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ORIGINAL ARTICLE

Effects of low-level He–Ne laser irradiation on the gene expression of IL-1β, TNF-α, IFN-γ, TGF-β, bFGF, and PDGF in rat's gingiva

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Abstract Biostimulatory effects of laser irradiation on cell proliferation and wound healing has been reported. However, little is known about the molecular basis of the mechanism. Interleukin 1β (IL-1β), tumor necrotic factor-α (TNF-α), and interferon-γ (IFN-γ) play an important role in inflammation, while platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β) and blood-derived fibroblast growth factor (bFGF) are the most important growth factors of periodontal tissues. The aim of this study was to investigate the effect of low-level He–Ne laser on the gene expression of these mediators in rats' gingiva and mucosal tissues. Twenty male Wistar rats were randomly assigned into four groups (A_{24}, A_{48}, B_{24}, B_{48}) in which A_{24} and A_{48} were cases and B_{24}, B_{48} were controls. An incision was made on gingiva and mucosa of the labial surface of the rats' mandibular incisors. Group A_{24} was irradiated twice with 24 hours interval, while the inflamed tissues of group A_{48} was irradiated three times with continuous He–Ne laser (632.8 nm) at a dose of 7.5 J/cm2 for 300 s. An energy of 5.1 J was given to the 68 mm^2 irradiation zone. Rats were killed 30 min after the last irradiation of case and control groups, then excisional biopsy was performed. Gene expression of the cytokines was measured using reverse transcriptase-polymerase chain reaction (RT-PCR) technique. Results were analyzed with Kruskal–Wallis and Mann–Whitney *U* tests. The gene expression of IL-1β and IFN-γ was significantly inhibited in the test groups ($P<0.05$), while the gene expression of PDGF and TGF-β were significantly increased ($P<0.05$). The case and control groups did not have a significant difference in the gene expression of TNF-α and bFGF ($P>0.05$). These findings suggest that low-level He-Ne laser irradiation decreases the amount of inflammation and accelerates the wound healing process by changing the expression of genes responsible for the production of inflammatory cytokines.

Keywords Low-level He–Ne Laser · Cytokine · Growth factor · Gene expression

Introduction

Inflammation of tooth supporting structures accompanied by insufficient repair process usually leads to severe damages of the tissues such as bone loss and subsequent tooth loss [1]. Several cellular and molecular events contribute to the clinical manifestations of these damages [1]. IL-1β, TNF-α, and IFN-γ are key mediators involved in different immune responses and in the acute phase of inflammation process [2, 3]. IL-1β and TNF-α are known

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to stimulate bone resorption [2, 3]. Recently, high levels of IL-1β were reported in the gingiva and crevicular fluid of patients with periodontitis, implicating the role of this potent cytokine in the disease process [2]. PDGF, TGF-β, and bFGF are known to have anti-inflammatory and wound healing effects [3, 4]. Anti-inflammatory effect and stimulation of the wound healing process have been reported as biological effects of low-level laser irradiation [5].

Kana et al. [6] reported the enhancement of wound healing by laser irradiation using an in vivo experimental system. Shimizu et al. [7] showed that laser irradiations caused the inhibition of PGE_2 and IL-1β and plasminogenin activator production in mechanically stressed human periodontal ligament (PDL). Funk et al. [8] reported that cytokine content will change after He–Ne laser irradiation. Reddy et al. [9] reported that He–Ne laser irradiation has better effects on healing impaired diabetic rat wounds than Ga–As laser laser irradiation. Al-Watban et al. [10] compared the effects of laser therapy on wound healing using different wavelengths (He–Ne 632.8 nm, He–Cd 442 nm, Ar 514 nm, 670 nm, 780 nm, 830 nm, and CO_2) and found that the optimum effect belongs to He–Ne laser with 632.8 nm, which is used in this study.

Also, Enwemeka et al. [11] reported the positive effects of various wavelengths of laser light on tissue repair, with 632.8 nm having the highest treatment effect. Al-Watban et al. compared the effects of continuous wave (CW) and pulsed lasers on wound healing and found that the effects of treatment using CW laser was higher than pulse frequency [12]. Neiburger [13] reported optimum tissue healing rates at helium–neon laser exposure ranges between 1 and 20 J/cm^2. However, little is known about the biological mechanism behind the anti-inflammatory effect of laser irradiation. Control of inflammation mediators such as IL-1β, TNF-α, and INF-γ in particular, and also enhancement of growth factors such as PDGF, TGF-β, and bFGF in particular, seems to be a suitable approach to reduce the amount of inflammation and accelerate the healing process. In accordance with all the above, the authors decided to perform a research to evaluate the effect of low-level He–Ne laser on gene expression of different cytokines and growth factors, all together to have a better image of their interactive role influenced by the laser irradiation in the inflammation process. Based on this hypothesis, the response of gene expression of IL-1β, TNF-α, IFN-γ, PDGF, TGF-β, and bFGF to low-level He–Ne laser irradiation were analyzed in rats' gingiva and mucosal tissues.

Materials and methods

Twenty healthy male Wistar rats with the mean weight of 222.25 g (ranging from 200 to 250 g) were randomly assigned into four groups (n=5 for each group; A_{24}, A_{48}, B_{24}, B_{48}). A_{24} and A_{48} were case groups and B_{24} and B_{48} were the controls. The proposal of this study was approved by the ethics committee of Center for Dental research, Shaheed Beheshti University of Medical Sciences, Tehran, Iran. General anesthesia was performed with diethyl ether to sedate the rats. A 7-mm incision with the depth of 2 mm was created on the gingival and mucosal tissues of the labial surface of mandibular incisors in all groups, using a No.15 surgical blade.

The He–Ne laser (25 LHP 925-230, Melles Griot, Carlsbad, CA, USA) was used in this study. Three hours after incision, the rats were sedated again and the samples of the case groups (A_{24} and A_{48}) were exposed to continuous non-contact laser irradiation with the wavelength of 632.8 nm at the dose of 7.5 J/cm^2 for 300 s, with the power output of 17 mW. The beam diameter was 0.96 mm. The wound was irradiated with a constant sweeping motion of the hand piece that followed the contour of the incision. The outer limits of the irradiation zone (13.6 ×5 mm) were determined by a rectangular plastic limiter specially designed for this study.

The incision was in the center of the irradiation zone. The distance between the tip and the gingival tissue was maintained at 1 mm at all times. The rats of control groups (B_{24}, B_{48}) did not receive laser irradiation. Twenty-four hours after primary incision, the rats in both of the case groups were irradiated for the second time with the same procedure. Thirty minutes after completion of the laser therapy, the rats belonging to A_{24} and B_{24} were killed. Forty-eight hours after primary incision, the rats belonging to A_{48} group were irradiated with the same procedure and 30 min after completion of irradiation, the rats of A_{48} and B_{48} groups were killed. The rats in the control groups (B_{24}, B_{48}) were not exposed to laser irradiation. Oval excisional biopsy was taken from the site of incision and frozen samples were used for future lab procedures.

Ribonucleic acid (RNA) extraction Ribonucleic acid (RNA) extraction was conducted using RNX^{plus} buffer, as described by the manufacturer (CinnaGen, Iran). In brief, 100 µl of frozen sample were mixed with 200 µl RNX^{plus} buffer (CinnaGen, Iran), and incubated for 24 h at room temperature. Fifty microliters of chloroform was added and centrifuged at 12,000 rpm for 15 min at 4°C. Total RNA was ethanol-precipitated, and dissolved in 10 µl diethyl pyrocarbonate-treated water (DEPC).

cDNA synthesis Reverse transcription (RT) was performed as previously described [14]. In brief, template RNA (equivalent to 50 µl of sample) was incubated in a 20-µl reaction mixture containing 20 pmol oligo (dT) 100 units reverse transcriptase (RT) (Fermentas, Lithuania), 20 units

Table 1 Mean±SD of band density (Kpixel/cm^2) for different cytokines obtained from different groups

Cytokine/Growth Factor	Group	Mean±SD of band density		P value/Significance
		24 h	48 h	
BFGF	Case	16.05±4.12	20.29±8.03	0.209
	Control	13.49±2.36	2.11±0.48	
IFN-γ	Case	30.63±12.49	30.41±7.07	0.010
	Control	61.98±6.83	70.11±11.18	
IL-1β	Case	6.07±1.58	2.35±0.72	0.010
	Control	35.31±5.54	16.24±2.29	
PDGF	Case	27.37±12.37	37.74±6.08	0.008
	Control	2.73±0.49	7.07±1.74	
TGF-β	Case	51.22±14.57	56.15±14.03	0.017
	Control	11.14±3.43	21.38±4.55	
TNF-α	Case	75.59±17.15	26.68±5.31	0.076
	Control	82.94±16.85	75.36±11.5	

RNasine (Fermentas, Lithuania), 1× RT buffer, and 0.2 mM dNTP, for 1 h at 42°C.

PCR reactions Nested-PCR for the each gene was set up. The first PCR reactions contained: 1 μg of synthesized cDNA, 40 pmol concentration of each forward and reverse gene-specific primers 1.5 mM MgCl2, 0.1 mM dNTP, 1X PCR buffer, 1.5 units of Taq DNA polymerase (CinnaGen, Iran) in 50 μl total volume. The PCR process was carried out with 30 cycles in thermocycler machine (Eppendrof, personal model) under the following conditions: denaturation at 94°C for 30 s, annealing temperature based on gene-specific primer for 60 s, and extension at 72°C for 40 s. Before PCR cycle's initiation, reaction was incubated at 94°C for 5 min, and after PCR cycle's termination, reaction was incubated at 72°C for 5 min [15]. The second PCR reactions were conducted similarly for each gene using the first PCR product as template DNA.

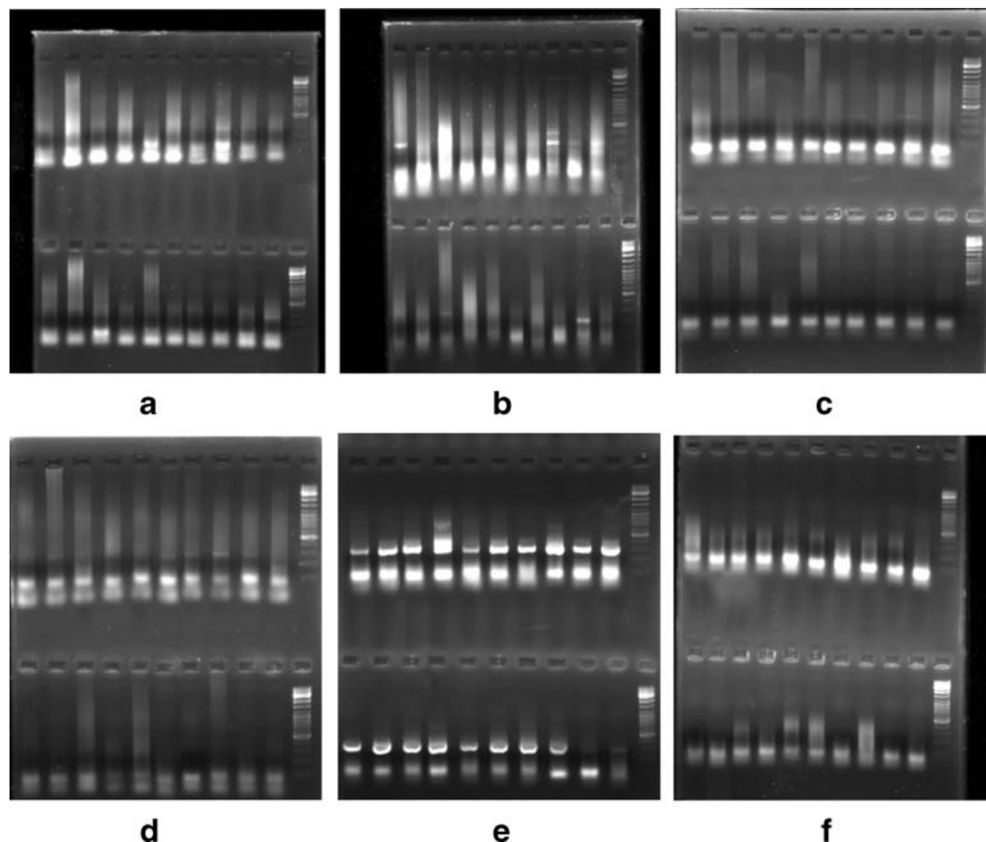

Fig. 1 Final electrophoresis results of RT-PCR analysis for gene expression of different cytokines and growth factors: **a**) PDGF, **b**) TGF-β, **c**) IL-1β, **d**) IFN-γ, **e**) TNF-α, **f**) bFGF. The five upper right bands belong to A_{24} group, five upper left bands belong to A_{48} group, five lower right bands belong to B_{24} group and five lower left bands belong to B_{48} group.

Detection of the PCR product The second PCR products were electrophoresed on 2% agarose gel and stained using ethidium bromide. The DNA band was observed by UV light with an UV Tranilluminator [16].

The mean band density on agarose gel was defined with the aid of Labwork software with the aid of Labwork V.8.0 software compared to PCR product of beta actin gene as house-keeping gene, which was carried out with each PCR reaction.

All the values were calculated as mean±standard deviation (SD) for the density of each band defined by the software for all the evaluated cytokines and growth factors using the SPSS software version 9.0 (SPSS Inc, Chicago, IL, USA). Kruskal–Wallis and Mann-Whitney *U* tests were used for statistical analysis. Statistically significant differences were set at $P<0.05$.

Results

Results are demonstrated in Table 1 and Fig. 1. A significant increase in the gene expression of PDGF and TGF-β was seen in the case groups, compared to the controls ($P=0.008$ and $P=0.017$, respectively, with Mann–Whitney *U* test), and a significant decrease in the gene expression of IL-1β and IFN-γ was observed in the case groups, compared to the controls ($P=0.01$ with Mann–Whitney *U* test) Also, no significant difference was observed in the gene expression of TNF-α and bFGF between the case and control groups ($P=0.076$ and $P=0.209$, respectively). Moreover, no significant difference was detected between the groups killed 24 or 48 h after primary incision in any of the observed variables ($P>0.05$ with Kruskal–Wallis *U* test) (Table 1).

Discussion

The biostimulatory mechanisms of laser irradiation are not fully understood and this fact has been questioned in its wide use in clinics. Therefore, it is important to clarify the mechanisms of the biostimulatory effects of laser irradiation by molecular and cell-biology-based studies to provide new treatment possibilities with laser therapy [2].

In the present study, low-level He–Ne laser irradiation significantly inhibited the gene expression of IL-1β and IFN-γ and also significantly increased the gene expression of PDGF and TGF-β.

IL-1β induces signaling pathways that lead to a transient expression of transcription factors of inflammatory mediators [2]. IL-1β plays an important role in bone resorption [7]. Increased levels of IL-1β is detected in gingival tissue and gingival fluid during periodontal disease [17, 18]. IL-1β and TNF-α activate leukocytes and stimulate the secretion of chemokines. IL-1β and TNF-α also stimulate bone resorption synergistically. IFN-γ plays important role in the inflammatory processes. IFN-γ is an important activator of macrophages [3].

Briefly, IL-1β, TNF-α, and IFN-γ are key mediators in inflammatory processes, and therefore inflammation may be controlled by laser irradiation via decreased production of these mediators. Growth factors such as PDGF exert potent effects on wound healing including the regeneration of periodontium. Moreover, the use of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) mixed with bone allograft results in robust periodontal regeneration in both class II furcations and interproximal infrabony defects [19]. PDGF increases the secretion of other growth factors by stimulating macrophages. TGF-β is an important signal in stopping immune and inflammatory responses and has potent effects on wound healing processes. TGF-β also antagonizes some lymphocytic responses. bFGF stimulates the growth of fibroblasts, which have an important role in healing processes [3]. Briefly, PDGF, TGF-β, and bFGF are among the most important growth factors of periodontal tissues, and therefore healing processes may be promoted by laser irradiation via increased production of these growth factors.

The anti-inflammatory effect of low-level laser irradiation has been reported. Maiya et al. demonstrated that He–Ne laser irradiation, as used in the present study, decreased the inflammation and accelerated wound healing in diabetic rats [20]. Enwemeka et al. [11] demonstrated that He–Ne laser irradiation has the highest treatment effect on tissue repair. Furthermore, low-level laser therapy is widely used clinically for rheumatoid arthritis with satisfactory results [21].

Funk et al. [8] reported that cytokine content will change after He–Ne laser irradiation. Shimizu et al. [7] showed that laser irradiations caused the inhibition of PGE_2 and IL-1β as well as plasminogenin activator production in mechanically stressed human PDL. Nomura et al. [2] demonstrated that expression of IL-1β was inhibited after low-level laser irradiation.

Conclusion

In general, the findings of the present study suggest that the inhibitory effect of low-level laser irradiation on IL-1β and IFN-γ production and the stimulatory effect of the laser on TGF-β and PDGF can be responsible for its anti-inflammatory effects and its positive effect on the wound healing process.

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References

1. Newman MG, Takei HH, Carranza FA (2002) Clinical periodontology, chapter 7. W.B. Saunders Company,
2. Nomura K, Yamaguchi M, Abiko Y (2001) Inhibition of interleukin-1beta production and gene expression in human gingival fibroblasts by low-energy laser irradiation. Lasers Med Sci 16(3):218–223
3. Abbas AK, Lichtman AH, Pober JS (1999) Cellular and molecular immunology, chapter 3. W.B. Saunders
4. Heldin CH, Westermark B (1999) Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 79 (4):1283–1316
5. Walsh LJ (1997) The current status of low level laser therapy in dentistry, Part 1, Soft tissue application. Aust Dent J 42(4):247–254
6. Kana J, Hutschenreiter G, Haina D, Waidelich W (1981) Effect of low-power density irradiation on healing of open skin wounds in rats. Arch Surg 116:294–296
7. Shimizu N, Yamaguchi M, Goseki T, Shibata Y, Takiguchi H, Iwasawa T, Abiko Y (1995) Inhibition of prostaglandin E2 and interleukin 1-beta production by low-power laser irradiation in stretched human periodontal ligament cells. J Dent Res 74 (7):1382–1388
8. Funk JO, Kruse A, Kirchner H (1992) Cytokine production after helium-neon laser irradiation in cultures of human peripheral blood mononuclear cells. J Photochem Photobiol B 16(3–4):347–355
9. Reddy GK (2003) Comparison of the photostimulatory effects of visible He-Ne and infrared Ga-As lasers on healing impaired diabetic rat wounds. Lasers Surg Med 33(5):344–351
10. Al-Watban FA, Zhang XYA (1996) Comparison of the effects of laser therapy on wound healing using different wavelength. J Laser Ther 8:127–135
11. Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Sanford LE, Woodruff LD (2004) The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomed Laser Surg 22(4):323–329
12. Al-Watban FA (2004) The comparison of effects between pulsed and CW lasers on wound healing. J Clin Laser Med Surg 22 (1):15–18
13. Neiburger EJ (1999) Rapid healing of gingival incisions by the helium–neon diode laser. J Mass Dent Soc 48(1):8–13
14. Pfeffer U (1998) One-tube RT-PCR with sequence-specific primers in RNA isolation and characterization protocols, chapter 22. Humana, pp. 143–151
15. Pherson MC, Moller MJ (2000) PCR, the basics from background to bench, Chapter 2. Bios Scientific Publishers, pp 9–21
16. Boffey SA (1984) Agarose gel electrophoresis of DNA. In: Methods in molecular biology. vol. 2, chapter 7. Humana, pp 43–50
17. Stashenko P, Jadinski JJ, Fujiyoshi P (1999) Tissue levels of bone resorptive cytokines in periodontal disease. J Periodontol 199 (62):504–509
18. Charon JA, Luger TA, Mergenhagen SE (1982) Increased thymocyte-activating factor in human gingival fluid during gingival inflammation. Infect Immun 38:1190–1195
19. Nevins M, Camelo M, Nevins ML, Schenk RK, Lynch SE (2003) Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. J Periodontol 74(9):1282–1292
20. Maiya GA, Kumar P, Rao L (2005) Effect of low intensity helium-neon laser irradiation on diabetic wound healing dynamics. Photomed Laser Surg 23(2):187–190
21. Nishida J, Satoh T, Satodate R, Abe M, Oyamada Y (1990) Histological evaluation of the effect of He-Ne laser irradiation on the synovial membrane in rheumatoid arthritis. Jpn J Rheumatoid 2:251–260