SHORT-TERM LOW-LEVEL LASER THERAPY ATTENUATES INFLAMMATION AND PRODUCTION OF INTERLEUKIN-1, BUT ELEVATES THE LEVEL OF MATRIX METALLOPROTEINASE 9 IN CHRONIC PERIODONTITIS

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Abstract

Background / Purpose: Low- level laser therapy (LLLT) has been used as an adjunct to the treatment of chronic periodontitis (CP), but its efficacy has not been well-documented. Therefore, the aim of this study was to investigate the effect of LLLT on clinical parameters in CP patients and their relationship with concentrations of biohumoral markers of inflammation in gingival crevicular fluid (GCF). .

Materials and methods: Thirty-six patients were randomly assigned to control and experimental groups after scaling. The experimental group was treated with a diode laser (wavelenght: 635 nm; power density: 100 mW/cm2), by applying the laser beam to diseased teeth for 9 days, whereas the control group did not receive LLLT. Clinical examination was performed at baseline and 10 days after the treatment. GCF samples were collected from the same periodontal site before and after therapy. The levels of interleukin 1 (IL-1) and matrix metalloproteinase 9 (MMP-9) in GCF were measured by ELISA.

Results: LLLT decreased clinical parameters of CP. The levels of IL-1α and IL-1β in GCF were decreased ($p< 0.05$), but the level of MMP-9 was increased ($p<0.01$). After LLLT, the level of IL-1α correlated positively with MMP-9 ($p< 0.05$) and the MMP-9 levels correlated negatively with plaque index ($p< 0.05$) and papillary bleeding index ($p< 0.01$).

Conclusion: LLLT attenuated periodontal inflammation in CP patients, as judged by clinical parameters and decreased levels of IL-1 in GCF. It remains to be studied whether elevated levels of MMP-9 in GCF might be beneficial for reparation processes.

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Introduction

Periodontitis, a chronic inflammatory disease of periodontal tissue, is characterized by the breakdown of tooth-supporting structure[1]. It is generally accepted that the host immune-inflammatory response to the periodontogenic microorganisms from the dental plaque, is a key pathogenic mechanism involved in the development and progression of the disease[2,3].

The immune-inflammatory response is coordinated by different infiltrating cells of both innate and adaptive immunity (neutrophils, macrophages, lymphocytes) and stromal cells. These cells produce a number of cytokines, enzymes and other biomolecules, which are associated with the tissue destruction. Simultaneously, anti-inflammatory mediators attenuate the disease progression [2-4].

Among different pro-inflammatory cytokines, interleukin-1 (IL-1) has been attributed as a key marker of periodontal inflammation and disease progression, including bone loss [5-7]. It has been demonstrated that the levels of both isoforms (IL-1α and IL-1β) and IL-1 receptor

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antagonist are significantly increased in gingival crevicular fluid (GCF) in patients with gingivitis and periodontitis, compared to healthy controls[6-9].

IL-1β is predominantly produced by monocytes, macrophages, neutrophils, epithelial cells and fibroblasts, whereas the production of IL-1α is mostly connected with gingival epithelial cells where it performs local functions[5, 10].

The family of matrix metalloproteinases (MMPs) consists of more than 26 proteolytic enzymes with zinc endopeptidase activity, which degrades extracellular matrix and basement membrane components. They share homologous protein sequences containing conserved and specific domain structures. MMPs participate in a number of physiological and pathological events, including collagen breakdown during periodontal tissue destruction. The enzymes also control the activity and bioavailability of cytokines and growth factors. [11-14]. MMP-9, known as gelatinase B, catalyzes the cleavage of all types of denaturated collagens and native components of the basement membrane. Recently, fibrinogen, α1-proteinase inhibitor, IL-1 and transforming growth factor-β (TGF-β) have been also identified as MMP-9 substrates [11, 15, 16].

It has been described that MMP-9 coordinates and effects the epithelial regeneration and interferes with the production of epithelial-associated IL-1α [17]. Increased levels of several MMPs, including MMP-9, have been described in blood, periodontal tissue and GCF in chronic periodontal diseases [15, 16, 18-21].

Low-level laser therapy (LLLT) was introduced as a therapeutic modality for the treatment of periodontal diseases, mostly in combination with other non-surgical procedures, but its efficacy has not been always documented [22, 23]. The beneficial effect of LLLT in periodontitis is based on the reduction of pain, edema and inflammation, but the mechanisms are complex and largely unknown [23]. Generally, the therapy activates photoreceptors in the electron transport chain within the membrane of mitochondria and other intracellular structures. Absorption of light activates respiratory chain components, stimulates adenosine triphosphate production and nucleic acid synthesis. These processes are followed by promotion of cellular proliferation (fibroblasts, epithelial cells), synthesis of collagen and secretion of growth factors [24-26]. However, therapeutic results are different, depending on the type of laser, duration of the treatment and clinical stages of the disease [25-27].

Up to now, very few studies have examined the effect of low-level diode laser on clinical efficacy in chronic periodontitis (CP) and they are mainly focused to the long-term effects. Based on our good experience with LLLT in everyday clinical practice, manifested by a significant reduction of symptoms and signs in CP patients, even after a short period of therapy, we wondered whether and how such laser treatment influences the production of IL-1 and MMP-9, the biomolecules postulated as markers of severity and progression of CP [8, 16]. In this context, we hypothesized that the production of IL-1α and IL-β isoforms after LLLT may be different, due to their different source and local functions. In addition, the relationship between IL-α, MMP-9 and clinical parameters in CP has not been examined yet.

Materials and methods

Patients and study design

The study was performed from June 2010 to October 2010 at Dental Polyclinic „Dr B. Ismaili“, Gostivar, FYR of Macedonia, according to the Declaration of Helsinki: ethical principles for medical research involving human subjects (World Medical Association). The study protocol and collection of GCF samples was approved by the Scientific Committee of Medical Faculty of Foca, University of East Sarajevo, BH. After written informed consent, 36 patients with untreated CP (19 men and 17 women), were selected for the study. The mean subject age was 51.6 years (range, 42-62 years). Of them 40% were tobacco smokers (Table 1).

Parameter	Control group	LLLT group	Total
N	18	18	36
Sex (men / women)	8/10	11/7	19/17
Age (years; mean ± SD)	52.3 ± 4.6	49.9 ± 8.2	51.6 ± 6.4
Smookers (men / women)	6 (3/3)	8 (6/2)	14 (9/5)
Lateral incisors* (n)	3	5	8
Canines* (n)	6	6	12
Premolars* (n)	9	7	16

Table 1. Demographic data of patients with chronic periodontitis.
* = sampling of gingival crevicular fluid; LLLT = low – level laser therapy.

The diagnosis was assessed according to clinical parameters and radiography. Patients who underwent antibiotic, anti-inflammatory and immunomodulatory therapy within the last two weeks or who had acute systemic illness, hematological and autoimmune diseases, cancer, as well as pregnant women were not included in the study. Exclusion criteria were also periodontal treatments within last 3 months prior to the study.

The subjects went through a complete anamnesis, impression and received radiographic and periodontal examinations. The following clinical parameters were determined: plaque index (PI) [28]; papillary bleeding index (PBI) [29]; probing pocket depth (PPD) (expressed as mm) [29]; and gingival recession (GR) (expressed as mm) [30]. Before determination of PBI and PPD, GCF was collected. After that, removal of hard deposits (mechanical scaling) was done. Patients were than randomly divided into control (18 subjects) and experimental (18 subjects) groups. The control group did not receive any other therapy, whereas the experimental group was treated with LLL. Both groups of patients received oral hygiene instructions within next 9 days and after that they were invited to the second clinical examination and GCF collection.

Low-level laser therapy

LLLT was performed with a diode laser (Scorpion Dental Optima, Sofia Bulgaria), by using instructions from the manufacturer for the treatment of CP. The laser beam was applied to all diseased teeth within 9 consecutive days. The laser therapy was as follows: wavelength: 635 nm; initial laser power: 25 mW; exposure per irradiation area involving one tooth and one intertooth area: 4 min. The size of the aperture was 2mm in diameter allowing the power density of about 100 mW/cm^2.

GCF collection

GCF samples were collected from one disease active site which was the same as before therapy. Each sample site was carefully isolated using cotton rolls to avoid saliva contamination. The fluid collection was done by a paper strip (DiaDent Dia-Prot, DiaDent Group International, Choong-Chong Buk, Korea), which was placed for 30 seconds in the gingival pocket until resistance was felt. Blood-contaminated samples were discarded. The volume of GCF was determined by measuring the strip weight before and after fluid collection. The strips were placed in Eppendorf vials containing 100 µl of physiological saline and kept under -70ºC.

Determinations of biomarkers in GCF

After thawing, GCF samples were centrifuged at 12 000 rpm for 5 min and diluted 1:2 with sample buffer. The levels of IL-1α, IL-1β and MMP-9 were determined at the Medical Faculty of Foca, using commercial enzyme-linked immunosorbent assay (ELISA) plates (R&D, Inc. Minneapolis, MN, USA), according to manufacturer's instructions. After the color development was stopped, the optical density was measured using a plate computerized reader set to a wavelength of 450 nm. The levels of these biomolecules were determined based on the standard curves. The concentrations were calculated and expressed as pg / µl of GCF.

Statistics

The clinical parameters and biomolecule levels were expressed as means ± standard deviations (SD). Differences between the pre- and post-treatment values within each group and the differences between the changes of the pre- and post- values among groups were compared using Wilcoxon matched-pairs signed rank test. The probability value for statistical significance was set at $p < 0.05$. Correlations between the levels of biomarkers as well as between the levels of biomarkers and clinical parameters were performed using the Spearman's correlation test.

Results

Effect of low-level laser therapy on clinical parameters in patients with chronic periodontitis.

The control and experimental group of CP patients before therapy did not significantly differ in neither of examined clinical parameters ($p>0.05$) (Fig.1). The laser therapy resulted in statistically significant decrease of PI ($p<0.01$), PBI ($p<0.05$) and PPD ($p<0.05$), compared to the baseline values. In contrast, no significant changes in GR were noticed ($p>0.05$). A slight decrease in PBI after 10 days ($p<0.05$) was also found in the control group of patients, compared to the corresponding baseline value (Fig.1).

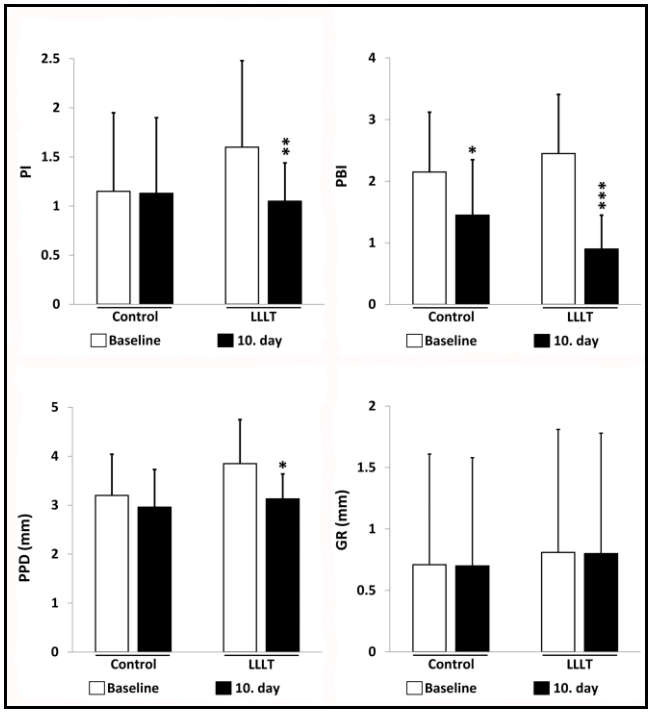

Figure 1. Effect of low- level laser therapy on clinical parameters in patients with chronic periodontitis.
Values are given as mean ± SD for n = 18 (control group) and n = 18 (LLLT group).
* = p < 0.05; ** = p < 0.01; *** = p < 0.005 compared to the corresponding baseline values (before therapy).

Effect of low-level laser therapy on the levels of IL-1α, IL-1β and MMP-9 in GCF of patients with chronic periodontitits.

The concentrations of IL-1α, IL-1β and MMP-9 were detected in all GCF samples. The baseline levels of all examined biomolecules did not differ significantly between the groups (p>0.05). LLLT significantly reduced the mean concentrations of both IL-1α and IL-1β in GCF of CP patients (p<0.05), compared to the mean cytokine levels before therapy. In contrast, the mean level of MMP-9 was significantly elevated (p<0.01). No significant differences in the levels of these biomolecules (baseline *versus* 10 days) in the control group (p>0.05) were found (Fig. 2).

Correlations between the levels of biomolecules in GCF and clinical parameters in patients with chronic periodontitis.

Table 2 shows the correlations between the mean levels of biomolecules in GCF and mean clinical parameters of CP. It can be seen that baseline values of IL-1β in both control and experimental groups correlated positively with PBI and PPD (p<0.05). The correlations remained significant after 10 days. Other correlations were not statistically significant.

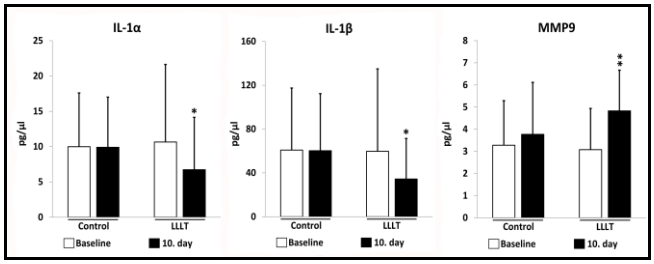

Figure 2. Effect of low- level laser therapy on the levels of IL-1α, IL-1β and MMP-9 in GCF of patients with chronic periodontitis.
Values are given as mean ± SD for n = 18 (control group) and n = 18 (LLLT group).
* = p < 0.05; ** = p < 0.01 compared to the corresponding baseline values (before therapy).

	CONTROL GROUP		LLLT GROUP	
PARAMETERS	BASELINE (n=18)	10. DAY (n=18)	BASELINE (n=18)	10. DAY (n=18)
IL-1α:MMP-9	r=0.34	r=0.37	r=0.36	r=0.47*
IL-1β:MMP-9	r=-0.26	r=-0.27	r=-0.25	r=-0.39
IL-1α:PI	r=0.26	r=0.28	r=0.28	r=0.26
IL-1α:PBI	r=0.31	r=0.32	r=0.29	r=0.39
IL-1α:PPD	r=0.27	r=0.28	r=0.31	r=0.26
IL-1β:PI	r=0.40	r=0.38	r=0.39	r=0.38
IL-1β:PBI	r=0.52*	r=0.50*	r=0.49*	r=0.58**
IL-1β:PPD	r=0.48*	r=0.46*	r=0.52*	r=0.61**
MMP-9:PI	r=-0.29	r=-0.30	r=-0.31	r=-0.47*
MMP-9:PBI	r=-0.25	r=-0.32	r=-0.26	r=-0.53**
MMP-9:PPD	r=-0.22	r=-0.24	r=-0.16	r=-0.27

Table 2. Correlations between the levels of biomarkers in GCF and clinical parameters in patients with chronic periodontitis.
* = p < 0.05; ** = p < 0.01; (Spearman correlation test)

After LLLT, a positive correlation between the levels of IL-1α and MMP-9 was established (p<0.05). In addition, negative correlations between the mean level of MMP-9 and mean values of PI (p<0.05), as well as MMP-9 and PBI (p<0.01), were observed. Such correlations were not statistically significant in the control group (Table 2).

Discussion

This is the first study examining the short-term effect of LLLT on clinical parameters and biomolecules of inflammation in CP patients, using a diode laser with wavelength of 635 nm. The laser is designed for its biostimulatory effect and in this context the therapy was conducted by

a protocol which was generally accepted in dentistry for the treatment of periodontitis [25].

To check the effect of LLLT alone, only scaling before the therapy was performed, as recommended by the manufacturer. Namely, the reflection of laser beam from hard dental deposits may limit the efficacy of therapy. Therefore, the patients treated with scaling only, served as a control group. Although this study was limited to the relatively small number of patients, the advantage of this study protocol was its prior – post experimental design, where each subject was his / her own control. Such approach may overcome interindividual variations in the levels of examined biomolecules.

The presented results clearly showed that short term LLLT suppressed inflammation in the periodontal tissue, as judged by decreased values of PI, PBI and PPD. The decrease of PI could be due to the direct antibacterial effect of LLLT, as documented for diode lasers [26, 31], and due to better oral hygiene performed by patients during the examination period. In addition, the reduction of PPD could be also due to the reduction of edema, as a main clinical parameter of the effectiveness of LLLT [22, 23]. Scaling performed before LLLT, might have some effects on decrease of PBI, since a slight reduction of this bleeding index was also noticed in the control group.

The results of clinical examinations are in agreement with significant decrease of IL-α and IL-1β levels in GCF. A number of clinical and experimental studies demonstrated that the levels of IL-1β was increased in gingivitis and CP and suggested that this cytokine could be a marker of disease severity and its progression [6-9].

The positive correlation between the levels of IL-1β and clinical presentation of the disease observed in this and other studies [7, 9, 26, 32] supports this hypothesis. The publications related to IL-1α in CP are very scarce. This work showed that the levels of IL-1α in GCF of investigated patients were lower compared to IL-1β, but its reduction after LLLT was almost the same. To our knowledge this is the first report showing the effect of LLLT on both isoforms of IL-1. The effect of LLLT on the reduction of IL-1β levels in GCF or expression of mRNK for IL-1β in the gingival tissue has also been reported by other authors [26, 33].In contrast, Lopes *et al.,* [34] and Qadri *et al.,* [35] did not find any changes of IL-1β levels in GCF, when Er-YAG laser was applied in the treatment of CP together with scaling and root planing. The only report showed a significant decrease of both IL-1α and IL-1β levels in GCF of CP patients after 6-8 weeks following two rounds of scaling and root planing [36].

The mechanisms by which LLLT decrease the levels of IL-1 are not known, but it can be postulated, on the basis of the regulation of IL-1 production at the molecular levels [5], that the suppressive effect of this laser therapy is related to the interference of laser photoenergy with cellular signaling molecules. One of the candidates could be nuclear factor kappa B (NF-kB), a well-known transcription factor for different pro-inflammatory cytokines [3-5]. LLLT could also block posttranslational modification, including caspase-dependent cleavage of IL-1 from its precursor [5], or acceleration of its degradation. Reduction of number of infiltrating phagocytic cells, as suggested by same histological studies [25], as a dominant source of IL-1 β, is also possible. Tobacco smoking has been considered as a causing factor of more aggressive forms of CP [2, 3]. However, this study did not show any significant differences in the inflammatory parameters between smokers and non-smokers (data not-shown).

MMPs are involved in the pathogenesis of periodontal diseases [15, 16, 18-21]. Elevated levels of MMP-9 were highly correlated with clinical loss of attachment and bleeding on probing in periodontitis [37]. Smith *et al.,* [16] detected in situ the expression of MMP-9 in junctional and pocket gingival epithelial cells, polymorphonuclear neutrophils and along connective tissue of periodontitis affected gingival tissue. The expression of pro-MMP-9 was mostly restricted to the pocket epithelium of inflamed gingiva. Macrophages are also a potent source of MMP-9 [11]. Marcaccini *et al*., [18] have been recently described that the levels of circulating MMP-8 and MMP-9 are increased in chronic periodontal disease and decreased three months after the non-surgical treatment (scaling and root planning). Based on the anti-inflammatory properties of LLLT [24, 27, 29, 38, 39] and the significance of MMPs for pathogenesis of periodontal diseases [15, 16, 18-21], it can be expected that LLLT would reduce the level of MMP-9 in GCF. Such a phenomenon was published in a study for MMP-8 [39]. Namely, Qadri *el al*., [39]

examined short-term effects of LLLT, as adjunct therapy in the treatment of CP and showed a slight decrease of MMP-8 level in GCF, whereas the IL-1β level was not significantly changed. However, the present study showed quite opposite results, that the levels of MMP-9 after LLLT were elevated. The differences may be related to different MMPs, different type of laser used, different regime of laser beam application or different timing of enzyme measurement [40].

What could be the explanation for increased levels of MMP-9 after LLLT and what could be the biological role of this metalloproteinase? It is known that after secretion by different cells, predominantly by neutrophils and epithelial cells, MMP-9 binds to extracellular matrix proteins [11, 14]. LLLT could detach this enzyme, resulting in its increased levels in GCF. Alternatively, LLLT could act on the synthesis of pro-MMP-9. The therapy could also induce apoptosis of infiltrating neutrophils, which are one of the main sources of MMPs [11, 14, 16], thus enabling the passive release of MMP-9.

Several findings in this work support the hypothesis that increased levels of MMP-9, triggered by LLLT, could be beneficial. At first, MMP-9 is involved in the acceleration of epithelization and promotion of cell migration by cleaning and remodeling of the extracellular matrix [11, 14, 16].

The increased concentration of MMP-9 is in agreement with its role in regulation of the inflammatory response, partly by the cleavage and inactivation of IL-1 and other pro-inflammatory cytokines [4, 5].

Negative correlations between MMP-9 and clinical parameters of periodontitis after LLLT also support the beneficial effect of MMP-9 in the early period after laser therapy. Although LLLT increased MMP-9 and simultaneously decreased IL-1β levels, no significant correlation was found. Interestingly, a positive correlation between the levels of IL-1α and MMP-9 was observed suggesting that the signaling pathways involved in the regulation of these biomolecules by LLLT are not the same.

This finding could be associated with the postulated role of MMP-9 in controlling the levels of IL-1α in the epithelial compartments, bearing in mind co-localization of MMP-9 and IL-1α in the gingival epithelium [17]

Conclusions

This study shows that LLLT could be a beneficial adjunct to the non-surgical treatment of CP. The benefits can be observed in terms of reduction of standard clinical periodontal parameters and biohumoral markers of periodontal inflammation (IL-1α and IL-1β). An increase in the levels of MMP-9 in GCF, followed by negative correlations with PI and PBI and a positive correlation with IL-1α, could be associated with remodeling of extracellular matrix and stimulation of the regenerative processes. The explanation of this hypothesis could be a future challenge.

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Declaration of Interest

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