Laser Treatment of Rosacea

A Pathoetiological Study

Solbritt Lonne-Rahm, MD, MSc, PhD; Klas Nordlind, MD, PhD; Desiree Wiegleb Edström, MD, PhD; Anne-Marie Ros, MD, PhD; Mats Berg, MD, PhD

Objective: To study the effect of laser treatment on rosacea, a common facial skin disease with symptoms of blushing, redness, telangiectasis, papules, pustules, and diffuse swelling of the skin, we focused on the stinging sensation and performed immunohistochemical evaluation of nerve density and neuropeptide expression.

Design: Clinical investigation as well as the lactic acid (stinger) test was performed before and 3 months after the treatment with flashlamp pulsed dye laser, when skin biopsy specimens were also taken.

Setting: University hospital.

Patients: Thirty-two patients with rosacea, all with positive results from the lactic acid “stinger” test, were treated by flashlamp pulsed dye laser.

Main Outcome Measures: The biopsy specimens were taken from the stinger-positive areas in the nasolabial folds, fixed in Lanas fixative (10% formalin and 0.4% picric acid), and analyzed for the expression of protein gene product 9.5 (general nerve marker), substance P, calcitonin gene–related peptide, and vasoactive intestinal polypeptide, using a biotinylated streptavidin technique.

Results: Thirty-one patients who were stinger positive before treatment showed decreased scores after treatment, and 1 patient had the same stinger test score before and after treatment. The number of protein gene product 9.5–positive fibers in the epidermis (P < .05) as well as the papillary dermis (P < .01) was decreased. This was also the case for substance P in the papillary dermis (P < .001), whereas no evident difference was noted for vasoactive intestinal polypeptide and calcitonin gene–related peptide. No difference was found for contact between nerves and vessels (factor VIII positive).

Conclusions: Laser treatment of rosacea that destroys small vessels has a good medical relevance because it reduces the unpleasant symptoms of the sensitive skin. A neurogenic etiology of stinging may be possible.

Arch Dermatol. 2004;140:1345-1349

Rosacea is a common disease, especially in fair-skinned women aged 30 to 50 years. The patients often complain of increased skin sensitivity. The pathoetiology of the disease is so far unknown, but angiogenic and sebaceous factors have been suggested. The clinical signs are permanent presence of erythema accompanied by telangiectasis, with a frequent admixture of facial blushing, papules, pustules, diffuse swelling, and nodules. The skin of patients with rosacea is often vulnerable to chemical and physical stimuli such as hot drinks, spices, alcoholic beverages, and the use of cosmetics and may also sting, burn, or itch. This sensitivity can be examined objectively by the stinger test, using lactic acid and water.

The skin is liberally supplied with sensory nerves, and some end in free nerve endings in the papillary dermis or epidermis. They release neuropeptides that have specific biological effects.

Substance P is often associated with blood vessels, hair follicles, and Meissner corpuscles. It is less abundant in the epidermis. This neuropeptide is involved in inflammatory responses. Together with calcitonin gene–related peptide (CGRP), it is commonly found in afferent C-fibers, which are implicated in the perception of itching and pain in humans. They coexist in nerve endings in the human skin. Vasoactive intestinal polypeptide (VIP) is distributed in the deeper parts of the dermis, near the blood vessels and sweat glands. The major neuropeptides mediating vasodilation of the microvasculature are in fact substance P, CGRP, and VIP.

In rosacea, an increased number of immunoreactive fibers and serum concentration of substance P has been reported, in addition to a more dense distribution of VIP receptor–positive cells within the endothelium as well as perivascular large cells. It was also hypothesized that epinephrine promotes a bradykinin release responsible for the vasodilation.

Flashlamp pulsed dye laser uses the principle of selective photothermolysis in the treatment of vascular skin lesions. The
The treatment resulted in purpura lasting 5 to 14 days. Vascular lesions such as telangiectasia is found in patients with rosacea, laser treatment has proven effective in treatment of vascular lesions such as telangiectasia. In patients with rosacea, laser treatment has been reported to give good or excellent reduction of telangiectasia and erythema. 

The aim of the present study was to investigate the effect of laser treatment on stinger-positive patients with erythematotelangiectatic rosacea with regard to stinging and nerve density, using the protein gene product (PGP) 9.5 panneuronal marker and expression of the neuropeptides substance P, CGRP, and VIP. In addition, contacts between nerves and vessels were studied.

**METHODS**

**SUBJECTS**

The study population comprised 32 patients with rosacea (31 women and 1 man). The mean age was 50 years (range, 31-67 years). All patients had diagnostic criteria of rosacea, with a clinical picture of telangiectasia and erythema, and some patients also had a few papules and pustules in the central parts of their faces. Only patients who had positive results form the stinger test were enrolled in the study. This test is used to elicit sensory or subjective irritation. Skin biopsy specimens, 3 mm, were taken before and 3 months after laser treatment. All subjects had given informed consent, and the study was approved by the ethics committee of the Karolinska Hospital, Stockholm, Sweden.

**FLASHLAMP PULSED DYE LASER**

A high-power flashlamp in the power source producing a true pulsed beam with 5-mm spot size, a duration of 450 microseconds and a wavelength of 585 nm, was used. The treatment resulted in purpura lasting 5 to 14 days.

**THE LACTIC ACID (STINGER) TEST**

The facial areas below the eyes were cleaned with soap, with a soft paper towel and water, rinsed well with water, and patted dry. Facial sweating was induced by exposure to a commercial facial sauna (Silhouet-Tone 50126; Silhouet-Tone [Canada] Ltd, Quebec, Ontario) for 15 minutes. A solution of 5% lactic acid in water was applied with a swab in a gentle circular rubbing motion to one side of the cheek from the side of the upper lip upward across the cheek. Water was applied as a placebo control in the same manner to the opposite cheek. The studied persons were asked after 2, 4, and 5 minutes to describe the presence and intensity of any skin sensation. The following scale was used: 0 = no skin symptoms, 1 = slight, 2 = moderate, and 3 = severe. If the cumulative score of the grades is 3 or more, the subject is judged to be a “stinger.”

**SKIN BIOPSY**

The biopsy specimens were taken from a stinger-positive area, alternating between the nasolabial fold or under the eye after an injection of lidocaine hydrochloride. The biopsy specimens were taken before and 3 months after laser treatment. The biopsy specimens were fixed in Lanas fixative (10% formalin and 0.4% picric acid) (4°C) for 2 hours. They were then rinsed in cold phosphate buffer with 10% sucrose (4°C) for at least 48 hours, snap frozen, and stored at –70°C before being further processed.

**IMMUNOHISTOCHEMICAL ANALYSIS**

Cryostat sections of 14 µm were made on a Dittes cryostat. The slides were incubated overnight (4°C) in a humid chamber with primary polyclonal antibodies, all diluted 1:20000, against PGP 9.5 (polyclonal, rabbit; Ultraclone, Cambridge, England), substance P, VIP, and CGRP (all from Bachem, St Helens, England) in phosphate-buffered saline (PBS) containing 0.3% Triton X-100 (Sigma-Aldrich Co, St Louis, Mo) and 0.3% bovine serum albumin. The sections were rinsed in PBS and incubated with biotinylated conjugated anti-goat IgG (diluted 1:200; Vector, Burlingame, Calif) for 40 minutes. The primary antibodies were visualized by incubating the sections with the fluorochrome Cy2 (dilution 1:2000; Amersham Pharmacia Biotech, Uppsala, Sweden).

As a control, the primary antibody (PGP 9.5) was omitted or the antisera samples (substance P, CGRP, and VIP) were preabsorbed at a concentration of the peptides (Bachem) at 10⁻³ mol/L overnight at +4°C. These control experiments resulted in omitted immunoreactivity.

For double staining, to study contact between nerves and vessels, the sections were incubated with the monoclonal antibody against factor VIII (diluted 1:250; Dakopatts, Copenhagen, Denmark) followed by a biotinylated anti-goat IgG (dilution 1:200) and Texas Red (diluted 1:2000) (both from Vector). This was followed by incubation with the polyclonal antibody against PGP 9.5 (diluted 1:4000), and incubation with a fluorescein isothiocyanate−conjugated swine-antirabbit antibody (diluted 1:40; Dakopatts).

The sections were then rinsed in PBS, mounted in gelatin/glycerol, and examined with epifluorescence using a Nikon epifluorescence microscope (Eclipse E800; Nikon, Yokohama, Japan).

The fluorochrome Cy2− and fluorescein isothiocyanate−fluorescent structures were visualized with a filter cube with excitation at 465 to 495 nm, while Texas red−fluorescent elements were seen with a filter cube with excitation at 540 to 580 nm. Photographs were taken using a video camera system (Nikon digital camera DXM 1200; Nikon) attached to the fluorescence microscope and connected to a personal computer.

The slides were coded before examination, which was done blindly by 1 observer (S.L.-R.). Labeled nerves were counted in the whole epidermis and papillary dermis. In each biopsy...
specimen, a count was made of 2 sections and another 2 sections 70 µm from the first one.

STATISTICAL ANALYSIS

The total sum of stinger test scores for persons in the groups before and after treatment was compared using the Kruskal-Wallis 1-way analysis of variance, and maximal scores in any of the 3 instances (2, 4, and 5 minutes) were compared with the Kruskal-Wallis exact test with Monte-Carlo estimation.

For the analysis of a difference in the total number of nerve fibers and neuropeptide-containing nerve fibers in the groups before and after treatment, the Wilcoxon signed-rank test was used. The level of significance was set at $P < .05$.

RESULTS

THE LACTIC ACID (STINGER) TEST

Of the 32 patients who were stinger positive before treatment, 24 became stinger negative after laser treatment. Seven patients scored with less skin symptoms, and 1 patient did not show any change. The median ± SD cumulative score was $6 ± 1.9$ before treatment and $1 ± 1.6$ after treatment ($P < .001$) (Figure 1).

PROTEIN GENE PRODUCT 9.5

There were several nerves in the epidermis, also reaching the stratum corneum, and in the dermis, separate and in bundles especially around hair follicles, sweat glands, and vessels (Figure 2).

There was a decrease in the number of PGP 9.5–positive nerve fibers in the epidermis ($P < .05$) and papillary dermis ($P < .01$), with a median ± SD of $39 ± 14.6$ per section before and $34 ± 17.7$ per section after laser treatment. In the papillary dermis the median ± SD was $46 ± 28.7$ before and $35 ± 21.3$ after laser treatment (Figure 3).

SUBSTANCE P

Substance P immunoreactivity was observed in thin fibers in the epidermis and dermis. However, only a few fibers were seen in the epidermis (Figure 2B). Some fibers were seen in proximity to blood vessels and hair follicles. In the dermal papillae there was a decrease ($P < .001$) in the number of substance P–positive fibers, with a median ± SD of $3 ± 2.2$ before and $1 ± 1.5$ after treatment (Figure 4).
Fibers positive for CGRP were observed mostly as thick, and only seldom thin, short fibers in the dermis but not in the epidermis (not shown). They could be seen in connection with hair follicles, but not many of them were close to vessels. In the dermal papillae, the median ± SD number of positive fibers was 1 ± 0.1 before and 0 ± 0.1 after treatment (P = .05).

VASOACTIVE INTESTINAL POLYPEPTIDE

Vasoactive intestinal polypeptide–positive fibers were observed in the papillary and reticular dermis as thin pearl-like fibers, sometimes approaching or in contact with the epidermis and acrosyringium. They were close to the deeper part of the hair follicle and in contact with the sebum glands (Figure 2C). Vasoactive intestinal polypeptide could also be seen in fibers around the sweat eccrine coils as well as in connection with deep vessels. In the dermal papillae there was no difference in the median ± SD number of positive fibers (1 ± 4.9 before and 1 ± 4.2 after treatment).

CONTACT BETWEEN NERVES AND VESSELS

There was no difference in the number of contacts between nerves and vessels (Figure 2D), with a median ± SD of 6.5 ± 4.6 before and 5 ± 3.6 after the laser treatment.

COMMENT

The present investigation shows a decreased facial skin sensitivity after laser treatment in patients with rosacea. Flashlamp pulsed dye laser uses the principle of selective photothermolysis in the treatment of vascular skin lesions. It produces a true pulsed beam with a duration of 450 microseconds and a wavelength of 585 nm, with a spot size of 5 mm. The target material is hemoglobin, and superficial vessels are selectively and completely coagulated. The studies on wavelengths exceeding 585 nm show that it destroys vessels in the upper part of the dermis. Superficial vessels containing blood will absorb laser light before it reaches deeper target vessels. Thus, the main affected tissue is expected to be that of vessels but, in addition, melanocytes that absorb the laser light (418–542 nm) in the basal epidermis may be damaged somewhat when subjected to 577-nm irradiation of approximately 5 mm from the dermal epidermal junction.

In this investigation, nerve fiber density and number of substance P immunoreactive nerve fibers were decreased, which might speak for a contributing neurogenic component to the rosacea/stinging condition. This decrease in number was also true for superficial nerves. However, nerve fibers might be destroyed directly by the laser treatment as an effect of the damage to the basal epidermal level. Another parameter that might speak in favor of a direct effect on the nerves is that there was no significant decrease in the number of contacts between nerves and vessels before and after laser treatment. The biopsy specimens were taken 3 months after the laser therapy. However, treatment of the skin with flashlamp pulsed dye laser may decrease the number of nerve fibers for a substantial time. Excimer laser therapy of the cornea has been shown to cause nerve degeneration for at least 1 year.39

Figure 3. Number of protein gene product (PGP) 9.5–positive nerve fibers in the epidermis (A) and in the papillary dermis (B) before and after laser treatment. The box plot shows the median, 10th, 25th, 50th, 75th, and 90th percentiles; the dots represent outliers.

Figure 4. Number of substance P–positive nerve fibers in the papillary dermis before and after laser treatment. The box plot shows the median, 10th, 25th, 50th, 75th, and 90th percentiles; the dots represent outliers. VIP indicates vasoactive intestinal polypeptide.

CALCITONIN GENE–RELATED PEPTIDE

Fibers positive for CGRP were observed mostly as thick, and only seldom thin, short fibers in the dermis but not in the epidermis (not shown). They could be seen in connection with hair follicles, but not many of them were close to vessels. In the dermal papillae, the median ± SD number of positive fibers was 1 ± 0.1 before and 0 ± 0.1 after treatment (P = .05).
In this investigation, there was no effect on the number of VIP- and CGRP-positive fibers. Although fibers positive for substance P and CGRP are colocalized in the trigeminal ganglion cells, some ganglion cells have only CGRP expression.7 Calcitonin gene–related peptide is also found in A fibers, and it might be that these are more resistant to the effects of laser. One may thus speculate that sensory nerves have an effector function in stinger-positive skin. This hypothesis is supported by the fact that injection of substance P into the human skin produces a flare and wheal reaction comparable with that seen following a similar injection of histamine.8

In conclusion, we have shown that flashlamp pulsed dye laser may be used as an effective treatment of the sensitive skin in rosacea. This effect might be achieved via the effect on superficial C-fibers, either directly or indirectly via effect on vessels.

Accepted for Publication: June 7, 2004.
Correspondence: Solbritt Lonne-Rahm, MD, MSc, PhD, Unit of Dermatology and Venereology, Department of Medicine, Karolinska Hospital, S-171 76 Stockholm, Sweden (sol-britt.lonne-rahm@kus.se).
Funding/Support: This work was supported by the Welander/Finsen Foundation, Stockholm, Sweden.
Acknowledgment: The technical assistance of Anna-Lena Kastman, BMA, is gratefully acknowledged.

REFERENCES