Treatment of Benign and Atypical Nevi With the Normal-Mode Ruby Laser and the Q-Switched Ruby Laser

Clinical Improvement but Failure to Completely Eliminate Nevomelanocytes

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Objective: To evaluate the effect of normal-mode and Q-switched ruby laser light (694 nm) on nevomelanocytes of benign, atypical, and congenital nevi.

Design: Half of the lesion of each of 31 nevi was treated with either the Q-switched ruby laser or the normal-mode ruby laser or both; the other half of the lesion was covered with aluminum foil and was not treated.

Setting: A university-affiliated, hospital-based laser center.

Patients: Sixteen patients with a total of 31 melanocytic nevi were enrolled in the study.

Interventions: All nevi were evaluated by at least 2 dermatologists to assess the degree of clinical atypia. Photographs were taken before and immediately after treatment and at each follow-up visit. The digital imaging system was used to evaluate the number of melanocytes in a measured length of basement membrane zone.

Main Outcome Measure: Three individual readings (number of melanocytes per unit length) were taken on both the control and treated halves and then compared to quantitate treatment effect. All analyses used averages from 3 measurements. A Student paired t test was used to compare the treated and untreated sides.

Results: Sixteen (52%) of the nevi showed a clinically visible decrease in pigment on the treatment side at the 4-week follow-up visit.

Conclusion: No lesions had complete histologic removal of all nevomelanocytes. Therefore, 1 or 2 laser treatments are not sufficient to cause complete removal of a lesion either clinically or histologically.

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PATIENTS AND METHODS

Sixteen patients with a total of 31 melanocytic nevi were enrolled in the study. Patients were recruited from the dermatology, pigmented lesion, and laser clinics of Massachusetts General Hospital, Boston. Inclusion criteria were age 18 to 75 years, consent to participate in the study, and a desire to have a mole removed. Patients were excluded who were mentally incompetent, prisoners, pregnant, under 18 or over 75 years old, or immunocompromised or who had an active infection or a photosensitivity disorder.

All nevi were evaluated by at least 2 dermatologists to assess the degree of clinical atypia. Clinical atypia was defined by irregular pigmentation or border, a recent change in the lesion, asymmetry, and size greater than 5 mm. Only benign-appearing, congenital, or mildly to moderately atypical nevi were selected for the study. Nevi of severe atypia clinically or with any concern for melanoma or nevi on the face, palms, soles, or genital area were excluded from the study. In addition, nevi were selected that were relatively symmetric; lesions with eccentric pigment dots or a sharply asymmetric border were excluded. If a nevus was deemed appropriate for the study, a consent form was reviewed with and signed by the patient. This study was approved by the Subcommittee of Human Studies at Massachusetts General Hospital.

Photographs were taken before and immediately after treatment and at each follow-up visit. Each nevus was marked to designate half of the lesion for laser treatment and half of the lesion for no treatment (control side). As the lesions selected were relatively symmetric, the control side reflected the clinical appearance of the treatment side.

Half of each nevus received laser treatment. The lasers used were either the QSRL (Spectrum Medical Technologies Inc, Palomar Medical Products Inc, Lexington, Mass) or the NMRL (Epilaser; Spectrum Medical Technologies Inc, Palomar Medical Products Inc). The QSRL, with a pulse duration of 40 to 60 nanoseconds and wavelength of 694 nm, was used at a fluence of 7.5 to 8.0 J/cm² with a spot size of 5 mm. The NMRL, with a pulse duration of 3 milliseconds and wavelength of 694 nm, was used with a spot size of 5 mm. The NMRL handpiece had a sapphire tip cooled to 10°C to limit epidermal heating. Partial overlap of the pulses, ranging in number from 3 to 8, avoided skip areas within the treatment site. A strip of metal foil covered the control area to prevent any irradiation of the untreated half of the nevus. Wound care included bacitracin ointment applied twice daily for 3 to 5 days.

Following placement of foil to cover half of each of the lesions, 4 nevi (group 1) were treated with the QSRL and 22 nevi (group 2) were treated with the NMRL. Three nevi (group 3) were treated first with the NMRL, immediately followed by treatment to the same half of the lesion with the QSRL. Two nevi (group 4) were treated with the QSRL, healed for 2 weeks, and then received treatment to the same half of the lesion with the NMRL.

Four weeks following the last laser treatment, the entire lesion was photographed and excised using a standard surgical elliptical excision procedure and 3-mm margins. One nevus was excised 3 months following laser treatment. The laser-treated side was identified by a suture. Patients had follow-up skin evaluations 2 weeks, 6 months, and 1 year following the excision. Clinical response was based on comparison of pretreatment and post-treatment (4 weeks) photographs of the nevi. Grading of clinical response was based on a “positive response” or “no response” dichotomous scale.

Tissue sections were cut 3 mm thick, deparaffinized in xylene, rehydrated in alcohol, and washed with phosphate-buffered saline (PBS). No proteolytic digestion was performed. The monoclonal anti–tyrosinase-related protein antibody (Mel-5) (Signet Laboratories Inc, Dedham, Mass) was used to detect melanocytes at a dilution of 1:40. Sections were incubated with the Mel-5 antibody overnight in a humidified chamber at 4°C. After being rinsed 3 times in PBS, the sections were incubated with biotin-labeled anti–mouse IgG antibody for 30 minutes and then washed again 3 times in PBS. The sections were subsequently incubated with alkaline phosphatase–labeled avidin, washed again 3 times in PBS, and colorized with new fuchsin substrate system (Dako Corp, Carpinteria, Calif). Sections were then counterstained with hematoxylin. Immunohistochemical control sections were incubated with secondary antibody alone.

The control halves of the nevi were analyzed to render a pathologic diagnosis and to compare with the treated halves. The treated and untreated halves were evaluated for the presence of fibrosis and/or other reparative changes and changes in the junctional component (referring to lentiginous melanocytic hyperplasia), the dermal component, dermal pigmented type A cells, pronounced dermal nests, pigmented melanophages, and depth of the dermal nests (measured from the overlying granular cell layer). Nevi for which a decrease in the junctional and/or dermal component was noted by light microscopy were evaluated with a digital imaging system (IPLab Spectrum, version 3.0, Signal Analytics Corp, Vienna, Va) to quantitate nevomelanocyte reduction. The digital imaging system was used to evaluate the number of melanocytes in a measured length of basement membrane zone. Three individual readings (number of melanocytes per unit length) were taken on both the control and treated halves and then compared to quantitate the treatment effect. The thickness of the dermal component was evaluated by measuring from the granular cell layer to the top and bottom of the dermal nests in 3 different areas of both the control and treatment sides. All analyses used averages from 3 measurements and then a Student paired t test to compare the treated and untreated sides.

The objective of this study was to evaluate the clinical and histologic effect of NMRL and QSRL on nevomelanocytic lesions, including acquired, atypical, and congenital nevi. The degree to which the 2 lasers remove melanocytes was compared. Histologic characteristics of nevi were correlated with treatment efficacy.
average total surface area of the nevi was 41 mm². Anatomic locations of the nevi were 74% (n = 23) on the trunk, 16% (n = 5) on the arm, and 10% (n = 3) on the leg. None of the patients had noted a recent change in the study moles. Table 1 describes the 4 different treatment modalities for the 31 nevi.

Immediately following laser treatment, 15 (48%) of the study sites developed whitening or lightening of the pigment. The QSRL left more notable tissue whitening than the NMRL. The rest of the nevi showed no significant clinical change. Sixteen (52%) of the nevi showed a visible decrease in pigment on the treatment side at the 4-week follow-up visit (Figure 1); 14 of these nevi had received only 1 laser treatment. One study site in group 4 had a 100% clinical response to the 2 laser treatments (Figure 2). Treatment responses are shown in Table 2 and Table 3.

Pathologic examination revealed that 65% (n = 20) of the nevi had fibrosis as well as a mild mucinous change in the stroma in the treatment site. For the 13 nevi that had a decrease in the junctional lentiginous melanocytic hyperplasia component, the number of melanocytes per basement membrane zone distance decreased by 47% percent. For the 12 nevi with a decrease in the dermal component, there was a 38% percent decrease in the thickness of the dermal nevomelanocytic nests. Representative histologic sections of nevi with a positive treatment effect are shown in Figure 3.

At first, nests appeared deeper in the nevi that did not show a histologic decrease in the dermal component, yet the difference did not reach statistical significance (P = .20). In general, a larger number of pigmented macrophages on the untreated vs treated side appeared to be associated with a positive treatment effect, yet the correlation was not statistically significant. There were 4 nevi that had no pigmented macrophages on the untreated side; none of these nevi had a decrease in the dermal component following laser treatment. Seventeen (55%) of the treatment sites had a flattened rete ridge pattern, and 16 (62%) of 26 had a decrease in the junctional nevomelanocytic nests as well as a decrease in the lentiginous component. Three (50%) of the 6 nevi that had pigmented type A cells in the papillary dermis showed a decrease in these cells following laser treatment. One nevus had postinflammatory hyperpigmentation following treatment, and 2 atypical nevi, 4 weeks following treatment with the NMRL, had a small focus of junctional nests that was interpreted as a benign-appearing recurrence.

There are many different types of pigmented lesions, all of which have distinct clinical features, biological behavior, time of onset, and risk for malignant transformation. Cutaneous laser surgery has generally been used for lesions with negligible concern for malignancy. Benign epidermal pigmented lesions that have been successfully treated with Q-switched lasers include café au lait macules, lentigenes, and ephelides.7,9,11 Dermal melanocytoses, such as nevi of Ota or Ito, or exogenous pigments, such as tattoos, can also be treated with Q-switched lasers. On the other hand, dermal melasma, postinflammatory hyperpigmentation, blue nevi, and some Becker nevi and nevus spilus have not been uniformly responsive with the present-day lasers.15,16

Histologically, nevomelanocytic lesions differ from other types of dermal or epidermal pigmented lesions in that they contain melanocytes that form into nests. The natural history and risk for malignant transformation differ depending on the type of nevus. Having an increased number of nevi is associated with an
increased risk of developing malignant melanoma, but an individual benign acquired nevus is not usually worrisome for malignant transformation. Removal of benign nevi is often for cosmetic purposes.

Dysplastic nevi have cellular and/or architectural atypia in addition to other features and may be associated with or may be a marker for increased risk of melanoma development. Though the treatment of people with dysplastic nevus syndrome is under ongoing discussion, complete surgical removal of atypical nevi is often recommended if the lesion is moderately or severely atypical or has changed in appearance. Ultraviolet light exposure may contribute to neoplastic changes in a nevus, yet the exact stimuli that trigger formation of a melanoma have not been established. Congenital nevi occur in approximately 1% of newborns. Small (<20 cm) congenital nevi have a reported lifetime melanoma risk of 1% to 2%. Giant (>20 cm) congenital nevi carry a 3.8% to 18% lifetime risk of developing melanoma. Removal of these lesions may be recommended, yet complete or partial excisions often leave a noticeable scar, and complete removal may be impossible.

Concern has been raised that laser irradiation of nevomelanocytic lesions could induce a neoplastic change in cellular behavior, that a partially removed...
lesion would repigment in a clinically and histologically atypical manner, or that laser removal does not permit a histologic specimen to be evaluated, which is not appropriate for lesions of equivocal clinical appearance. Some researchers have hypothesized that a partially removed lesion is lighter and therefore has lost some of its natural protection from ultraviolet light exposure. An increased incidence of squamous cell carcinoma in burn scars has been reported, and nonlethal heating of a cell may induce the production of stress proteins. The concept of debulking part of a congenital nevus in an attempt to decrease the risk that the lesion will develop melanoma is controversial. A partially treated lesion may be more difficult to monitor for neoplastic changes, and the effect on melanocytes of sublethal irradiation exposure is unknown. To date, there has been no direct correlation between laser treatment and malignant transformation of a dysplastic nevus following treatment with a laser. However, lentigo maligna treated with a ruby laser recurred, and a lentigo maligna melanoma arose in a lentigo maligna treated with a laser. It is unclear whether this change was directly related to the laser treatment.

Besides surgical excision, other treatment modalities for nevi, such as cryotherapy, dermabrasion, electrosurgery, and argon and carbon dioxide lasers, have been explored, yet their efficacy and clinical use are limited. In a preliminary study of a series of QSRL treatments of benign nevi, of 8 nevi showed a mild decrease in the density of nevus cells. For the 2 congenital nevi treated, there was no decrease in nevus cells following treatment. Goldman et al studied treatment of congenital and benign acquired nevi with 4 different types of lasers (QSRL, Q-switched neodymium:YAG [QSYAG] laser, Q-switched alexandrite laser, and the short pulsed-dye laser at 510 nm) either alone or sequentially. For the congenital nevi, of 10 patients achieved at least a 50% reduction in the pigmentation of the lesion. Of 23 benign acquired nevi, 9 showed 25% to 50% reduction in pigment and 8 had no response to the treatment. The short pulsed-dye laser was ineffective, and there was no difference in treatment efficacy among the Q-switched lasers.

Treatment of congenital nevi has been attempted with both QSRLs and NMR lasers. Waldorf et al treated 18 small to medium congenital nevi with the QSRL. There was a 76% lightening after 8 treatments. Five patients had more than 90% clearing after 13 sessions. After discontinuation of therapy for an average of 4.9 months, of 12 patients had partial repigmentation. Grevelink et al treated defined segments of 5 congenital nevi with both the QSRL and the QSYAG laser (1064 nm). There was partial lightening in all lesions. The maximum depth of destruction was 0.20 mm for the QSRL and 0.40 mm for the QSYAG laser. Normal-mode ruby laser treatment has been demonstrated, in case reports, to successfully lighten congenital nevi.

In one study, benign acquired nevi treated with a QSRL showed a complete response in 67% of cases and a partial response in 33% of cases. The depth of QSRL destruction was 0.10 to 0.40 mm from the granular cell layer. The QSYAG and Q-switched alexandrite (755 nm) lasers have also been demonstrated to lighten benign melanocytic nevi.

The results of this study are consistent with the findings of previous studies of laser treatment of nevi. Fifty-two percent of the nevi (16/31) showed lightening of

Figure 3. A, Untreated half of a dysplastic nevus, showing lentiginous melanocytic hyperplasia, several junctional nests, and perirete concentric eosinophilic fibrosis. B, Treated half of the lesion 4 weeks after treatment with the normal mode ruby laser, showing flattening of rete, papillary dermal mucinous amphophilic fibrosis, and reduced but residual lentiginous and nested melanocytic hyperplasia (hematoxylin-eosin, original magnification ×20). C, Untreated half of a compound nevus, showing lentiginous melanocytic hyperplasia, junctional nests, and intradermal nests stained for tyrosinase-related protein-1 (Mel-5) and unstained deep intradermal nests. D, Treated half of the compound nevus 4 weeks after treatment with the normal-mode ruby laser, showing marked reduction but not ablation of the lentiginous and nested melanocytic hyperplasia and the intradermal nests of melanocytes with tyrosinase-related protein-1 (Mel-5) (immuno-avidin-biotin–alkaline phosphatase with new fuchsin substrate [see the “Patients and Methods” section], original magnification ×20).
pigmentation. Lack of a clinical or histologic response may be due to a low pigment concentration in the lesion or the need for a series of treatments. Because of their 694-nm wavelength, both the QSRL and the NMRL are targeted at melanin, which can be in melanocytes, keratinocytes, or macrophages. A higher concentration of pigment will cause more absorption of the laser energy and more cellular destruction. In this study, a positive treatment response trend was noted with the presence of more pigmented macrophages in the papillary dermis. Following treatment, there tended to be fewer pigmented macrophages, as the laser may have caused fracture or dispersion of the pigment. Raised lesions and those with a deeper, less pigmented dermal component had less of a response.

In theory, the longer pulse duration of NMRL compared with QSRL better targets groups or nests of cells rather than individual organelles or pigment particles. The longer pulse duration of NMRL allows thermal conduction to heat and injure cells adjacent to the pigmented cells. Treating first with the QSRL to diminish the superficial pigment followed by NMRL irradiation may enhance the penetration of the NMRL light. In the present study, although the sample size was small, after treatment both with the QSRL and 2 weeks later with the NMRL, both the junctional and the dermal components were reduced.

Besides having less of a reduction in the dermal component, atypical nevi did not respond differently than benign nevi. Of note, the 2 nevi that showed histologic recurrence within 4 weeks were both dysplastic nevi. No malignant transformations occurred, with the longest follow-up duration of an atypical nevus being 3 months. Atypical nevi had more of a junctional than a dermal response, whereas congenital nevi showed more dermal than junctional reduction.

**CONCLUSIONS**

Treatment of 31 benign, atypical, and congenital nevi with QSRL and NMRL resulted in a partial response following 1 treatment and possible increased efficacy using both lasers in sequential sessions. No lesions had complete histologic removal of all nevomelanocytes. Therefore, 1 or 2 laser treatments are not sufficient to cause complete removal of a lesion. Lasers may be a treatment option for lesions that cannot be surgically resected, yet at this point lasers should not be considered the standard of care for atypical melanocytic nevi, especially nevi that are atypical and/or cannot be followed up clinically. No malignant transformations occurred following laser treatment during the 2-week to 3-month study period. Future research efforts should focus on increasing the follow-up duration, using a series of sequential laser treatments, and combining lasers with different parameters to target both superficial and deep melanocytic components. It is the view of the authors that since the present generation of lasers cannot assure complete removal of all nevomelanocytic nests/nevomelanocytes, lasers should only be regarded as a treatment option for atypical lesions that cannot be surgically excised.

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