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Effect of Er, Cr: YSGG laser combined with oral basic treatment on inflammatory injury and apoptosis in patients with periodontitis

Wei−Xing Jin[1]✉, Zheng−Zhi Guan[2]

[1.] *Stomatology Department, Chengguan Township Health Center of Yunmeng County Hubei Province, Xiaogan, Hubei Province, 432500*

[2.] *Stomatology Department, Yunmeng County People's Hospital in Hubei Province, Xiaogan, Hubei Province, 432500*

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ABSTRACT

Objective: To study the effect of Er, Cr: YSGG laser combined with oral basic treatment on inflammatory injury and apoptosis in patients with periodontitis. **Methods:** Patients with chronic periodontitis who were treated in dental clinic of our hospital between February 2015 and March 2017 were selected as the research subjects and randomly divided into the group A who accepted ultrasonic dental cleaning, hand scaling combined with Er, Cr: YSGG laser treatment and the group B who received ultrasonic dental cleaning and hand scaling treatment. The contents of inflammatory response cytokines, protease molecules and apoptosis molecules in gingival crevicular fluid were detected before treatment and 1 week after treatment. **Results:** TNF-α, hs-CRP, IL-1β, IL-6, IL-8, EMMPRIN, CyPA, MMP2, MMP9, Smac, Bax, Fas and FasL contents in gingival crevicular fluid of both groups of patients 1 week after treatment were significantly lower than those before treatment, and TNF-α, hs-CRP, IL-1β, IL-6, IL-8, EMMPRIN, CyPA, MMP2, MMP9, Smac, Bax, Fas and FasL contents of group A 1 week after treatment were significantly lower than those of group B. **Conclusion:** Er, Cr: YSGG laser combined with oral basic treatment can inhibit the inflammatory injury and apoptosis in periodontal tissue of patients with periodontitis.

1. Introduction

Periodontitis is the most common type of periodontal disease in China, which affects patients′ chewing function and daily life. The main non-operative therapy for periodontitis is the removal of dental plaque, tartar, diseased cementum and other infected tissues in periodontal pocket by manual instrument or ultrasonic dental descaler[1,2]. Persistent plaque in periodontal tissue will activate inflammatory response and increase the release of inflammatory factors, which on the one hand, directly causes tissue damage through inflammation, and on the other hand, causes tissue damage through activation of apoptosis. However, the effect of routine treatment to remove plaque is not ideal, plaque clearance is not complete and periodontal inflammation is easy to relapse. Erbium, chromium: yttrium-scandium gallium-garnet (Er, Cr: YSGG), also known as water laser, stimulates water molecules via the laser with 2 780 nm wavelength and forms tissue micro-explosion, which effectively removes the smear layer on tooth surface without causing damage to the deep pulp tissue, and has good security[3,4]. In the following studies, we specifically analyzed the effect of Er, Cr: YSGG laser combined with oral basic treatment on inflammation and apoptosis of patients with periodontitis.

2. Case information and research methods

2.1 General case information

Patients with chronic periodontitis who were treated in dental clinic of our hospital between February 2015 and March 2017 were selected as the research subjects, all patients conformed to the diagnosis of periodontitis and with probe depth ≥ 4 mm and clinical attachment loss ≥ 1 mm of more than 2 teeth, and they did not receive periodontal treatment within the previous six months. Patients who

✉Corresponding author: Wei-Xing Jin, Stomatology Department, Chengguan Township Health Center of Yunmeng County Hubei Province, Xiaogan, Hubei Province, 432500.
Tel: 18772128888
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were using immune preparations and had used antibiotics in the past 3 months were excluded, and a total of 76 cases were enrolled. The patients with chronic periodontitis were divided into group A and group B by random number table, 38 cases in each group. Group A received ultrasonic dental cleaning, hand scaling combined with Er, Cr: YSGG laser treatment, including 22 men and 16 women that were 38-61 years old; group B received ultrasonic dental cleaning and hand scaling, including 23 men and 15 women that were 37-62 years old. There was no significant difference in general information between the two groups of patients.

2.2 Therapy

Both groups of patients received supragingival scaling by ultrasonic dental descaler, and accepted subsequent treatment 2 weeks later. Group A: ultrasonic dental descaler was used to remove subgingival calculus, and the root surface was shaved by hand scaling; then RFRT5-14 tip was inserted in the periodontal pocket for laser treatment, the power meter was 1.0-1.25 W, water 30%-40% and air 25%, and the tip was horizontally and vertically moved along the root surface. Group B: ultrasonic dental descaler was used to remove subgingival calculus, and the root surface was shaved by hand scaling.

2.3 Gingival crevicular fluid collection and index detection

Before treatment and 1 week after treatment, appropriate filter paper strip was put into the bottom of periodontal pocket and maintained there for 30 s, gingival crevicular fluid was obtained, placed in 1.5 mL EP tube, joined by 0.5 mL PBS buffer, shaken and centrifuged for 3 min to separate supernatant liquid, and enzyme-linked immunosorbent assay kit was used to detect TNF-α, hs-CRP, IL-1β, IL-6, IL-8, EMMPRIN, CyPA, MMP2, MMP9, Smac, Bax, Fas and FasL contents in supernatant liquid.

2.4 Statistical methods

SPSS 20.0 software was used to input and analyze data, differences in data between two groups were analyzed by t test and $P<0.05$ indicated statistical significance in differences in test results.

3. Results

3.1 Inflammatory cytokine contents in gingival crevicular fluid

Before treatment and 1 week after treatment, analysis of inflammatory cytokines TNF-α, hs-CRP, IL-1β, IL-6 and IL-8 contents in gingival crevicular fluid between group A and group B was as follows: TNF-α, hs-CRP, IL-1β, IL-6 and IL-8 contents in gingival crevicular fluid were not significantly different between group A and group B before treatment ($P>0.05$); TNF-α, hs-CRP, IL-1β, IL-6 and IL-8 contents in gingival crevicular fluid of both groups of patients 1 week after treatment were significantly lower than those before treatment ($P<0.05$), and TNF-α, hs-CRP, IL-1β, IL-6 and IL-8L contents of group A 1 week after treatment were significantly lower than those of group B ($P<0.05$).

3.2 Protease molecule contents in gingival crevicular fluid

Before treatment and 1 week after treatment, analysis of protease molecules EMMPRIN (ng/mL), CyPA (ng/mL), MMP2 (pg/mL) and MMP9 (pg/mL) contents in gingival crevicular fluid between group A and group B was as follows: EMMPRIN, CyPA, MMP2 and MMP9 contents in gingival crevicular fluid were not significantly different between group A and group B before treatment ($P>0.05$); EMMPRIN, CyPA, MMP2 and MMP9 contents in gingival crevicular fluid of both groups of patients 1 week after treatment were significantly lower than those before treatment ($P<0.05$), and EMMPRIN, CyPA, MMP2 and MMP9 contents of group A 1 week after treatment were significantly lower than those of group B ($P<0.05$).

Table 1.

Changes in inflammatory cytokines in gingival crevicular fluid before and after treatment (pg/mL).

Groups	n	Time	TNF-α	hs-CRP	IL-1β	IL-6	IL-8
Group A	38	Before treatment	3.42±0.45	6.94±0.88	2.16±0.32	1.93±0.22	1.41±0.17
		After treatment	1.67±0.22*#	2.55±0.36*#	0.98±0.11*#	0.76±0.08*#	0.75±0.09*#
Group B	38	Before treatment	3.48±0.42	7.02±0.91	2.20±0.35	1.98±0.24	1.44±0.15
		After treatment	2.21±0.28*	3.94±0.51*	1.47±0.22*	1.33±0.18*	1.03±0.16*

*: comparison of indexes in gingival crevicular fluid within group between before and after treatment, $P<0.05$; #: comparison of indexes in gingival crevicular fluid between group A and group B after treatment, $P<0.05$.

Table 2.

Changes in protease molecules in gingival crevicular fluid before and after treatment.

Groups	*n*	Time	EMMPRIN	CyPA	MMP2	MMP9
Group A	38	Before treatment	6.52±0.93	3.21±0.42	157.4±20.3	205.6±27.5
		After treatment	2.89±0.35	1.25±0.16	64.6±8.7	89.5±10.3
Group B	38	Before treatment	6.61±0.88	3.17±0.38	160.1±19.5	204.6±24.7
		After treatment	4.04±0.57	1.92±0.22	98.5±11.3	141.2±22.7

*: comparison of indexes in gingival crevicular fluid within group between before and after treatment, $P<0.05$; #: comparison of indexes in gingival crevicular fluid between group A and group B after treatment, $P<0.05$.

Table 3.

Changes in apoptosis molecules in gingival crevicular fluid before and after treatment.

Groups	*n*	Time	Smac	Bax	Fas	FasL
Group A	38	Before treatment	1.77±0.20	5.42±0.78	126.7±17.4	158.3±19.4
		After treatment	0.78±0.09*#	2.19±0.31*#	47.5±6.7*#	70.3±9.5*#
Group B	38	Before treatment	1.81±0.22	5.37±0.72	129.1±15.8	160.1±18.8
		After treatment	1.33±0.18*	3.42±0.41*	79.7±9.4*	103.5±12.6*

*: comparison of indexes in gingival crevicular fluid within group between before and after treatment, $P<0.05$; #: comparison of indexes in gingival crevicular fluid between group A and group B after treatment, $P<0.05$.

3.3 Apoptosis molecule contents in gingival crevicular fluid

Before treatment and 1 week after treatment, analysis of apoptosis molecules Smac (ng/mL), Bax (ng/mL), Fas (pg/mL) and FasL (pg/mL) contents in gingival crevicular fluid between group A and group B was as follows: Smac, Bax, Fas and FasL contents in gingival crevicular fluid were not significantly different between group A and group B before treatment ($P>0.05$); Smac, Bax, Fas and FasL contents in gingival crevicular fluid of both groups of patients 1 week after treatment were significantly lower than those before treatment ($P<0.05$), and Smac, Bax, Fas and FasL contents of group A 1 week after treatment were significantly lower than those of group B ($P<0.05$).

4. Discussion

Manual or ultrasonic scaling is a major means of the clinical treatment of periodontitis, which can remove plaque, tartar and diseased cementum in periodontal pocket, and help restore healthy periodontal tissue. However, a layer of amorphous tissue will be formed on the root surface after conventional scaling, including residual odontolith and cementum as well as bacteria and bacteria-related products. The remaining amorphous tissue is known as the smear layer, which can adversely affect the healing of periodontal tissue. Er, Cr: YSGG laser is the periodontal therapy developed in recent years, which makes water molecules rapid evaporate through the high absorption of water molecules in periodontal tissue to laser energy, and also forms micro blasting in local tissue to help remove the tartar, plaque and other lesions adhered in periodontal tissue[5,6]. At present, studies about Er, Cr: YSGG laser believe that the roughness of root surface tissue increases significantly after laser treatment, and rough root surface tissue is conducive to the periodontal ligament fibroblast attachment and not conducive to the attachment of pathogenic microorganisms, which can promote plaque removal and periodontal tissue healing[7,8]. In recent years, Er, Cr: YSGG laser value for periodontitis treatment has received more and more attention, but there is no clear report about the effect of laser treatment on the pathological changes of periodontitis.

Continuous inflammatory reaction activation is an important pathological feature of local periodontitis, and incomplete plaque removal will cause inflammation in active state and lead to the massive synthesis and secretion of a variety of inflammatory cytokines[9]. TNF-α has the function of initiating inflammatory response, and is mainly synthesized and secreted in early inflammatory response[10]; hs-CRP is the acute phase protein directly related to the activation of inflammatory reaction[11]; IL-1β can not only mediate the activation of inflammatory reaction directly, but can also promote the secretion of various adhesion molecules and chemokines; IL-6 and IL-8 are cytokines with multiple biological functions, which can activate periodontal inflammation and promote periodontal tissue destruction[12]. Er, Cr: YSGG laser has stronger plaque removal effect, and in order to define the degree of inflammatory response activation in the periodontal tissue after laser treatment, the corresponding inflammatory cytokine contents in gingival crevicular fluid were analyzed in the study, and the results showed that TNF-α, hs-CRP, IL-1β, IL-6 and IL-8 contents in gingival crevicular fluid of both groups of patients significantly decreased after treatment, and TNF-α, hs-CRP, IL-1β, IL-6 and IL-8L contents of group A 1 week after treatment were significantly lower than those of group B. It means that regular basic oral treatment can remove plaque and reduce periodontal inflammation to a certain extent, and combination of Er, Cr: YSGG laser is more effective than routine oral basic treatment in inhibiting periodontal tissue inflammation.

The persistent periodontal tissue inflammation in patients with periodontitis can lead to the progressive development of periodontal attachment loss and the continuous alveolar bone resorption, and severe cases can develop into tooth loss. In the process of alveolar bone resorption, inflammatory response activation caused by plaque infection can result in the loss and degradation of bone matrix by the degradation of extracellular matrix. MMPs is a group of important molecules that regulate the synthesis and degradation of extracellular

matrix. MMP2 and MMP9 in this family have biological activities of degrading elastin, collagen and laminin. In the sclerotin of the alveolar bone, type I collagen is the primary collagen component, and the collagen degradation by MMP2 and MMP9 can lead to the alveolar bone resorption[13,14]. EMMPRIN is an inducible factor of MMPs, which can increase the expression and secretion of various MMPs by interacting with CyPA, thus promoting the degradation of extracellular matrix and the resorption of alveolar bone[15,16]. In order to further clarify the activation of periodontal tissue inflammation after laser treatment, the corresponding protease molecule contents in in gingival crevicular fluid were analyzed in the study, and the results showed that EMMPRIN, CyPA, MMP2 and MMP9 contents in gingival crevicular fluid of both groups of patients significantly decreased after treatment, and EMMPRIN, CyPA, MMP2 and MMP9 contents of group A 1 week after treatment were significantly lower than those of group B. It means that regular oral basic treatment can inhibit the alveolar bone resorption to a certain extent, and the combination of Er, Cr: YSGG laser is more effective than routine oral basic treatment in inhibiting the alveolar bone resorption.

The inflammatory response of periodontal tissue can not only directly cause inflammatory tissue damage and alveolar bone resorption, but can also activate apoptosis and cause periodontal tissue injury. Bax is a very important molecule that regulaties mitochondrial pathways of apoptosis, which can forme Bax channels on mitochondrial membrane, promote cytochrome C release from the mitochondria into the cytoplasm, then activate cascade activation reaction mediated by downstream caspase and promote apoptosis; at the same time, Bax can increase the expression of Smac, and the highly expressed Smac can be combined with various anti-apoptotic proteins to weaken their anti-apoptotic activity, and thereby promote apoptosis[17,18]. Fas/FasL are the key molecules that regulate the death receptor apoptosis pathway, and the two can identify each and then initiate the apoptosis mediated by caspase through FADD domain[19,20]. In order to define the effect of laser treatment on apoptosis in periodontal tissue of patients with periodontitis, the corresponding apoptosis molecule contents in gingival crevicular fluid were analyzed in the study, and the results showed that Smac, Bax, Fas and FasL contents in gingival crevicular fluid of both groups of patients significantly decreased after treatment, and Smac, Bax, Fas and FasL contents of group A 1 week after treatment were significantly lower than those of group B. It means that regular oral basic treatment can inhibit periodontitis tissue apoptosis to a certain extent, and the combination of Er, Cr: YSGG laser can be more effective than conventional oral basic treatment in inhibiting periodontitis tissue apoptosis.

The effect of Er, Cr: YSGG laser on the inflammatory injury and apoptosis of periodontitis tissue was mainly analyzed in the study, and analysis of the results can reach the preliminary conclusion that Er, Cr: YSGG laser combined with oral basic treatment of periodontitis can be more effective than routine oral basic treatment in inhibiting the inflammatory injury and apoptosis of periodontitis tissue.

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