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The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation

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Abstract

Objectives: The aim of this split-mouth, double-blind controlled clinical trial was to study the effects of irradiation with low-level lasers as an adjunctive treatment of inflamed gingival tissue.

Materials and Methods: Seventeen patients with moderate periodontitis were included. After clinical examination, all teeth were scaled and root planed (SRP). One week after SRP, we took samples of gingival crevicular fluid (GCF) and subgingival plaque. The laser therapy was started 1 week later and continued once a week for 6 weeks. One side of the upper jaw was treated with active laser and the other with a placebo. The test side was treated with two low-level lasers having wavelengths of 635 and 830 nm. The patients then underwent another clinical examination with sampling of GCF and plaque. The GCF samples were analysed for elastase activity, interleukin-1β (IL-1β) and metalloproteinase-8 (MMP-8). We examined the subgingival plaque for 12 bacteria using DNA probes.

Results: The clinical variables i.e. probing pocket depth, plaque and gingival indices were reduced more on the laser side than on the placebo one ($p < 0.01$). The decrease in GCF volume was also greater on the laser side, 0, 12 μl, than on the placebo side, 0.05 μl ($p = 0.01$). The total amount of MMP-8 increased on the placebo side but was slightly lower on the laser side ($p = 0.052$). Elastase activity, IL-1β concentration and the microbiological analyses showed no significant differences between the laser and placebo sides.

Conclusion: Additional treatment with low-level lasers reduced periodontal gingival inflammation.

Key words: low-level laser; periodontal inflammation; protease activity; therapeutic laser

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Lasers have been used in dentistry since the beginning of the 1980s. In oral surgery, the carbon dioxide laser (CO_2) has become an accepted method for the removal of superficial layers without damaging underlying tissues and for its excellent coagulating effects. More recently the Er:YAG laser was introduced because of its versatile properties e.g., the ablation of hard and soft tissues. Several lasers have been used to sterilize root canals and periodontal pockets. The Nd:YAG laser is useful for debridement of calculus and reduction of endodontic microbes inter alia (Gutknecht et al. 1996). While surgical lasers such as these are routinely used in modern dentistry, low-level lasers (also known as therapeutic lasers) have been utilized less frequently. Low-level lasers do not cut or ablate but are based on photobiological processes (Karu 2003). Unlike the powerful surgical lasers that require >1 W, these lasers function in the milliwatt range with wavelengths usually in the red and near-infrared spectrum and can be used to change intra-cellular photoreceptors, e.g. endogenous porphyrins, flavoproteins and cytochrome c-oxidase in the respiratory chain (Karu 2003). The absorption leads to a cascade of photobiological events, which could have advantageous effects on periodontal healing. For example an increased cell metabolism and collagen synthesis have been shown in fibro-

blasts, and an increased activity of leukocytes and release of growth factors have also been suggested. Cells in a reduced state respond best to laser irradiation (Yamamoto et al. 1996, Karu 2003). Low-level lasers have been used for more than 30 years and no adverse effects have been reported. The US Food and Drug Administration lists these lasers as *non-significant risk Class III medical devices* and several of these have been approved. No noticeable increase in temperature occurs and patients readily accept the therapy.

In this study we used two lasers, Indium–Gallium–Aluminium–Phosphide (InGaAlP, 635 nm) and Gallium–Aluminium–Arsenide (GaAlAs, 820 nm). The InGaAlP laser was chosen because this wavelength seems to have good effects on the mucosa and gingiva (Loevschall & Arneholt-Bindslev 1994) and because of the 10 year's experience of one of the authors (T. Q.) concerning this wavelength for treatment of gingivitis and periodontitis. The GaAlAs laser was added to improve the penetration of light into the periodontal and bony areas (Saito & Shimizu 1997).

The positive effects of therapeutic lasers in dentistry have been reported for such diverse conditions as mucositis (Bensadoun et al. 1999), paresthesia (Khullar et al. 1996), HSV-1 (Schindl & Neumann 1999), temporomandibular disorders (Kulekcioglu et al. 2003), dentine hypersensitivity (Kimura et al. 2000) and osseointegration (Dörtubak et al. 2002). In vitro studies have primarily concentrated on the fibroblast. Several authors report stimulation of gingival fibroblast proliferation after the use of low-level laser (Yu et al. 1996, Almeida-Lopes et al. 2001) and have shown that the stimulated fibroblasts are better organized, in parallel bundles (Almeida-Lopes et al. 2001).

No study has been done on the value of low-level laser irradiation as an adjunct to conventional scaling and root planing (SRP). We therefore investigate the clinical use of a combination of two therapeutic lasers on gingival inflammation.

Material and Methods

Participants and study design

Seventeen patients (10 women), mean age 53 (35–70) years, with moderate chronic periodontitis were selected for this study. To be included the patients had to be 35 years of age or older, have no ongoing general disease and be on no medication. Those who had taken an antibiotic during the last 4 weeks, had teeth with a mobility rate of II, III or pockets deeper than 7 mm in the areas studied were excluded. As it turned out, none of the participants had taken any antibiotics during the last 6 month. Patients with an acute condition in the mouth or partial dentures in the upper jaw were also excluded. Five patients were smokers. Some of the participants had had periodontal treatment earlier but none had received laser treatment before.

Initially, all participants received basic periodontal treatment including scaling, root planing and oral hygiene instructions. Baseline measurements of the probing pocket depth (Perio Wise, Premier, Canada), gingival index (GI, Silness & Löe 1967) and plaque index (PI, Löe 1964) were recorded before the SRP. Gingival cervicular fluid (GCF) samples, for analyses of elastase, IL-1β and metalloproteinase-8 (MMP-8), and subgingival plaque samples were taken 1 week after SRP. One of the authors (T. Q.) did both baseline and follow-up examinations as well as the SRP on all patients. After another week a laser therapist started the low-level laser therapy.

The test or control areas comprised teeth 13, 14, 15, 16, 17 and 23, 24, 25, 26, 27. One side was treated with the active laser and the other with the placebo laser once a week for 6 weeks. One week after the last laser irradiation, the clinical examination and GCF/plaque sampling were done in the same way as at baseline. The laser therapist randomly allocated the quadrants for active laser or placebo. The clinical examiner did not know which side had been treated with active laser until the completion of the study. This study was approved by the Ethics Committee of Huddinge Hospital, Sweden.

Laser treatment

We employed a handheld battery-operated Combilaser (Lasotronic AG, Baar, Switzerland), which has two wavelengths that can be used together or separately. In this study the wavelengths were utilized separately. Two identical units were used. In the placebo unit the laser diode was replaced by a very low-powered red LED diode. The laser wavelengths were 635 (visible) and 830 (invisible) nm and the outputs, controlled daily with an analogue power metre (Lasotronic AG, Baar, Switzerland), 10 and 70 mW. Since all battery-powered tools lose power as the batteries deteriorate, the batteries were changed after each day of use. We treated (1) the buccal papillae with 635 nm laser for 90 s (0.9 J) and (2) 6 mm more apically with 830 nm for 25 s (1.75 J), from the buccal and lingual sides.

The energy densities were 4.5 and 8.75 J/cm^2 and the power densities 50 and 350 mW/cm^2. The treatment was given during slight contact with the tissue.

Samples

In all patients, two GCF samples were taken from each side of the upper jaw after removal of supragingival plaque from the sites to be sampled. These had been isolated with cotton rolls and gently dried with an air syringe before sampling. GCF was collected with prefabricated paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA), which were inserted into the pockets until resistance was felt and kept there for 30 s.

Blood-contaminated samples were discarded. We measured GCF volume with a calibrated Periotron™ 8000 meter (Oraflow Inc.). The two samples from each side of the upper jaw were pooled together and diluted in phosphate buffer saline (PBS) up to 1 ml. After elution for 15 min., the strips were removed and the samples frozen at -20°C pending analysis. Subgingival plaque was sampled from the same sites with sterile paper points (size30), which were inserted for 30 s. The paper points from each side were then pooled together in sterile transport vials and sent to a laboratory for bacterial DNA-probe analysis.

Laboratory analyses

IL1-β was measured as described elsewhere (Figueredo et al. 1999). Briefly, a monoclonal antibody to IL1-β (MAB 601, R&D Systems, Minneapolis, MN, USA), diluted 125 times in carbonate buffer, was coated onto microtitre plates (Nunc Maxisorb, Nanc a/s, Roskilde, Denmark) overnight at $+4^{\circ}$C. These were washed once, with PBS+0.05% polyoxyethylenesorbitan monolaurate (Tween® 20, Sigma Chemical, St. Louis, MO, USA), and blocked with 1% HSA for 1 h at room temperature. After four washings, a standard curve (2 –200 pg/ml) and undiluted samples (100 μl) were added to the plates. They were incu-

bated at +37°C while shaking for 45 min. and then washed four times. The detection antibody (BAF 201, R&D Systems), a biotinylated polyclonal goat antibody diluted 250 times, was incubated as described above. After washing, the horseradish peroxidase conjugated streptavidin, diluted 200 times in PBS+0.1% HSA, was added to the plates and incubated in the same way as the detection antibody. The plates were washed again and the undiluted substrate (TMB, Sigma Chemical) added. The reaction was stopped with 1 M H_2SO_4 after 15 min. and the absorbency read at 450 nm in a spectrophotometer (Millenia Kinetic Analyser, Diagnostic Product Corporation, Los Angeles, CA, USA).

The total elastase activity was measured with a chromogenic substrate specific for granulocyte elastase. One hundred microlitres of undiluted sample was mixed with 65 μl of substrate S-2484 (L-pyroglutamyl-L-propyl-L-valine-*p*-nitraniline, mw 445.5 Da, Heamochrome Diagnostica, Mölndal, Sweden) on a 96-well microtitre plate (Nunc Maxisorb, Nunc a/s,). The mixture was shaken for 5 min. and the absorbency at 405 nm was read in a spectrophotometer. After 2 h of incubation at 37°C, the absorbency was read for the second time. The total elastase activity is expressed in mAbs (milliabsorbances).

MMP-8 was analysed with a commercial kit (Quantikine®, R&D Systems Inc.) in accordance with the manufacturer's instructions. Briefly, a monoclonal antibody specific for MMP-8 had been pre-coated onto a microplate. Samples diluted 10 times and a standard curve were pipetted into the wells and incubated at room temperature for 2 h. The plates were then washed and a monoclonal antibody against MMP-8 conjugated to horseradish peroxidase was added and incubated again as before. After another washing procedure, the substrate solution was added and the reaction stopped after 15 min. with a stop solution. The absorbency at 450 nm was read within 20 min. in a spectrophotometer.

The subgingival microbiota was analysed using a checkerboard DNA–DNA hybridization method. The 12 microorganisms tested with the DNA probe in the subgingival samples were: *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythensis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Treponema denticola, Peptostreptococcus micros, Campylobacter rectus, Eikeinella corrodens, Selenomonas noxia* and *Streptococcus intermedius*. We used standard procedures for the checkerboard DNA–DNA hybridization method (Papapanou et al. 1997) and recorded the frequencies of positive sites and of sites with $\geqslant 10^6$ of these bacteria.

Statistical analysis

The unit of analysis was the subject. The significance of the differences in treatment effect between placebo and laser was calculated with the Student paired *t*-test or the Wilcoxon-signed rank test. The frequencies of positive subjects and of subjects with $\geqslant 10^6$ of the analysed bacteria were calculated with Fisher's exact test.

Results

Baseline probing depth was 4.7 (0.7) mm on the laser side and 4.7 (0.6) mm on the placebo side. After treatment the probing depth was 3.8 (0.6) mm on the laser side and 4.5 (0.6) mm on the placebo side. The probing depth reduction was significantly larger on the laser side (Table 2). Baseline and follow-up values of gingival and plaque are shown in Table 1. Both gingival and plaque index were reduced more on the laser-treated side ($P<0.001$).

The changes in the laboratory variables after laser or placebo treatments are shown in Table 3. After treatment, the GCF volume was reduced by 0.14 μl on the side given additional treatment with laser, while the volume was reduced by 0.04 μl on the placebo-treated side.

We found a tendency to a reduction in MMP-8 on the laser-treated side ($p = 0.052$). On the laser side, the mean amount of MMP-8 fell by 100 pg, but increased by 274 pg on the placebo side. No significant differences were observed in elastase activity and the amount of IL-1β (Table 3).

As regards the subgingival microbiota, no differences were detected between laser and placebo sides in the frequencies of positive subjects or of subjects with $\geqslant 10^6$ of the 12 bacteria analysed (Table 4).

Discussion

In this study we showed that additional treatment with low-level laser reduced the gingival inflammation after non-surgical treatment. Both gingival index and probing pocket depth declined more on the side given such treatment. Another marker of inflammation, the GCF volume (Oliver et al. 1969), also fell more on the laser side. One explanation may be that laser irradiation reduces prostaglandin PGE_2 (Sakurai et al. 2000). The stimulation of cellular ATP (Karu 2003) could be another contributory factor.

The decrease in plaque index was also greater on the laser side, which agrees with an earlier animal study

Table 1. Gingival and plaque index at baseline and after scaling, root planing and adjuctive treatment with active or placebo laser

	Gingival index (median (range), mean (SD))		Plaque index (median (range), mean (SD))	
	baseline	follow-up	baseline	follow-up
Placebo ($n = 17$)	2 (1–3), 2.2 (0.5)	2 (0–3), 1.7 (0.7)	1 (0–3), 1.4 (0.6)	1 (0–2), 1.1 (0.7)
Laser ($n = 17$)	2 (1–3), 2.3 (0.6)	1 (0–2), 0.9 (0.8)	2 (0–2), 1.6 (0.6)	1 (0–2), 1.0 (0.6)

Table 2. Mean values (SD) of probing pocket depth and GCF volume before and after treatment with active laser or placebo

	Probing pocket depth (mm)			GCF volume (μl)		
	baseline	follow-up	change	baseline	follow-up	change
Placebo ($n = 17$)	4.7 (0.6)	4.5 (0.6)	0.1 (0.3)	0.41 (0.15)	0.41 (0.15)	−0.05 (0.13)
Laser ($n = 17$)	4.7 (0.7)	3.8 (0.6)	0.9 (0.4)	0.44 (0.15)	0.29 (0.13)	−0.12 (0.11)
p^*	0.84	<0.001	<0.001	0.56	0.41	0.02

*p values calculated with Student's paired *t*-test. GCF, gingival crevicular fluid.

Table 3. Mean values (SD) of elastase activity, total amounts of IL-1β and MMP-8 between samples taken before and after treatment with active laser or placebo

	Elastase activity (mAbs)			IL-1β (pg)			MMP-8 (pg)		
	baseline	follow-up	change	baseline	follow-up	change	baseline	follow-up	change
Placebo ($n = 17$)	45 (3–324)	34 (2–611)	9 (− 576 to 252)	20.7 (5.1–49.7)	17.2 (1.3–71.3)	1.7 (57.9 to 24.7)	415 (0–1040)	465 (210–2940)	90 (2180 to 585)
Laser ($n = 17$)	17 (3–337)	32 (2–269)	32 (23 to 160)	21.0 (5.6–123.3)	21.0 (6.1–65.4)	0.8 (24.4 to 82.8)	500 (160–1600)	425 (0–1015)	70 (510 to 1145)
p*	0.80	1.0	0.15	0.80	0.80	0.45	0.15	0.15	0.052

*p-values calculated with Wilcoxon's signed-rank test. MMP-8, metalloproteinase-8; mAbs, milliabsorbances.

Table 4. Percentage of positive samples (A) and of samples with $\geqslant 10^6$ bacteria (B) of indicated species, before and after treatment with laser or placebo. $N = 17$ subjects.

	Laser				Placebo			
	before		after		before		after	
	A	B	A	B	A	B	A	B
P. gingivalis	17.6	0	11.8	0	17.6	0	11.8	0
P. intermedia	29.4	11.8	29.4	5.9	29.4	5.9	35.3	0
P. nigrescens	41.2	5.9	35.3	0	35.3	5.9	35.3	0
T. forsythensis	47.0	0	41.2	0	41.2	0	35.3	0
A. actinomycetemcomitans	11.8	0	5.9	0	11.8	0	5.9	0
F, nucleatum	17.6	0	23.5	0	29.4	0	41.2	0
T. denticola	52.9	0	64.7	0	64.7	0	35.3	0
P. micros	64.7	0	64.7	0	82.4	0	76.5	0
C. rectus	17.6	0	5.9	0	11.8	0	0	0
E. corrodens	23.5	0	23.5	0	23.5	0	17.6	0
S. noxia	5.9	0	5.9	0	11.8	0	11.8	0
S. intermedius	64.7	0	64.7	0	70.6	0	76.4	0

There were no significant differences between the laser and placebo sides.

(Iwase et al. 1989). It is uncertain whether this is because of a reduction in the degree of inflammation or the laser irradiation per se. However, the microbial analyses showed no differences between the laser and placebo sides in prevalence of subjects with positive findings or of those with $\geqslant 10^6$ of each bacteria. A previous in vitro study of the effect of laser irradiation on microorganisms has found that the growth of *Streptococcus mutans* is stimulated by laser (Kim et al. 1992). However, in another clinical and histological study by the same authors (Kim & Lee 1987) the number of motiles and spirochetes declined while that of the non-motiles increased. This finding was not confirmed by our study. Some authors have reported that a combination of low-level laser light with various dyes, such as toluidine blue O (TBO), significantly reduces the number of subgingival microorganisms. In such cases the laser activates the bactericidal effects of the dye and does not act directly on the microorganisms (Wilson et al. 1995).

We found that additional irradiation with low-level laser was better than scaling and root planing alone. Its effect was greatest on the gingival index and probing pocket depth. The beneficial effect on gingival inflammation was also shown by the marked decrease in the volume of GCF. In a study by Yilmaz et al. (2002), laser alone did not affect the inflammatory response more than instructions about oral hygiene. Mechanical subgingival debridement was necessary. However, the outcome in the group receiving subgingival debridement and laser was only slightly better than in the group given subgingival debridement alone.

Our analyses of GCF showed a slight decrease in the amounts of MMP-8 on the laser side and an increase on the placebo side. MMP-8 is stored in the secretory granula of neutrophilic granulocytes and released from the cells to the inflammatory lesion during migration (Bentwood & Henson 1980). It can therefore be regarded as a surrogate marker of the number of neutrophils in the area and as a marker of the severity of inflammation. In vitro irradiation of peripheral neutrophils affects neutrophil functions such as the generation of reactive oxygen species and phagocytosis (Luza & Hubacek 1996, Fujimaki et al. 2003).

In the present study, no effect was found on neutrophil phagocytosis, measured as elastase release, i.e. degranulation of primary granula.

Some data suggest that laser irradiation affects the production of cytokines (Shimizu et al. 1995), but our study did not confirm the occurrence of inhibition of IL1-β, which has been reported by others (Shimizu et al. 1995). This may be because the previously cited studies were done in vitro and the actual energy density at the target was therefore considerably higher.

It is not always possible to select the optimal laser and treatment parameters for laser therapy because of the lack of adequate studies. The parameters used in this study seem to have been within the ''therapeutic window'' of dosage but not necessarily optimal. Many studies have failed to find this window,

especially in studies performed in the 1980s and early 1990s (Tuner & Hode 1998). Many authors used doses in the range of 0.001–0.01 J/cm^2 (Masse et al. 1993) although it had been suggested by Mester et al. as early as 1971 that doses of about 1–2 J/cm^2 are necessary to heal wounds.

Some of the effects of laser therapy may be because of an increase in the microcirculation in the irradiated area (Schaffer et al. 2000). In the study of gingival microcirculation using healthy volunteers with experimental gingivitis, no effects were seen (Rydén et al. 1994), but other authors have shown that low-level laser affected the microcirculation in mildly inflamed gingiva, but not in uninflamed or severely inflamed gingiva (Kozlov et al. 1995). On the other hand, when the microcirculation in the masseter muscle was studied (Tullberg et al. 2003), no increase in microcirculation occurred in tender areas, but a significant increase was noted in similar locations in healthy volunteers.

A suggested aspect of laser therapy is the so-called systemic effect, which implies that if a pathological condition on one side of the body is irradiated, a small but noticeable effect would be obtained on a similar condition on the other side of the body (Rochkind et al. 1989). The design of our present study does not allow us to investigate this effect.

In conclusion, the additional treatment with therapeutic laser reduced the periodontal inflammation, as assessed by the gingival index, probing pocket depth, GCF volume and MMP-8 levels.

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