the ET-1/NO imbalance.

The inflammation in the periodontal tissue is directly mediated by a variety of inflammatory factors, and the disequilibration between ET-1 and NO causes local tissue inflammation through regulating the expression of a variety of inflammatory factors. NLRP3 inflammasome is the important structure to adjust the degree of inflammation and the secretion of inflammatory factors, and the inflammasome is made up of NLRP3, ASC and caspase-1. In the cascade activation of inflammatory response, the activation of NLRP3 can recruit ASC and crack the pro-caspase-1 into active caspase-1[13]; under the catalysis of active-caspase-1, the pro-IL-1β and pro-IL-18 are cut into mature IL-1β and IL-18, and then IL-1β and IL-18 can mediate inflammation cascade activation and increase the secretion of TNF-α and IL-18 through the caspase amplification of the inflammatory response[14,15]. In the study, the expression of NLRP3 inflammasome in periodontal tissue was analyzed at first, and the results showed that NLRP3, ASC, Caspase-1, IL-1β and IL-18 expression in gingival tissue of smoking group were significantly higher than those of non-smoking group, positively correlated with ET-1, and negatively correlated with NOS and NO. The NLRP3 inflammasome activation can directly increase the secretion of IL-1β and IL-18, which activate the secretion of TNF-α and IFN-γ through the caspase amplification of the inflammatory response[16,17]. In the study, analysis of the content of these cytokines in gingival crevicular fluid showed that IL-1β, IL-18, TNF-α and IFN-γ content in gingival crevicular liquid of smoking group were significantly higher than those of non-smoking group, positively correlated with ET-1, and negatively correlated with NOS and NO. The above results show that increased ET-1 generation and decreased NO generation from NOS catalysis in the periodontal tissue can cause NLRP3 inflammasome activation, which further increases the synthesis and secretion of a variety of inflammatory cytokines.

Based on above discussion, it can be concluded that smoking can cause increased EA and ET-1 as well as decreased NOS and NO in gingival tissue of patients with chronic periodontitis, increased EA will increase the expression of MMPs, and increased ET-1 as well as decreased NOS and NO will increase the expression of NLRP3 and the secretion of inflammatory factors, eventually causing periodontal tissue inflammation and structure damage.

References