Clinical and Pathophysiologic Correlates of 1064-nm Nd:YAG Laser Treatment of Reticular Veins and Venulectasias

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Background: The goal of sclerotherapy, laser therapy, and intense pulsed-light therapy is to produce long-term, cosmetically significant elimination of disfiguring leg veins. This study examines the histologic and clinical effects of using a 1064-nm Nd:YAG laser system on lower extremity vessels.

Design: A single treatment using the following parameters: wavelength, 1064 nm (multiple synchronized pulsing); spot size, 6 mm; pulse duration, 14 milliseconds (single pulse); and fluence, 130 J/cm².

Setting: Private dermatology practice.

Patients: Thirteen women (mean age, 38.5 years) with blue venulectasia, 0.5 to 1.5 mm in diameter (class 2), and reticular veins, 1.5 to 3.0 mm in diameter (class 3), on the thighs.

Main Outcome Measures: Examination of treated and untreated areas by 2 masked observers using macrophotography (1, 2, 3, and 6 months after treatment), Doppler, and optical chromatographic changes. Findings from three 2-mm punch biopsies from treated (immediately and 4 weeks after treatment) and untreated sites. Routine histologic examination; special stains (for elastic and connective tissue and for mucopolysaccharides); and immunohistochemical analysis for expression of the heat shock protein hsp70, tie2 (an endothelial cell–specific receptor tyrosine kinase), and transforming growth factors β1 and β2.

Results: Eight patients (62%) manifested 75% to 100% clearing of treated vessel surface area. Treated areas revealed perivascular hemorrhage, thrombi, fragmentation and homogenization of elastic fibers, and eosinophilia of vessel walls. Expression of hsp70 and transforming growth factor β was increased in treated vessels.

Conclusions: Our data confirm the effectiveness of 1064-nm Nd:YAG laser treatment in clearing dilated lower extremity veins, probably by heat-induced vessel damage and subsequent fibrosis. Maintenance of clearing was achieved for up to 6 months. However, the presence of recanalized thrombi in some of the specimens suggests the potential for long-term vessel reappearance.

Arch Dermatol. 2001;137:613-617

The long-pulsed Nd:YAG (1064-nm) laser has gained increasing popularity during the past 3 years for treatment of disfiguring leg veins (blue venulectasia and reticular veins up to 4 mm in diameter) and more recently for the effective removal of unwanted body hair.1,4 Attractive aspects of this technology include deeply penetrating wavelength and long-pulse options, which produce an “epidermal bypass” that targets the desired chromophore (hemoglobin for lower extremity vessels and melanin for pilosebaceous photoepilation) while protecting the epidermis. The 1064-nm wavelength also allows darker skin types to be treated with minimal risk to the epidermis because of decreased interaction with melanin, thus minimizing the potential for irregularities in epidermal contour and pigmentation.5

The present study examined the histologic and clinical effects of using a 1064-nm Nd:YAG laser system (Vascu-Light; ESC Sharplan, Norwood, Mass) on lower extremity vessels. We hypothesized that clinical clearing of ectatic vessels might be accomplished by induction of specific vascular injury secondary to heating, with consequent fibrotic closure of the abnormal vessels. To test this hypothesis and study potential mechanisms, we examined immunohistochemical expression, by irradiated vessels, of the heat shock protein hsp70, a marker of cellular stress that might also play a role in collagen remodeling. In addition, we examined treated and control skin specimens for increased expression of recep-
MATERIALS AND METHODS

Thirteen women aged 32 to 58 years (mean, 38.5 years) with skin types II through V were entered into the present study: type II, 6 patients; type III, 1 patient; type IV, 4 patients; and type V, 2 patients. In all patients, it was documented that there had been no UV-B exposure for 8 weeks and no oral contraceptive use for 12 weeks before treatment.

Treated vessels with blue venulectasia, 0.5 to 1.5 mm in diameter (class 2) (72%), and reticular veins, 1.5 to 4.0 mm in diameter (class 3) (28%), were included. All veins were located on the inner or outer thighs.

A 5-cm² surface area was outlined by means of a clear vinyl mapping guide, using the iliac crest and medial knee (suprapatellar notch) as reference points.

Deep, axial, and perforator reflux were ruled out by means of Doppler and photoplethysmographic evaluation in all patients.

A single treatment was carried out using the following treatment parameters: wavelength, 1064 nm (multiple synchronized pulsing); spot size, 6 mm; pulse duration, 14 milliseconds (single pulse); and fluence, 130 J/cm².

Improvement was judged by double-masked observers using macrophotography (Yashica Medical Eye II; Yashica, Tokyo, Japan) equipped with a macro lens, a shooting area of 2.4 x 3.60 cm, and a shooting distance of 15.5 cm, using identical lighting and patient positioning with respect to anatomic landmarks 1, 2, 3, and 6 months after treatment and correlated with optical chromatographic changes (Minolta-Colorimeter model R10; Minolta, Ramsey, NJ).

Control sites contiguous to treatment areas were left untreated. No compression was used after treatment. At each visit, patients were examined for the adverse effects of pigment dyschromia, bruising, and epidermal surface irregularities.

Vessel closure was defined by Doppler analysis (Mini-Doppler D500; Hunt Leight Technology Medical Division, Luton, Okla) at each visit (8-MHz probe).

From each subject, 3-mm punch biopsy specimens were taken: 2 immediately after therapy (1 from the treated site and 1 control from a nontreated area) and 1 after 3 months from a treated site. Specimens were studied for routine histologic (hematoxylin-eosin stain), elastic tissue (Verhoeff–van Gieson technique), connective tissue (Masson trichrome stain), and mucopolysaccharide (periodic acid–Schiff stain) composition. In selected cases, immunohistochemical examination was used to analyze the degree of heat-reduced damage with anti-hsp70 antibody (Dako, Carpinteria, Calif).

Biopsy specimens were also analyzed for expression of the signaling receptors TGF-β1 and TGF-β2 (Santa Cruz Biotechnology, Santa Cruz, Calif). Antibodies specific to the receptor were detected with the avidin-biotin peroxidase method using diaminobenzidine as chromagen. Biopsy specimens were analyzed semiquantitatively using light microscopy.

RESULTS

CLINICAL EVALUATION

Eight patients (62%) manifested 75% to 100% clearing of treated vessel surface area as measured by clinical evaluation and Doppler closure (Table). Treated patients manifested immediate intravascular pigment darkening and perivascular erythema as end points of therapy, including type 2 and 3 vessels. Optical chromatography revealed a mean erythema index of 45.08 after a single treatment, confirming skin lightening (Figure 1 and Figure 2). Hyperpigmentation was noted in 3 (23%) of 13 patients; however, it resolved within 6 weeks in all cases. Telangiectatic matting occurred in 1 patient (8%).

Clinical and Pathophysiologic Effects of Using a 1064-nm Nd:YAG Laser System on Lower Extremity Vessels in 13 Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Skin Type</th>
<th>Vessel Type</th>
<th>Maximum Diameter, mm</th>
<th>% Clearing Grade at 3 mo/6 mo</th>
<th>Chromatography</th>
<th>Adverse Effects</th>
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<td>4/4</td>
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<td>3</td>
<td>2</td>
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<td>50/100 + dl</td>
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</table>

* dl indicates redness lightening.

Five categories of improvement, based on either reduction in size or reduction in number of vessels remaining, was assessed by one of us (N.S.S.) and a masked observer (J.N.) and graded as follows: worsening (grade 0); 0% to 25% improvement (grade 1); 25% to 50% improvement (grade 2); 50% to 75% improvement (grade 3); and 75% to 100% improvement (grade 4).

Biopsy specimens were also analyzed for expression of the signaling receptors TGF-β1 and TGF-β2 (Santa Cruz Biotechnology, Santa Cruz, Calif). Antibodies specific to the receptor were detected with the avidin-biotin peroxidase method using diaminobenzidine as chromagen. Biopsy specimens were analyzed semiquantitatively using light microscopy.
Biopsy specimens taken immediate after treatment revealed intravascular hemorrhage and fragmentation and homogenization of elastic fibers (Figure 3) and eosinophilia of smooth muscle cells in the vessel walls of large dermal veins (Figure 4).

Small dermal capillaries contained coagulated red blood cells without evident thrombosis. Expression of hsp70 by the large damaged vessels was increased compared with control and follow-up (3-month) specimens in all treated patients (Figure 5).

At 3-month follow-up, the large dermal veins showed thrombosis, fragmented elastic fibers, extravasated red blood cells, hemosiderin, and thrombosis.
Small vessels showed minimal evidence of thrombosis (Figure 6).

Elevated levels of TGF-β2 were found in visibly thickened regions of the vein consistent with active regions of sclerosis in all specimens examined (Figure 7).

Levels of TGF-β1 receptor, which is found in some tissues even under normal conditions, also seemed to be increased in regions of active sclerosis. No evidence of apoptosis was found in posttreatment or 3-month follow-up specimens.

**COMMENT**

As supported by the results of the present study, the 1064-nm Nd:YAG laser induces efficient photocoagulation of blue venulectasia and reticular veins up to 4 mm in diameter. Lasers have been shown to induce endothelial cell injury. Reproducible shock waves leading to cellular cavitation have been documented. Multiple synchronized pulsing allows delivery of energy in a partitioned mode.

In the present study, strong clinical efficacy (75%-100%) clearing was noted in a 5-cm² area of vessel after a single treatment session and confirmed by Doppler closure. These results were corroborated by a decrease in the erythema index, as measured by optical chromatography. The increasing a1+ scale from 0 to 100 is a measure of increasing to decreasing redness of a given target, in this case cutaneous vascular erythema. In the study by Weiss and Weiss, using a similar clinical improvement scale, an improvement grade of 3.2 was achieved, with most sites clearing 75% clinically and a transient hyperpigmentation rate of 42% noted. As shown in the present pathophysiologic study, this is accomplished by induction of vascular thrombosis and damage to the vessel wall. Heat shock protein (hsp70) probably plays a role in repairing stress-damaged cells. In the present study, increased expression of hsp70 in the myocytes of the damaged vessels supports a heat-induced stress, which might play a role in laser-induced vascular destruction. The protein might also be involved in collagen remodeling, accounting for the rare occurrence of scarring after laser treatment of the lower extremity. As shown in the present study, programmed cell death or apoptosis does not play a role in laser-induced vascular injury.

It is also of interest that pan-vessel fibrosis does not occur on a regular basis despite clinical and Doppler evidence of vessel integrity interruption and closure as measured at 3 months. In addition, the presence of recanalized thrombosis in selected biopsy specimens suggests the
potential for vessel reappearance. Long-term studies examining this potential effect are in progress. Similar histologic findings might be noted after sclerotherapy.

Transforming growth factor β is a key profibrotic factor that controls wound repair and fibrosis.8-10 Two receptors, type 1 and type 2, are both required for complete signaling by TGF-β. Both receptors are transmembrane proteins that have cytoplasmic serine/threonine kinase activity.11-13 Most types of radiation, including laser light, will activate TGF-β.14,15 The concurrent activation of the type 1 and type 2 receptors is probably sufficient to initiate the fibrotic process noted in many of the patients examined histologically in this study.

CONCLUSIONS

The long-pulsed, millisecond-domain, 1064-nm Nd:YAG laser is an effective modality for closure and long-term clearance of blue venulectasia and reticular veins up to 4 mm in diameter. Intravascular thrombosis and selective pan-vessel fibrosis occur in most but not all patients. In the present study, pan-vessel fibrosis did not distinctly correlate with total vessel clearing. Maintenance of clearing was achieved for up to 6 months, as documented in the present study. The occasional presence of recanalized thrombus suggests the potential for long-term vessel reappearance. Finally, this study supports the potential role of biological modifiers and cytokines such as heat shock proteins (hsp70) and TGF-β in directing the light-induced heat injury to blood vessels and surrounding dermis as well as playing a modulatory role in wound healing and dermal remodeling, which lead to the efficacious clinical effects noted using this long-wavelength, extended–pulse duration technology.

Accepted for publication December 27, 2000.

Dr Sadick has received research support for this study. Ms Nicholson is an independent research nurse funded by ESC Sharplan for this study.

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