Long- and Short-term Histological Observations of Congenital Nevi Treated With the Normal-Mode Ruby Laser

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Objective: To evaluate histologically the long- and short-term changes associated with cosmetic improvement or failure of normal-mode ruby laser treatment of patients with congenital nevi.

Design: A biopsy of the laser-treated lesions of 10 patients with good or poor cosmetic results was performed at periods up to 8 years 10 months after treatment (mean, 4 years 9 months). Short-term findings were evaluated in 3 patients.

Setting: Ueda Setsuko Clinic and the Dermatology Unit of the Kyushu University, Fukuoka, Japan.

Patients: Of the 85 Japanese patients with relatively large congenital nevi who had been treated with the normal-mode ruby laser since 1990, 13 gave informed consent for biopsy and histological examination of the treated area.

Results: A long-term follow-up study of the 8 patients with good cosmetic results showed the presence of residual nevus cells 1.11 ± 0.35 mm (mean ± SD) (range, 0.63-2.05 mm) below the skin surface. Above these cells was a layer of connective tissue that formed a subtle microscopic scar that preserved the normal structure of the papillary dermis. Hair follicles were damaged at the base, and the hairs were attenuated. However, in the 2 patients with poor cosmetic results, nests of pigmented cells were commonly seen in the epidermis, and melanin was relatively abundant in basal keratinocytes. No malignant changes were observed in any patient. A short-term study in 3 patients showed damage to pigmented cells in the epidermis and upper dermis as observed following electrodesiccation.

Conclusions: Multiple treatments with the normal-mode ruby laser produced immediate thermal damage to the superficial nests of nevus cells and a subsequent remodeling of the superficial connective tissue. When the thickness of the subtle microscopic scar reached 1 mm, it masked the underlying residual nevus cells and achieved a good cosmetic result. Follow-up for at least 8 years after laser treatment showed no evidence of malignant change in the treated areas.

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The effectiveness of the normal-mode ruby laser in treating congenital nevi that show an abundance of melanin was previously demonstrated. Such treatment led to a marked reduction in deep pigmentation; in the number of thick, dense hairs; and in the rough surface of the lesion. The appearance and texture of the nevi improved at each irradiation treatment, and, when 4 to 6 laser treatments had been completed, the treated area resembled the normal surrounding skin.

Such treatment can improve the quality of the patients’ lives by erasing disfiguring skin lesions present since birth. However, it is necessary to evaluate the histological changes produced by repeated laser irradiation treatment.

Histological changes have been reported for congenital nevi following treatment with the Q-switched ruby laser but not with the normal-mode ruby laser. The former had been regarded as unsuitable for treating congenital nevi. It is, therefore, necessary to evaluate whether normal-mode ruby laser treatment may affect the risk of malignant changes within large congenital nevi or in regions that show residual nevus cells.

In the present study, we performed skin biopsies and studied the histological changes that occurred after the administration of normal-mode ruby laser treatment for periods as long as 8 years 10 months (mean, 4 years 9 months). Biopsy specimens of skin lesions from patients with good and
PATIENTS AND METHODS

PATIENTS

Of the 85 Japanese patients with relatively large congenital nevi who had been treated with the normal-mode ruby laser since 1990, informed consent was obtained from 13 patients or their guardian (4 males and 9 females; age range, 3-49 years) for the biopsy and histological examination of the treated area. Their clinical features, including age and sex, location of the lesion, characteristics of the hair present on the lesion, and results of follow-up with cosmetic outcome, appear in Table 1. This group includes 3 patients with giant hairy nevi (patients 1, 5, and 8).

Ten specimens were obtained; 8 were obtained from the skin lesions in the 8 patients with good cosmetic results (patients 1-8), and 2 were obtained from the lesions in the 2 patients with poor cosmetic results (patients 9 and 10) several years after the final treatment (mean, 4 years 9 months; range, 2 years to 8 years 10 months). The evaluation procedure used was previously described. Results were classified on a 5-point scale regarding the clearing of pigmentation (excellent, good, fair, poor, or no change) (Table 1). In patients with a successful outcome, the treated areas of the congenital nevi were free of scarring, and their appearance and texture resembled that of the surrounding normal skin (Figure 1, A). In contrast, poorly responsive lesions exhibited persistent deep pigmentation even after multiple treatments (Figure 1, B and C).

Specimens were obtained from 3 patients to study the short-term histological changes following a single laser treatment. A biopsy of the lesions was performed at 1 hour (patient 11); at 1, 4, and 7 days (patient 12); and at 3 months (patient 13) following treatment (Table 1).

LASER TREATMENT REGIMEN

The appropriate source and dose of laser for each particular lesion was determined as follows. A panel of 10 to 16 spots in the lesion was initially irradiated with the normal-mode ruby laser (Toshiba, Tokyo, Japan) at a wavelength of 694.3 nm, pulse durations of 0.3 and 1.0 × 10⁻⁹ seconds, and energy fluences of 10 to 30 J/cm². At the same time, we also tested the effects of the Q-switched ruby laser (Spectrum, Boston, Mass), used at a pulse duration of 28 × 10⁻⁹ seconds and energy fluences of 5 to 7 J/cm². We compared the results 3 months after such preliminary irradiation treatment. Based on the findings, we then administered the normal-mode ruby laser, using the best combination of variables that had led to a reduction in pigmentation without residual scarring. The interval between treatments ranged from 1 to 6 months.

In the 13 patients, the normal-mode ruby laser treatment was applied at a pulse duration of 1 × 10⁻³ seconds, energy fluences of 13 to 30 J/cm², and a spot size of either 10 × 10 or 15 × 15 mm (Table 1). In addition, the Q-switched ruby laser was used in 3 patients to eradicate the bluish color that persisted even after the deep pigmentation had been reduced by use of the normal-mode ruby laser (patients 6-8). The variables used for the Q-switched ruby laser were the same as those used for the test irradiation.

PREPARATION OF BIOPSY SPECIMENS

Skin specimens were fixed in 3% formaldehyde solution buffered with phosphate-buffered saline (pH 7.2), embedded in paraffin, sectioned 4 to 5 mm thick, and stained with hematoxylin-eosin. To detect nevus cells that contained small amounts of melanin in the cytoplasm, some specimens were evaluated immunohistochemically using antibodies against S100 (Nichirei, Tokyo) and HMB-45 (Dako, Carpinteria, Calif) proteins.

EVALUATION OF TISSUE SPECIMENS

We evaluated 16 histological variables related to the clinical appearance and the texture of the skin. The variables concerning the epidermis were (1) the presence and density of nests of nevus cells, (2) the density of melanin in the epidermal basal cells, (3) the thickness of the epidermis from the basal cell layer to the granular cell layer, and (4) the number of columnar keratinized cells. The variables concerning the epidermal-dermal interface were (1) the presence and density of the nevus cells; (2) the presence and density of the macrophages that had phagocytosed quantities of melanin; (3) the shape of the epidermal basal surface as flat, papillomatous, or digitated; and (4) the features of the papillary dermis as fibrillar or amorphous. The variables concerning the upper dermis were (1) the presence and density of nevus cells, (2) the presence and density of macrophages, (3) the amount of inflammatory cell infiltrate, and (4) the microscopic features of the connective tissue that should have been reconstructed following damage by the laser beam. The variables concerning the lower dermis were (1) the features of the residual nevus cells and (2) the distance from the epidermal surface to the residual nests of nevus cells. The variables regarding hairiness were the density and size of (1) the hair shafts and (2) the hair follicles. The data are summarized in Table 2.

The number of keratinized cell layers, the thickness of the granular-basal layer, and the distance between the nests of nevus cells and the epidermal surface were determined in tissue sections by measuring 10 different points under a light microscope (model BH-2; Olympus, Tokyo) equipped with a scaled ocular lens. Data are reported as mean ± SD. Calculations used SAS statistical software (SAS Institute Inc, Cary, NC). The density of pigmentation and the number of nevus cells or melanophages present were graded qualitatively as negative or absent, lightly pigmented or occasionally present, darkly pigmented or usually present, and densely pigmented or numerous.

Features that would suggest the presence of malignant changes in the laser-treated epidermis, connective tissue, or residual nevus tissue were evaluated in all 13 subjects, regardless of cosmetic outcome. These features included irregular cell shape, basophilic cytoplasm, irregular distribution of melanin, nuclear atypia, and mitosis of pigmented cells.

While poor cosmetic results were evaluated. We previously found that each laser treatment was followed by a temporary repigmentation that gradually lessened in 2 months. However, in some patients, the repigmentation persisted and interfered with the desired cosmetic effect. Therefore, we also studied the short-term changes that occurred between laser treatments in patients undergoing initial treatment.
RESULTS

The clinical features and outcome in the 13 patients are summarized in Table 1, and the histological findings are presented in Table 2. Patients underwent long-term (patients 1-10) or short-term (patients 11-13) evaluation.

### COSMETICALLY IMPROVED LESIONS OBSERVED 2 TO 8 YEARS AFTER TREATMENT

The areas of treated skin observed in 8 patients for up to 8 years 10 months after successful treatment appeared virtually normal. Clinically, there was a marked reduction in the amount of deep pigmentation and in the number of dense hairs. The previously rough surface of the lesion was smooth and unscarred, with only slight pigmentation remaining (Figure 1; A and Table 1). An examination of biopsy specimens with a light microscope showed the following. At low magnification, the epidermis appeared smoothly undulated, with intermittent grooves that corresponded to the “glyphics” of the skin surface. At medium magnification, the keratinocytes showed a uniform differentiation from basal cells toward keratinized cells. At high magnification, no atypical keratinocytes, melanocytes, or nevus cells were revealed. Keratinized cells were seen to be normally several layers thick (mean, 5.9 ± 2.4 layers; range, 1-12 layers). The epidermis was of moderate thickness, ranging from 0.04 to 0.07 mm. While the basal keratinocytes sometimes exhibited rather large amounts of melanin in the cytoplasm, the epidermis of the successfully treated lesions showed no residual nevus cells (Figure 2 and Table 2).

The papillary interface of the epidermis with the underlying dermis was well preserved in 7 of the 8 patients with successfully treated lesions. The exception was patient 1, who had developed a bacterial infection in the treated area. Nevertheless, the connective tissue matrix of the dermal papillae of the 8 patients appeared more amorphous than fibrillar, thus giving the impression of a subtle scar that was apparent only on microscopic examination. Macrophages containing melanin granules were usually present in the papillary dermis.

Residual nevus cells were present as large or small nests that were located approximately 1 mm (mean, 1.11 ± 0.35 mm; range, 0.63-2.05 mm) below the skin surface in all 8 patients. Such cells, particularly those close to the apex of the nest, exhibited melanin within the cytoplasm. The base of the hair follicles in the residual nevus tissue was damaged, and the hair shafts were attenuated. No marked changes were observed in the eccrine sweat glands or their ducts.

An unusual connective tissue was observed between the epidermis and the residual nevus tissue. Such tissue was composed of relatively fine collagen fiber bundles that were arranged parallel to the skin surface, thus giving the impression of a subtle microscopic scar of uniform thickness. It showed little interference with the epidermal appendages. The chronic inflammatory cells in this microscopic scar consisted largely of lymphocytes that occasionally infiltrated the area around the small blood vessels.

Immunohistochemical studies revealed the presence of cells positive for S100 protein, which were seen individually in the basal layer of the epidermis and as nests in the residual nevus lesion. Such cells were observed only occasionally in the microscopic scar. Nevus cells in the epidermis were positive for HMB-45 protein.

### LESIONS WITH POOR COSMETIC RESULTS OBSERVED 2 TO 5 YEARS AFTER TREATMENT

A clinical examination of the skin of the 2 patients with poor cosmetic results (patients 9 and 10) revealed deep pigmentation and a rough surface even after multiple treat-
ments with the normal-mode ruby laser and the Q-switched ruby laser (Figure 1, B and C; and Table 1). Microscopic examination results of the biopsy specimens obtained 2 to 5 years after the unsuccessful treatment of these 2 patients revealed scattered nests of pigmented nevus cells in the epidermis (Figure 3, A). Some of these nests contained a small amount of melanin. Around them were basal keratinocytes whose cytoplasm was abundant in melanin. Only a thin microscopic scar covered the nests of residual pigmented nevus cells, so that they were frequently observed in the papillary dermis, as is typical before laser therapy. The cytoplasm of such superficial nevus cells contained large amounts of melanin. While most nevus cells in the epidermis and dermis were positive for S100 protein, only the epidermal nevus cells were positive for HMB-45 protein, as is usually seen.

**ACUTE LESIONS FOLLOWING LASER TREATMENT**

Immediately following the laser treatment, the acute lesions (patients 11-13) exhibited a scorched appearance similar to a chemical burn (Figure 3, B-D). In 1 hour, the pigmented nevus cells and the pigmented basal keratinocytes in the epidermis were elongated and arranged perpendicularly to the skin surface as observed.
following electrodesiccation. The damaged cells had a string bean shape with elongated nuclei and a granular cytoplasm. Such cells were sometimes separated from the dermis to form clefts. Similar damage (elongated nuclei and granular cytoplasm) was also found in the nests of nevus cells in the papillary and upper dermis. Laser-damaged cells were present even at a depth of 0.6 mm from the skin surface, where the overlying epidermis bore little basal pigmentation or lacked nests of pigmented nevus cells. The matrix of the papillary and upper dermis appeared amorphous. The connective tissue cells, including the fibroblasts and capillary endothelial cells, showed no pyknotic changes in the nuclei or necrotic changes in the cytoplasm, suggesting that they had been spared from thermal damage.

In 24 hours, the damaged epidermis had become eroded, but only a small amount of exudate or a small number of inflammatory cells were present. In 4 days, the epidermis had begun to regenerate and had accumulated up to 3 layers of flattened keratinocytes. In 7 days, the regenerated epidermis had attained a nearly normal thickness. It was covered with a thin keratinized layer of cells with frequent parakeratosis. Only a few inflammatory cells, but numerous macrophages containing various amounts of pigment granules, were seen in the papillary dermis where the connective tissue exhibited little change.

The superficial pigment-laden nevus cells were damaged by each successive treatment and were finally eradicated. Therefore, as treatment progressed, nevus cells that had been present at the apices of the residual nevus tissue showed progressive damage. Between treatments, the tissue eradicated by the laser was replaced by a microscopic subtle scar composed of fine collagen fiber bundles. Simultaneously, the pigment-laden macrophages disappeared, being found only around the capillaries and the hair follicles. The cells within 0.2 to 0.4 mm of the apices of the residual nevus tissue were damaged by each treatment; the thickness of the microscopic scar was increased by 0.2 to 0.5 mm with each treatment. An estimated 4 to 6 laser treatments were required for the microscopic scar to achieve a thickness of 1 mm.

**ABSENCE OF MALIGNANT CHANGES**

During the immediate and short-term phases following laser treatment in the 3 patients thus observed (patients...
Histological findings showed that the color of the congenital nevi was improved by the eradication of the nests of pigmented nevus cells in the epidermis and the upper dermis. The development of a microscopic scar about 1 mm thick was required to mask the underlying residual pigmentation. Improvement in skin texture was attributed largely to the presence of this subtle scar that preserved the architecture of the upper dermis, including the papillary interface with the epidermis.

In congenital nevi, the nevus cells tend to cluster and form globular nests. The use of high-energy fluences (13-30 J/cm²) combined with pulse durations of $1.0 \times 10^{-3}$ seconds of the normal-mode ruby laser should effectively heat the region measuring about 10 to $30 \times 10^{-6}$ m around the heavily pigmented cells. This corresponds closely to the size of the nests of nevus cells in the epidermis and upper dermis.

The globular shape of the nevus nests would maximize their eradication while minimizing the extent of thermal damage to the surrounding connective tissue. Because of its small surface area, the globular nevus nest has only a minimal interface to conduct the thermal energy produced by laser exposure. Therefore, thermal energy will persist within an individual nest of cells and damage them, even though some nevus cells are spared from direct thermal damage. Some nevus cells contain a small amount of melanin within their cytoplasm and are thus spared from such thermal damage produced by the ab-
The different clinical and histological findings obtained with the normal-mode ruby laser vs the Q-switched ruby laser may be due to their differing photothermolytic effects on the pigmented nevus cells. That is, the normal-mode ruby laser produces nonselective thermal damage, while the Q-switched ruby laser produces selective photothermolysis. 

Because of the globular shape of the nevus nests, there is a minimal conduction of heat to the surrounding tissue that also helps to reduce the visible scarring following such thermal injury. The resulting microscopic scarring may help to remodel the upper dermis, thus improving the skin texture.

The resulting thermal damage also involved the hair bulb, where numerous melanocytes and an abundance of melanin were aggregated in a well-demarcated pattern. Although the hair follicles tended to regenerate after each treatment, the hair shafts became thinner with successive treatments as the hair bulb was repeatedly exposed to the laser. Such a reduction in the presence of dense hair contributed much to the desired cosmetic effect.

The removal of excess unwanted body hair has recently been carried out by using a long pulsed ruby laser or alexandrite laser at rather high-energy fluences (30-60 J/cm²). 

The normal-mode ruby laser (Toshiba) produces a high-energy fluence (up to 45 J/cm²) at a long pulse duration (1 × 10⁻³ seconds). Also, such large amounts of energy can be delivered to an area as large as 15 × 15 mm in 1 shot and, therefore, could be useful in reducing excess hair.

In the present study, the Q-switched ruby laser was also used to eradicate the bluish color that sometimes became evident after the original deep pigmentation had been reduced by the normal-mode ruby laser. The pigmented nevus cells of such lesions were no longer clustered in nests, but numerous pigment-laden cells, mostly macrophages, were scattered in the upper dermis. The Q-switched ruby laser could selectively damage such pigmentation, as previously reported for oculodermal melanosis and tattoos.

In our short-term studies in 3 patients, the pigmented cells in the epidermis showed considerable thermal damage following each laser treatment. Therefore, the nests of pigmented nevus cells seen in the epidermis of the poorly responsive lesions presumably represent the regeneration of nevus cells that contained little melanin and were thus spared from thermal damage. It also seems likely that cells were regenerated from these nevus cells beyond the reach of the laser beam, including those in the hair follicles. Such regenerated nests of nevus cells in the epidermis would absorb thermal energy during successive laser treatments on a “first-come, first-served” basis. Therefore, the major components of the congenital nevi in the dermis would be spared from damage since they were sheltered from the laser beam.

It was surprising to find that a microscopic or subtle scar served to remodel the treated area, including the papillary interface with the epidermis. This could result from the fibroblasts being spared from the laser-induced thermal damage. Fibroblasts contain no melanin to absorb the ruby laser beam. They can thus restore the extracellular matrix and rebuild the connective tissue by arranging their fibers in accordance with the tensile loads placed on the skin. 

This implies that nevus cells had filled the spaces left in the dermal connective tissue. The cells that form in congenital nevi are believed to migrate from the neural crest during embryonic development.

The short-term studies presented showed that multiple normal-mode ruby laser treatments produced immediate thermal damage to the superficial nests of nevus cells and a subsequent remodeling of the superficial connective tissue. When the thickness of the microscopic or subtle scar reached approximately 1 mm, it masked the underlying residual nevus cells and achieved a good cosmetic result. The long-term study showed no histological or clinical evidence of the development of such malignant neoplasms as melanoma, squamous cell carcinoma, or sarcoma in the treated areas up to 8 years after the laser treatment. Nevertheless, further follow-up is required to determine whether the regenerated nevus cells of poorly responsive lesions, residual nevus cells, or both may influence the risk of the development of malignant neoplasms. Routine palpation of the area is recommended on follow-up to try to detect any changes occurring below the microscopic scar. The microscopic scar could mask such changes and obscure the remaining nevi.

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