SWED DENT J 18: 35-41 (1994)

Effect of low level energy laser irradiation on gingival inflammation

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Key words: Low level energy laser, stereophotographic technique, plaque formation, gingival inflammation.

ABSTRACT

The effect of low level energy infra-red laser irradiation on gingival inflammation was studied. Gingivitis was induced in ten female dental students by refraining from all oral hygiene measures for 28 days. On days 21 and 24 the marginal gingiva, buccal to one of the lateral mandibular incisors, was exposed to 4 minutes of laser irradiation (total dose = 1J). Serving as a control site the gingiva of the contralateral incisors was exposed to ordinary light. There was no statistical difference between the laser exposed sites and the control sites related to either plaque formation or gingival bleeding. The gingivitis reaction was evaluated with the aid of a stereophotographic method by calculating changes in the number of gingival vessels. It was found that the number of vessels identified increased over time for both laser exposed and control sites. The difference between sites at day 28 was not statistically significant ($t = 0.82$, $P > 0.05$). These results suggest that low energy laser irradiation (LLLT) does not influence the inflammatory reaction of the gingiva.

SAMMANFATTNING

Effekten av bestrålning med infraröd lågenergilaser på gingival inflammation studerades. Gingivit åstadkoms på tio kvinnliga tandhygieniststuderande genom att de avstod från all munhygien i 28 dagar. Den buccala marginala gingivan på en av underkäkstvåorna belystes dag 21 och dag 24 med laserljus under 4 minuter (total dos = 1J). Ett kontrollområde belystes kontralateralt med vanligt ljus. Ingen statistisk skillnad förelåg mellan den laserbelysta sidan och kontrollsidan beträffande plackbildning eller blödning. Gingivitreaktionen utvärderades med en stereofotografisk metod för beräkning av antalet kärlförändringar i gingivan. Antalet identifierade kärl ökade med tiden för såväl den laserbelysta sidan som kontrollsidan. Skillnaden var dag 28 ej statistiskt signifikant ($t = 0.82$, $P > 0.05$). Resultaten ger inget stöd för att belysning med lågenergilaser (LLLT) skulle påverka den inflammatoriska reaktionen i gingivan.

INTRODUCTION

Low level energy laser treatment (LLLT), in the power range of 1 to 50 mW, were introduced into the field of medicine about two decades ago. Treatment with this type of laser, "Biostimulation", has increased significantly during the last ten years. Low energy lasers within the visible and invisible wavelengths as well as continuous and pulsed lasers have been used.

It has been claimed that invisible infra-red, e.g. gallium-arsenide laser light, penetrates biological tissues to a depth of a few millimetres while visible "soft" laser, e.g. helium-neon laser, has only a superficial effect (*Korytny* 1978, *Kana et al* 1981, *Lutsyk et al* 1981). It has also been proposed that both types of these low energy lasers stimulate wound healing (*Mester* 1985, *Dyson & Young* 1986). However, *Anneroth et al.* (1988) found that low energy infra-red laser irradiation did not improve the wound healing process.

Low energy lasers have been used in the treatment of a great number of different pathological conditions in the oral soft tissue. Positive results have been reported in the treatment of e.g. recurrent aphthous stomatitis (*Korytny* 1978, *von Alften* 1987), acute herpetic stomatitis, and exudative erythema multiforme (*Lutsyk et al* 1981). *Chomette et al* (1987) studied the effect of low energy laser irradiation on gingival tissue and found a powerful increase of the enzymatic activity. It has also been claimed that laser irradiation would rapidly improve the healing of gingival inflammation (*Kert & Rose* 1989). However, there is a lack of controlled experiments to justify such a conclusion.

The aim of this randomized double-blind study was to investigate the effect of low level energy laser irradiation on gingival inflammation using a stereophotographic technique for the evaluation of the vascular reaction.

MATERIAL AND METHOD

Ten healthy female dental students, ages 25–42 yr (mean 35.4 yr), volunteered to take part in this investigation. Gingivitis was induced by the subjects refraining from all oral hygiene measures for a period of 28 days. The experimental gingivitis induction area was limited to the mandibular anterior region from 33 to 43. Seven days before the experiment, the teeth in the region to be studied were professionally cleaned in order to obtain a clinically healthy gingival status. At day 28, the teeth were again cleaned and habitual dental hygiene routines were reinstituted. Gingiva and teeth were examined and photographed immediately prior to the start of the experiment (day 0), at days 21 and 28 during the experiment, and finally, at day 42, i.e. 14 days after the termination of the gingivitis induction experiment. The marginal gingiva was exposed to laser irradiation for 4 minutes at two occasions with a 3 day interval. This occurred on days 21 and 24.

The frequency was set at 500 Hz, giving the laser an average output power of 1 mW. The irradiation was focused on an area of 0.5 cm^2 at the marginal gingiva labial to the lateral incisors. The dose was 0.5 J/cm^2 on each occasion, i.e. a total dose of $1J/cm^2$. The probe was held in direct contact with the gingiva during the irradiation. The contralateral incisor area was sham exposed to ordinary light and served as control site. The selection of experimental and control sites was made randomly, while the exposure to laser or ordinary light was made blind. The study was approved by the Ethical Committee of Karolinska Institutet.

LASER EQUIPMENT

The following laser equipment was used:
IR CEB-UP MID Laser (Space Laser, Italy®) Infra-red pulsed gallium-arsenide (GaAs) laser
Average output power: 0.9–8.0 mW
Wave length: 904 nm
Pulse frequency: 500–4000 Hz
Pulse duration: 180 nsec.
Beam-diameter: 8 mm
Visible locating beam: ordinary light
Additional equipment: fibre optic and timing device

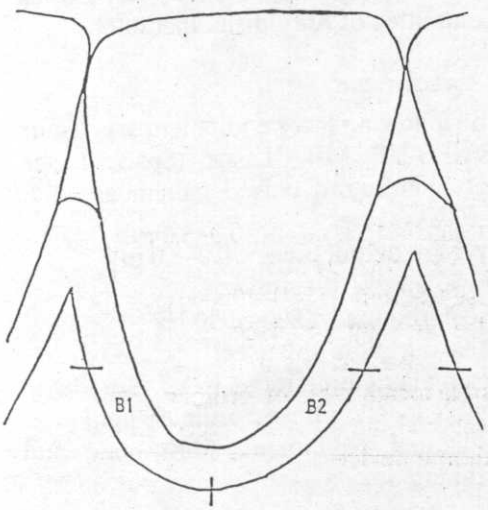
Two probes: A-probe (laser)
B-probe (photodiode, ordinary light)

The two probes were supplied by the manufacturer. Before experiment started the average output power of the laser probe was tested and stated.

STEREOPHOTOGRAPHIC EVALUATION OF VASCULAR REACTION

The dento-gingival region selected for this study was the marginal gingiva of the mandibular lateral incisors. Documentation was made by means of a camera system consisting of a stereomicroscope and two camera housings designed for synchronous exposure (*Bergström & Jonason* 1974). Utilizing this technique, stereopairs of photographs can be obtained for evaluation under a stereoscope. The negative scale was 1:0.22, and the stereopairs of photographs were evaluated under 10 x magnification. Within the marginal unit, the intensity of the inflammatory reaction was quantified from the number of visible vessel endings. Details of this method and its execution have been presented ealier (*Bergström* 1992).

Fig. 1. Labial aspect of marginal gingiva of one mandibular incisor. Counting of vessels was done within two basal zones (B_1, B_2).



The labial aspect of the marginal gingiva of one mandibular incisor constituted the area to be investigated. The inter-papillary marginal gingiva was subdivided into two basal zones (B_1, B_2) within which the counting of vessels was made (Fig 1). Every visible vessel within the area was recognized as a distinct vessel ending/vascular structure contrasting to the surrounding tissue. In gingiva exhibiting no or minimal inflammation, the vessel endings were identified as "points". A change in the structural forms of the vessels and/or an increased number of vessels were observed with ongoing inflammation. These vessel endings became dilated, more superficially located and they formed loops. The vessels were evaluated with regard to form and structure.

The width of this zone was 0.55 mm and the length 4 mm. Thus, the total examination area in the film plane was 2 mm^2, the depth dimension was not taken into consideration. The counting of vessels in the subject was always related to the same area throughout the observation period. This evaluation was performed blind with regard to the type of irradiation.

PLAQUE REGISTRATION

Registration of accumulated plaque was made from photographs at a magnification of six times, after staining with a disclosing solution (Diaplaque® Wallco AB, Kista, Sweden). The images of the teeth with dyed plaque were traced onto a transparant paper divided into square millimetres. The amount of plaque covering the labial surfaces of the mandibular incisors was calculated and expressed as a percentage of the total area of the labial surfaces. Intraindividual differences between laser and control sites were calculated and analysed. Repeated calculations showed a high degree of repro-

ducibility (90% within ±5%). The analysis with regard to laser or ordinary light was made blind.

GINGIVAL BLEEDING

Bleeding on probing was assessed with an electronic pressure sensitive probe (model 200, Vine Valley Research, Middlesex, New York, USA). The probing was made intracrevicularly with a pressure of 60 g and a probe tip diameter of 0.5 mm. Any bleeding within 30 seconds was considered as positive. Observations were made mesio-buccally, mid-buccally and disto-buccally to each of the 4 mandibular front teeth. Thus, a total of 12 sites was observed. The reproducibility of the method has been analysed previously (*Preber & Bergström* 1985).

STATISTICS

The two-tailed significance analysis of differences between means was employed using Student's paired t-test. The subject constituted the unit in the analysis. Significance was accepted at the P-level <0.05.

RESULTS

PLAQUE FORMATION

The amount of plaque increased over time for both laser irradiated and control sites during the experiment. The coverage was on average 40% and 36%, respectively, by day 28. At day 42, i.e. 14 days after termination, plaque had practically disappeared and reached pre-experimental levels. There was no statistical difference between laser exposed and control sites at day 21 ($t = 0.2$, $p > 0.05$), nor at day 28 ($t = 1.3$, $p > 0.05$). Table 1 and Figure 2.

Table 1. Plaque accumulation (%) for laser irradiated and control sites during the experiment (Mean and SEM).

	Plaque accumulation (%)			
	Laser irradiated site		Control site	
Day	Mean	SEM	Mean	SEM
0	1.5	0.50	1.7	0.57
21	31.8	7.10	31.6	6.70
28	40.1	5.30	35.4	4.06
42	2.4	0.79	2.7	1.04

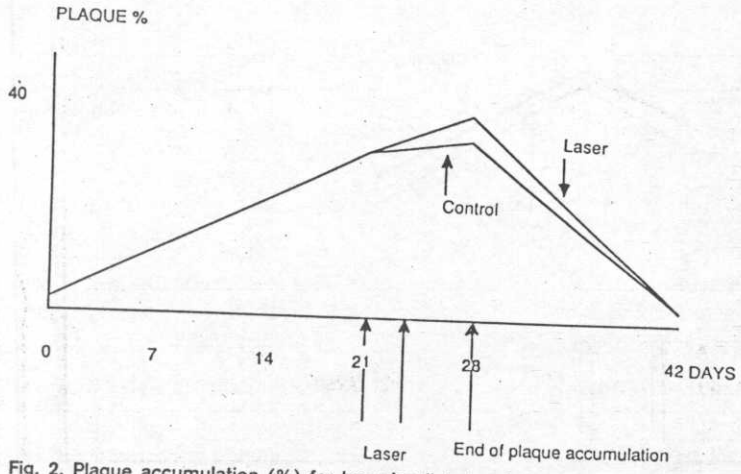


Fig. 2. Plaque accumulation (%) for laser irradiated and control sites during 28 days of experimentally induced gingivitis and 14 days after termination of experiment.

GINGIVAL BLEEDING

A gradual increase in the number of bleeding sites for both laser irradiated and control sites was observed during the course of the experiment, attaining the respective means of 2.5 and ±0.3 (mean ±SEM) bleeding sites, and 2.3 ±0.3 bleeding sites at day 28. There was no statistical difference between laser exposed and control sites at day 21 ($t = 1.33$, $p > 0.05$) nor at day 28 ($t = 0.57$, $p > 0.05$).

VASCULAR REACTION

The inflammatory reaction as measured by the number of visible vessels per unit area over time, in laser irradiated and control sites is presented in Figure 3 and Table 2. The means ±SEM number of vessels at the start of experiment was 23.9 ±5.70 and 26.0 ±3.53 in laser irradiated and control sites, respectively. This difference was not statistically significant ($t = 0.58$, $p > 0.05$). After 21 days of plaque accumulation, the laser irradiated and control sites, respective means ±SEM, were 36.3 ±4.77 and 40.9 ±3.95. The difference between these laser and control sites was not statistically significant ($t = 0.76$, $p > 0.05$). After exposure to laser irradiation, the mean ±SEM at day 28 was 46.6 ±7.86 and 51.6 ±6.22 for laser irradiated and control sites, respectively. The difference was not statistically significant ($t = 0.82$, $p > 0.05$). At day 42, 14 days after plaque elimination and reinstitution of oral hygiene routines, the mean ±SEM number of vessels was 25.9 ±5.04 and 31.4 ±5.60 for laser irradiated and control sites, respectively. The difference was not statistically significant ($t = 0.81$,

Table 2. Number of visible vessels identified during the course of the investigation in laser irradiated and control sites, (Mean and SEM).

	Number of vessels identified			
	Laser irradiated site		Control site	
Day	Mean	SEM	Mean	SEM
0	23.9	5.70	26.0	3.53
21	36.3	4.77	40.9	3.95
28	46.6	7.86	51.6	6.22
42	25.9	5.04	31.4	5.60

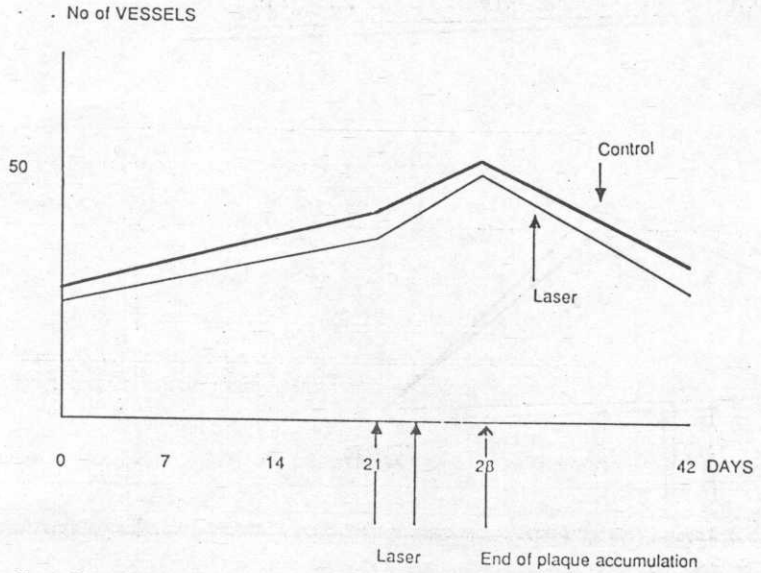


Fig. 3. Vascular changes during 28 days of experimentally induced gingivitis and 14 days after termination of experiment. Number of visible vessels in laser irradiated and control sites.

p>0.05). The number of vessels at day 42 in both laser irradiated and control sites showed no statistically significant differences as compared to the pre-experimental condition. However, the increase relative to baseline observed at day 21 and day 28 was statistically significant in both laser irradiation (t = 2.48 and 3.79, respectively, p >0.05) and control sites (t = 3.94 and 4.11, respectively, p >0.01).

DISCUSSION

Low level energy lasers have been ascribed a wide range of biological effects. However, no universally accepted theory has explained the mechanism of "laser biostimulation". In published studies the common scientific criterium are usually not fulfilled (*Basford* 1986, 1989). This is true for both basic as well as clinical studies.

It has been suggested that the effect of low energy laser irradiation depends on the fact that laser-light is absorbed by the tissues and the tissue fluid. The light energy may be absorbed where the concentration of fluid is highest, and thus, more easily absorbed by inflamed and oedematous tissue (*Dyson & Young* 1986). Therefore, using low level energy lasers for the improvement of the healing process in inflamed gingival tissue has been one of the most promoted measures in dentistry today. However, this benefit has not been supported by current findings. Neither bleeding index nor the vascular reaction was affected, as could be substantiated in this blind trail. The recommended optimal laser dose has previously been proposed to range from 0.5–1J/cm^2 (*Kert & Rose* 1989); the dose used in this study was 1 J/cm^2.

The photographic documentation of the gingival area was made using a stereophotographic evaluation, faciliating the possible visualization of the subepithelial vessels at the gingival margin. This method offers a greater capacity to identify vessel structure than two-dimensional viewing (*Bergström & Jonason* 1974, *Bergström et al* 1988, *Bergström* 1992). The gradual vascular reaction, i.e. rising vessel density is in response to the increased plaque accumulation. In gingiva exhibiting no or minimal inflammation, the vessels were identified as "points". With ongoing inflammation, and/or an increased number of vessels, a change in the structural forms of the vessels were observed. The vessel endings became dilatated, more superficially located, and formed loops. In the presence of mild inflammation, the loops were incomplete whereas with a longer duration of inflammation the loops became elongated and doubled. It is realized that with this method no information is obtained regarding the vessel density per unit area. However, the area observed within the subject remains the same during the whole observation period. The degree gingival inflammation was measured as the number of vessels identified increased over time in both the laser and in the control group.

The role of dental plaque in the development of gingivitis is well documented and has been for many years an accepted theory. If low level energy laser irradiation would have an inhibitory effect on dental plaque deposition as reported by *Iwase et al* (1989), it should also decrease the gingival inflammation. However, in the present study, not only the plaque accumulation but also the gradual increased gingival bleeding during the experimental period for both laser exposured and control sites was equivalent.

This study could not confirm that low level energy laser irradiation would affect either the plaque formation process or the gingival inflammation.

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