

Systemic Effects of Low-Intensity Laser Irradiation on Skin Microcirculation in Patients with Diabetic Microangiopathy

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Low-intensity laser irradiation has been shown to induce wound healing in conditions of reduced microcirculation, which is in part explained by systemic effects. We therefore investigated such a potential systemic effect of low-intensity laser irradiation on skin blood circulation in patients with diabetic microangiopathy. Patients with diabetic microangiopathy were randomized to receive either a single helium–neon (HeNe, 632.8 nm) low-intensity laser irradiation with a dose of 30 J/cm^2 or a sham irradiation over the forefoot region in a double-blind, placebo-controlled clinical study. Skin blood circulation by means of temperature recordings over forefoot regions was detected by infrared thermography. Following a single transcutaneous low-intensity laser irradiation, a rise in skin temperature in both feet of the subjects in the laser group was noted, whereas in both feet of the subjects in the placebo group a drop in skin temperature occurred. The baseline-adjusted skin temperature 15 min after the end of the irradiation was significantly higher in the laser-treated forefeet compared to the placebo-“treated” forefeet ($p < 0.0001$); the baseline-adjusted difference in the temperature was $1.94 \pm 0.35^\circ\text{C}$. Simultaneously, the baseline-adjusted skin temperature was significantly higher in the laser-untreated forefeet com-

pared to the placebo-“untreated” forefeet ($P < 0.0001$); the baseline-adjusted difference was $1.70 \pm 0.33^\circ\text{C}$. Our data show a significant increase in skin circulation due to athermic laser irradiation in patients with diabetic microangiopathy and point to the possibility of inducing systemic effects. © 2002 Elsevier Science (USA)

Key Words: capillaries; diabetes; laser; skin; systemic effects.

INTRODUCTION

Skin microangiopathy is a common complication in diabetic patients (Jorneskog *et al.*, 1995a,b). Recent research provides evidence that endothelial and smooth muscle cell dysfunction contribute to impaired microcirculation in patients with diabetes mellitus, the major functional abnormality being the marked limitation of microvascular vasodilatation to varied stimuli (Morris *et al.*, 1995; Jaap *et al.*, 1995). In association with neuropathy, disturbed microcirculation is responsible for the development of diabetic gangrene, ulcers, and infections of both skin and bone in long-term diabetic patients. The risk of diabetic microangi-

TABLE 1
Demographic and Clinical Baseline Characteristics of Patients with Diabetic Microangiopathy and Elevated Glycosylated Hemoglobin

	Placebo group (n = 15)	Laser group (n = 15)
Age, years [median (range)]	70 (31–80)	59 (48–81)
Sex (m/f)	7/8	9/6
Insulin-dependent diabetes mellitus	7	4
Non-insulin dependent diabetes mellitus	8	11
Duration of diabetes, years	9.9 ± 7.9	13.5 ± 11.1
Fasting serum glucose level, mg/dl	173.2 ± 74.1	171.7 ± 57.2
HbA _{1c} , mg%	8.5 ± 1.2	8.7 ± 2.2
Smokers	3	3
Macroangiopathy (diagnosed by angiography)	3	5
Patients with gangrene	5	6
Patients with ulcer (neuropathic and ischemic)	10	9
Patients with history of previous amputation	3	5
Hypertension (RR > 150/90 mm Hg)	12	11
Microalbuminuria (albumin excretion >30 mg/24 h) (Nathan, 1993)	6	7
Neuropathy (diagnosed clinically as peripheral, symmetric paraesthesia, worsening at night) (Nathan, 1993)	5	4
Retinopathy (fundus photography)	10	9

Note. Values are given in absolute frequencies for nominal variables, means and standard deviations for normally distributed variables, or medians and ranges for not normally distributed variables.

opathy has been shown to be correlated with the patients' glycemic control, as measured by glycosylated hemoglobin (Danne *et al.*, 1996; Klein, 1995). Among the various methods for investigating skin microcirculation, infrared (IR) thermography is considered a valuable noninvasive tool (Di Carlo, 1995; Seifalian *et al.*, 1994; Uchikawa *et al.*, 1992; Shindo *et al.*, 1991; Schindl *et al.*, 1998a,b). Low-intensity laser irradiation employing light sources that emit visible and near-IR light of powers in the mW range have been used in medical indications since the 1970s (Mester *et al.*, 1971). This athermic phototherapy has been shown to be effective in the treatment of impaired microcirculation, delayed wound healing, and pain syndromes (for review, see Schindl *et al.*, 2000). We recently reported that low-intensity laser irradiations are able to induce neoangiogenesis (Schindl *et al.*, 1995, 1999b) and to be of beneficial influence on diabetes and radiotherapy-associated recalcitrant ulcers (Schindl *et al.*, 1997a,b, 1999). In addition to a local effect, there are several lines of evidence for a possible systemic effect of topical laser therapy in processes of impaired microcirculation and delayed wound healing (Rochkind *et al.*, 1989; Braverman *et al.*, 1989; Tunér and Hode,

1998; Polo *et al.*, 1999). We therefore investigated the influence of a single, topical, low-intensity laser irradiation on systemic microcirculation in patients with diabetic microangiopathy in a randomized, double-blind, placebo-controlled study design.

MATERIALS AND METHODS

Patients

Patients with diabetic ulcers or gangrene were subjected to an initial IR thermography in order to evaluate skin circulation. Thirty patients showing a reduced temperature profile over their forefoot region (mean temperature below 29°C) and levels of glycosylated hemoglobin higher than 6% were included in the study. Clinical or blood chemical signs of infection, medication with drugs that might influence platelet aggregation, vasodilatation, or a combination of these were exclusion criteria. The patients' baseline characteristics were as described in Table 1 and included age, sex distribution, duration and type of diabetes, fasting

serum glucose level, percentage of glycosylated hemoglobin, smoking habits, and rate of diabetes-related complications (Table 1). After obtaining informed consent from the patients, the forefeet of the subjects were randomized into two groups ($n = 15$): Group 1 received a single session of topical low-intensity laser irradiation over one randomly chosen forefoot [laser-treated side (L/T)], the second side being left untreated [laser-untreated side (L/0)]. In group 2, the laser was positioned over one forefoot without being turned on [placebo-“treated” side (P/T)], the second side being left “untreated” [placebo-untreated side (P/0)].

Thermography Unit and Temperature Recordings

Temperature recordings were performed as described earlier (Schindl *et al.*, 1998). Briefly, a noncontact IR thermography camera (Thermo Tracer THI100, nbn Electronics, Graz, Austria) coupled to a microcomputer was used for the temperature recordings. This instrument measures the IR radiation emitted from the patient's skin with a sensitivity of 0.1°C. Analysis of the thermograms was performed with PicWinIris, Version 2.22, software, which allows measurements over a defined region of interest. In our study, the area distal of a line drawn between the medial and lateral malleolus was defined as the “forefoot region.” Thermograms were taken at 0 min and 20 and 50 min after the start and at 15 min, after the end of the irradiation and placebo-irradiation procedure, respectively. An examiner unaware of the study protocol analyzed the temperature recordings.

Laser Device and Irradiation Protocol

A continuous-wave helium–neon (HeNe) laser (wavelength, 632.8 nm; power output, 30 mW) was utilized for the light irradiation. The beam (original spot diameter, 5 mm) was diverged by the instrument's scanner, and the irradiation time was adjusted to receive an energy density of 30 J/cm² at the skin surface. Initial tests with these irradiation parameters (monitoring the temperature when irradiating a swab for a period of 50 min) revealed that the laser beam itself was athermic, i.e., it did not induce a rise in

temperature. After a period of 30 min in the supine position, for reaching equilibrium to room temperature, the patient's eyes were covered with wavelength-selective eyewear and temperature recordings as well as irradiations/sham irradiations were started. The ambient temperature was kept constant at 24°C during the intervention. For the sham irradiation, the laser was positioned in the same manner as for laser irradiation but was not turned on.

Statistical Analysis

Baseline characteristics for both groups of patients are given in absolute frequencies for nominal variables, means and standard deviations for normally distributed variables, or medians and ranges for not normally distributed variables. The laser effect was evaluated by comparing the skin temperature 15 min after the end of the irradiation procedure of the laser-treated forefeet to that of the placebo-treated forefeet by analysis of covariance (ANCOVA) using the respective baseline measurements as covariates. The systemic effect was evaluated similarly by comparing data from the laser-untreated forefeet to those of the placebo-untreated forefeet. *P* values <0.05 were considered as indicating statistical significance and are results from two-sided tests. The software used for statistical analysis was the SAS System for Windows (SAS Institute Inc., Cary, NC).

RESULTS

The changes in skin circulation as determined by thermographic measurement of the skin temperature over the forefoot regions after one single transcutaneous low-intensity HeNe-laser irradiation and sham-irradiation are shown in Table 2 and Fig. 1. The baseline-adjusted skin temperature 15 min after the cessation of laser irradiation was significantly higher in the laser-treated forefeet compared to that in the placebo-treated forefeet ($P < 0.0001$); the baseline-adjusted difference in the temperature was $1.94 \pm 0.35^\circ\text{C}$. Simultaneously, the baseline-adjusted skin temperature was significantly higher in the laser-un-

TABLE 2
Changes in Skin Temperature over Forefeet

Group	Time	Laser		Placebo	
		Mean	SD	Mean	SD
Treated	Baseline	26.79	2.69	27.05	2.83
Treated	20 min	27.47	2.51	26.83	2.97
Treated	End	27.91	2.39	26.43	3.02
Treated	15 min after end	27.36	3.69	26.34	3.13*
Untreated	Baseline	26.67	3.27	27.33	2.56
Untreated	20 min	27.14	3.15	27.18	2.65
Untreated	End	27.65	2.96	26.87	2.64
Untreated	15 min after end	27.87	2.86	26.77	2.68*

Note. Changes in skin temperature (means, SD) were determined by IR thermography in the course of a single topical laser and placebo irradiation.

* $P < 0.0001$.

treated forefeet compared to that in the placebo-untreated forefeet ($P < 0.0001$); the baseline-adjusted difference was $1.70 \pm 0.33^{\circ}\text{C}$. As can be seen in Fig. 1, the rise in skin temperature commenced earlier in the laser-treated feet than in the laser-untreated feet.

DISCUSSION

The present study addressed the possible induction of systemic effects by a single topical exposure to low

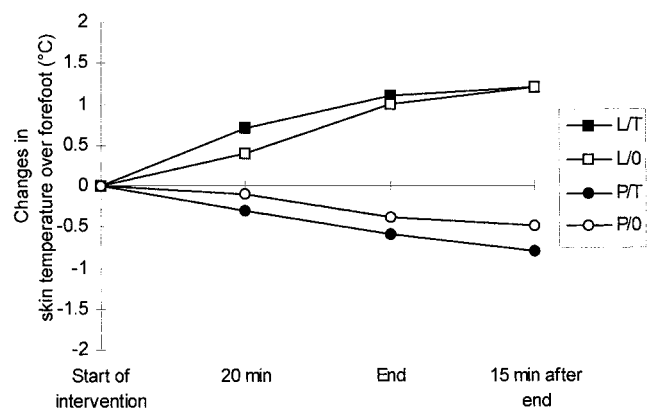


FIG. 1. Alterations in skin temperature over forefoot regions under laser and placebo irradiation in patients with diabetic microangiopathy. Filled squares indicate treated feet in the laser group (L/T), open squares untreated feet in the laser group (L/O); filled circles indicate treated feet in the placebo group (P/T), open circles untreated feet in the placebo group (P/O).

intensities of red laser light on skin microcirculation in patients with diabetic microangiopathy. For the evaluation of such effects, we utilized an IR-thermography camera as a noninvasive and convenient indirect method of quantifying skin blood circulation. Our results not only confirm previous findings about the improvement of microcirculation at the irradiation site (Schindl *et al.*, 1998a; Skobelkin *et al.*, 1990) but also suggest the possibility of causing a systemic effect on microcirculation as described earlier in an animal model (Schindl *et al.*, 1995). The systemic increase in skin temperature achieved after laser irradiation in the present study was found as early as 20 min after the initiation of light exposure and persisted up to 15 min after cessation. The degree of improvement of skin microcirculation achieved in the present study is comparable with that of previously published data (Schindl *et al.*, 1994) and with findings from studies dealing with the effects of other treatment modalities used to improve skin circulation in diabetic patients (Uchikawa *et al.*, 1992; Shindo *et al.*, 1991).

In contrast to the findings of the present study, Braverman failed to induce a significant rise in skin temperature in both irradiated and nonirradiated wounds compared to control animals in a rabbit model when using a HeNe and IR (904 nm) laser operated at energy densities of 1.65 J/cm^2 and 8.25 J/cm^2 . It has to be stressed that healthy animals without any impairment of microcirculation were irradiated (Braverman *et al.*, 1989).

The relatively short period of time within which the observed changes in skin temperature occurred in the present study seems to be due to a short-term vasodilation that is induced in addition to the long-term effect of low-power lasers on the proliferation of endothelial cells during angiogenesis, which has been demonstrated by our group (Schindl *et al.*, 1995, 1999b) and other researchers (Skobelkin *et al.*, 1990). The most likely explanation for triggering remote responses following a localized light exposure is the release of cytokines and growth factors into the circulation which are responsible for systemic vasodilation and formation of new capillaries. This hypothesis is supported by the findings of Funk *et al.* (1992, 1993), who reported the induction of increased levels of cytokines such as IL-1 α , IL-2, IFN- γ , and TNF- α after they exposed leukocytes to 19 J/cm² of a HeNe laser. More evidence comes from Dyson and co-workers, who demonstrated the secretion of growth factors by laser-irradiated macrophages. Addition of the supernatant of irradiated cultures of U-937 cells was found to stimulate fibroblast proliferation (Young *et al.*, 1989; Bolton *et al.*, 1992). O'Kane *et al.* (1994) reported on the low-intensity laser-induced release of the angiogenic cytokine IL-6 by U-937 and HL-60 cells at doses above 5.8 J/cm². This cytokine has been demonstrated to be the major component in wound fluid responsible for the induction of endothelial cell surface alkaline phosphatase, which dephosphorylates AMP to adenosine, a product of potent vasodilatory and anti-inflammatory activity (Mack *et al.*, 1997). In a publication by Bouma and co-workers (1996), however, no changes in the secretion rate of IL-6 and IL-8 were observed upon laser irradiation (904 nm, doses between 0.3 and 9.0 J/cm²) of cultures of human peripheral blood monocytes and human umbilical vein endothelial cells. Besides the relatively low doses applied in this study, the removal of the culture medium immediately after the completion of the irradiation might be considered an explanation for the negative results obtained.

The subcellular and molecular mechanism(s) of action of low-intensity laser irradiation are still a matter of ongoing research. However, thermal effects could largely be ruled out (Maegawa *et al.*, 2000; Schindl *et al.*, 1998b; Kipshidze *et al.*, 2001). Most likely, a direct or indirect interference of visible and infrared wave-

lengths with mitochondrial components of the respiratory chain is part of the signal transduction pathway (Tiphlova and Karu, 1989; Karu *et al.*, 1993; Lubart *et al.*, 1992). Recent studies have demonstrated alterations of laser-induced cell proliferation in the presence of reactive oxygen species (ROS) quenchers, which suggests that a photodynamic modulation of the quantity of ROS may be of importance (Grossman *et al.*, 1998; Jori *et al.*, 1996; Duan *et al.*, 2001; Polo *et al.*, 1999). Also, protein tyrosine kinases (PTKs) seem to be involved in the signal transduction pathway, as laser-induced respiratory bursts of neutrophils can be abolished by the PTK inhibitor genistein (Duan *et al.*, 2001).

In conclusion, in the present study we demonstrated the induction of systemic enhancement in skin circulation in patient feet suffering from diabetic microangiopathy. Regarding these results, in light of a steadily increasing incidence of diabetes, its late complications, often leading to severe handicap, and a failure to reduce the rate of amputations due to diabetic angiopathy (Stiegler *et al.*, 1998), low-intensity laser therapy might be able to partially contribute to an improvement of the situation. Results obtained in animal models and preliminary clinical trials seem to confirm this hypothesis (Yu *et al.*, 1997; Schindl *et al.*, 1998b, 1999b,c). Further research will address the underlying mechanisms and the clinical efficacy of a laser-induced increase in skin circulation in animal models and large prospective studies.

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