Damage to hair follicles by normal-mode ruby laser pulses

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Background: Although many temporary treatments exist for hirsutism and hypertrichosis, a practical and permanent hair removal treatment is needed.

Objective: Our purpose was to study the use of normal-mode ruby laser pulses (694 nm, 270 μsec, 6 mm beam diameter) for hair follicle destruction by selective photothermolysis.

Methods: Histologically assessed damage in ex vivo black-haired dog skin after the use of different laser fluences was used to design a human study; 13 volunteers with brown or black hair were exposed to normal-mode ruby laser pulses at fluences of 30 to 60 J/cm^2, delivered to both shaved and wax-epilated skin sites. An optical delivery device designed to maximize light delivery to the reticular dermis was used. Hair regrowth was assessed at 1, 3, and 6 months after exposure by counting terminal hairs.

Results: Fluence-dependent selective thermal injury to follicles was observed histologically. There was a significant delay in hair growth in all subjects at all laser-treated sites compared with the unexposed shaven and epilated control sites. At 6 months, there was significant hair loss only in the areas shaved before treatment at the highest fluence. At 6 months, four subjects had less than 50% regrowth, two of whom showed no change between 3 and 6 months. Transient pigmentary changes were observed; there was no scarring.

Conclusion: Selective photothermolysis of hair follicles with the normal-mode ruby laser produces a growth delay consistent with induction of prolonged telogen with apparently permanent hair removal in some cases.

(J Am Acad Dermatol 1996;35:889-94.)

Excess hair growth in unwanted areas may result from heredity, endocrine disease, or drug therapy.[1] Temporary methods of removal include shaving, cold or hot wax epilation, and chemical depilatories that frequently cause contact dermatitis.[2] Electrolysis, which may be permanent, is tedious and usually only partially effective. Regrowth of 15% to 50% of hair after electrolysis has been reported.[3] Hair removal by laser has been attempted for trichiasis and for grafts obtained from hair-bearing skin.[4-8] However, destruction of hair follicles based on the theory of selective photothermolysis[9] has not been previously described. Melanin in the hair shaft or follicles, or both, provides a chromophore absent in the dermis surrounding these follicles. At deeply penetrating wavelengths in the 600 to 1100 nm region, melanin absorption may therefore be used for selective photothermolysis of hair follicles.[10]

We studied follicular damage, hair removal, and hair regrowth after exposure to normal-mode ruby laser (NMRL) at varying fluences. The fluences used were based on a preliminary study in ex vivo black-haired dog skin.

METHODS

Laser and delivery apparatus

A normal-mode ruby laser (model 936R4H-2, Lasermetrics, Winter Park, Fla.) was used. It emits 270 μsec pulses at 694 nm wavelength at up to 1 Hz. The beam was steered via a glass prism into a delivery device placed directly and firmly against the skin, immediately after the skin had been wiped with isopropyl alcohol.

The contact device was designed to maximize delivery of light to the reticular dermis while minimizing epidermal injury, by a combination of optical and thermal manipulations. A sapphire lens was used to provide a convergent beam (approximately 20 mm in focal length) at

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Accepted for publication July 27, 1996.

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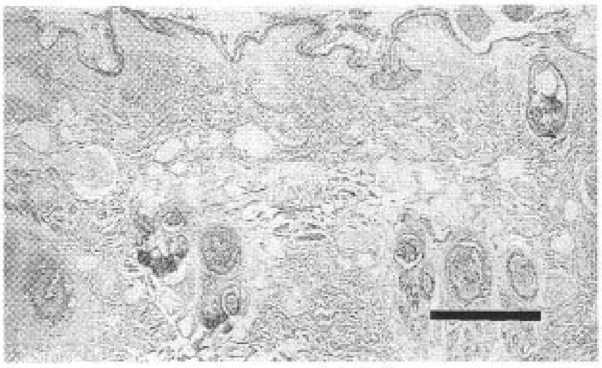

Fig. 1. Photomicrograph of black-haired dog skin after 40 J/cm^2 ruby laser pulse. There is minimal damage to follicular epithelium without adjacent collagen damage. *Bar* = 500 μm.

the skin surface and to increase beam coupling into the skin as compared with air as the external medium. In theory, a convergent beam as it enters the skin produces an increase in irradiance with depth greater than a collimated beam, to a depth of at least $1/\mu_s'$, where μ_s' is the transport scattering coefficient.[11] At 694 nm in skin, μ_s' is approximately 3 to 5 mm^{-1}.[12] The sapphire lens was cooled to 4° C to provide heat conduction from the epidermis before, during, and after each laser pulse. Delivered pulse energy into air was measured with a laser energy meter (model 351, Scientech, Boulder, Colo.).

Pilot histologic study in black-haired dog skin

Freshly clipped black-haired dog skin was exposed in vitro to varying fluences from the NMRL. Both the fluence and exposure spot size were varied. Laser exposure parameters were 40 J/cm^2, beam diameter 8 mm; 70 J/cm^2, beam diameter 6 mm; and 160 J/cm^2, beam diameter 4 mm. Punch biopsy specimens (3 mm) were obtained from exposed and adjacent unexposed sites and processed routinely for light microscopy.

Human study

Thirteen healthy adult volunteers (12 men, 1 woman) were recruited through newspaper advertisements. All had fair skin (Fitzpatrick's type I, II, or III) and brown or black hair. Exclusion criteria included photosensitivity, pregnancy, history of keloid formation, immunosuppression, or a history of poor wound healing.

After informed consent was obtained, an area was chosen on the back or posterior thigh, based on uniformity and density of terminal hair follicles. Eight 3 × 2 cm sites were mapped and photographs were taken. Hair counts were obtained and recorded from each site, by two independent observers. Before laser exposures, half of these sites were shaved and the remaining sites were epilated with cold wax (My-Epil, Ella Bache, New York, N.Y.).

One shaven site and one wax-epilated site served as unexposed controls. The remaining sites were irradiated with the NMRL (6 mm beam diameter at the skin surface) at fluences of 60 J/cm^2, 40 J/cm^2, and 30 J/cm^2. Adjacent nonoverlapping pulses were placed to treat each 3 × 2 cm area. In four subjects, treatment was done after local intradermal injection of lidocaine 1%. After exposures, bacitracin ointment was applied, and subjects were instructed to cleanse the sites gently twice a day. In four subjects, 3 mm punch biopsy specimens were obtained before treatment and within 2 hours after the laser exposures from both the wax-epilated and shaven sites and were processed routinely. Clinical evaluation, hair counts, and photographs were obtained at 1, 3, and 6 months after exposures. Histologic specimens were evaluated blindly by a dermatopathologist (T. F.).

Data analysis

Hair regrowth was defined as the percentage of terminal hairs present after treatment compared with the number before treatment. For each site at each follow-up visit, hair regrowth was calculated. Results for each experimental condition were pooled for all subjects. The mean and standard deviation for each condition were calculated. A paired t test for significant differences between posttreatment and pretreatment hair counts was done for each experimental condition at the 1-, 3-, and 6-month observation times.

RESULTS

Histologic study in ex vivo dog skin

Control skin showed no alterations in the follicular epithelium or dermal collagen. After irradiation at different fluences, damage to the follicular epithelium had occurred, seen as increased eosinophilia of the cytoplasm and condensation and elongation of nuclei. Collagen damage was seen as increased basophilic staining and homogenization of the fibrillar structure. At 40 J/cm^2, there was focal damage to the follicular epithelium and alteration of the periadventitial collagen with focal collagen damage in the reticular dermis immediately adjacent to the hair follicle (Fig. 1). At 70 J/cm^2, there was more extensive damage to the follicular epithelium and periadventitial dermis, with extension of damage to collagen in the interfollicular reticular dermis (Fig. 2). At 160 J/cm^2, there was confluent damage to dermal structures, reticular dermis, and adnexae.

Human study

Subjects described the exposures as moderately to strongly painful, and a "rotten-egg" smell occurred

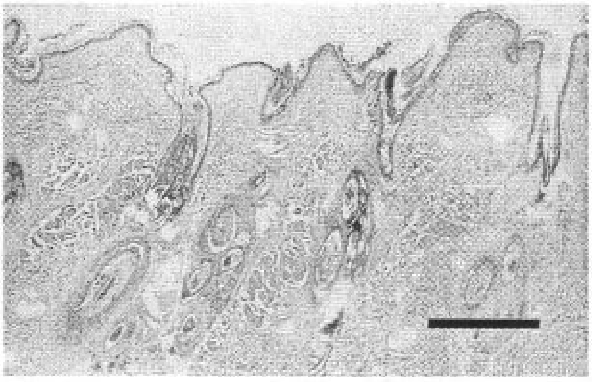

Fig. 2. Photomicrograph of black-haired dog skin after 70 J/cm^2 ruby laser pulse. There is coagulation of follicular epithelium as well as surrounding dermal collagen. *Bar* = 500 μm.

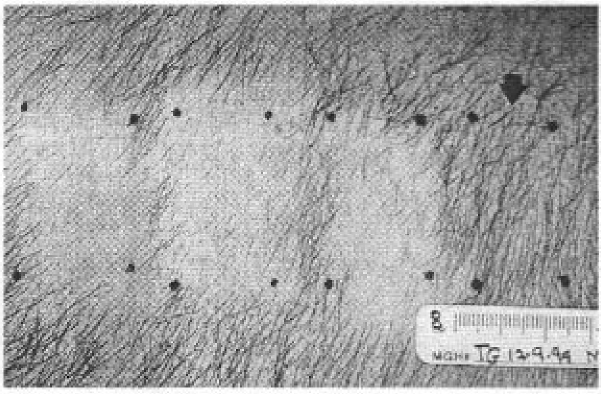

Fig. 3. Appearance of epilated sites treated with 60, 40, and 30 J/cm^2 pulsed laser *(left to right)* and untreated epilated control *(arrow)* 3 months after treatment. Hair regrowth is decreased at all laser-treated sites.

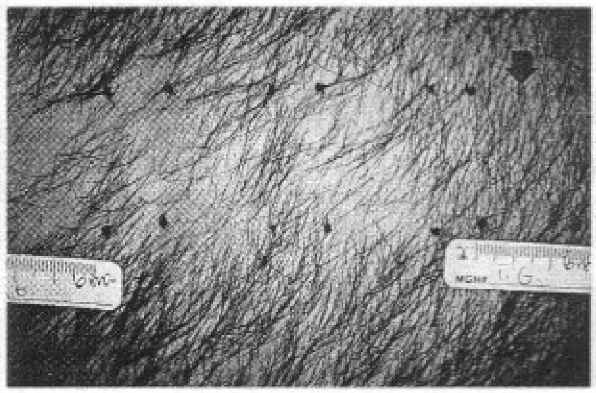

Fig. 4. Same sites and subject as in Fig. 6, 6 months after treatment. Hair regrowth is still decreased at laser-treated sites.

with exposures. Immediate responses to treatment were variable and fluence-dependent. All sites became erythematous and edematous. In addition to erythema, immediate whitening developed in one subject similar to that seen with Q-switched ruby laser exposure. One subject showed epidermal ablation at all fluences. Purpura confined to the exposure sites occurred in three subjects.

Figs. 3 and 4 illustrate regrowth of hairs 3 and 6 months after treatment, respectively. The subject illustrated is typical and did not show the greatest hair removal. A fluence-dependent growth delay is apparent at treated sites, with decreased hair growth at 3 months. At 6 months, much of the hair has regrown. In a few subjects, the regrowing hair was finer in laser-treated sites than in the adjacent untreated area. In addition, in one person the regrowing hairs in the treated site were lighter.

Fig. 5 shows the pooled percentage of hair regrowth at 1, 3, and 6 months as a function of fluence in epilated and shaven sites for all subjects. A statistically significant growth delay was seen at 1 and 3 months for all fluences at both shaven and epilated sites in comparison with the unexposed shaven and epilated control sites. At 6 months, there was significantly less hair only in the shaved sites treated with the NMRL at 60 J/cm^2 compared with the untreated control. At 6 months, 5 of the 13 subjects had complete regrowth of hair in the treated sites, and four still had less than 50% regrowth. Of these four, two showed no regrowth between 3 and 6 months.

Hyperpigmentation was present in three subjects and had cleared in all three by the 6-month follow-up visit. Two subjects had transient hypopigmentation. One subject failed to follow the postexposure care instructions; healing at one of the six exposure sites was prolonged, with erythema and hyperpigmentation still present at 6 months. No scarring was seen in any subject.

Histologic findings in human study

Untreated shaven and wax-epilated control biopsy specimens were compared with those from laser-treated sites. The follicular epithelium was intact in all control (unexposed) sites. Laser exposure caused damage to follicular epithelium in both wax-epilated and shaven sites, seen as increased eosinophilia and nuclear elongation. Hair shafts showed fragmentation and eosinophilia. In one case, focal thermal coagulation of perifollicular collagen in the dermis occurred at the 60 J/cm^2 fluence. In all cases, there was heterogeneous but widespread injury to follicular

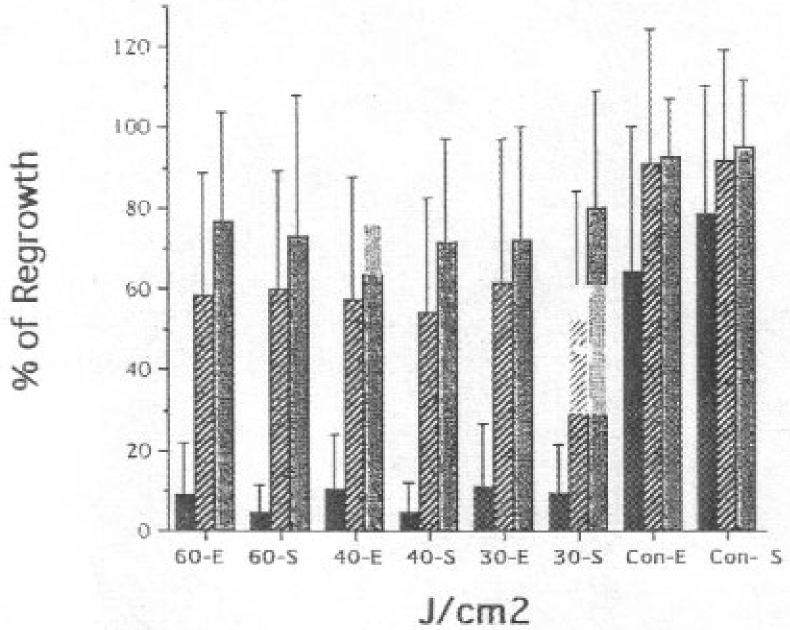

Fig. 5. Hair regrowth at 1, 3, and 6 months for each fluence (60, 40, and 30 J/cm²) in shaved *(S)* and epilated *(E)* sites, compared with unexposed control *(con-E, con-S)* sites. ■, 1 month; ▨, 3 months; ▩, 6 months.

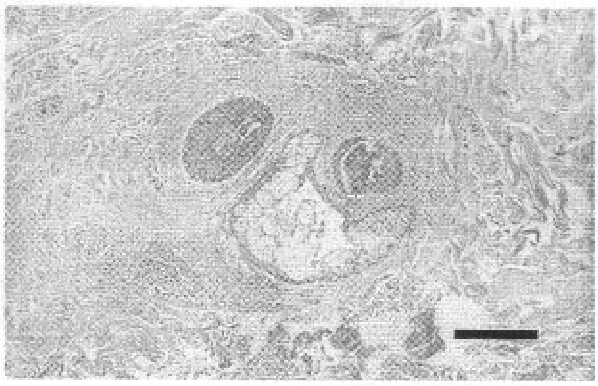

Fig. 6. Photomicrograph of human hair follicle in cross section after exposure to 60 J/cm² ruby laser pulse in site shaved before exposure. Thermal damage to the follicular epithelium and focal rupture are noted. *Bar* = 200 μm.

epithelium, seen as cytoplasmic eosinophilia and nuclear condensation. In all cases, the follicular epithelium had ruptured in isolated areas. Hemorrhage was not seen. Epidermal injury was difficult to evaluate in the horizontal sections obtained. Little or no apparent histologic difference was noted between specimens obtained from areas exposed to different fluences (30, 40, and 60 J/cm²). Fig. 6 shows a horizontal section of a thermally and mechanically damaged specimen, taken immediately after treatment of a shaven site with the 60 J/cm² fluence. The hair shaft shows fragmentation and increased eosinophilia, and the follicular epithelium shows increased eosinophilia. There is no damage to the perifollicular dermis.

DISCUSSION

Lasers have been used to treat trichiasis[4-6] and to remove hair from skin grafts.[7, 8] None of these case reports used wavelengths or laser pulses based on selective photothermolysis, which predicts that selective thermal damage of a pigmented target structure will result when a sufficient fluence at a wavelength preferentially absorbed by the target is delivered during a time equal to or less than the thermal relaxation time of the target.[9] This approach underlies the use of pulsed lasers for removal of microvascular lesions, pigmented lesions, and tattoos.

The use of photodynamic therapy using topical aminolevulinic acid followed by red light exposures for the destruction of hair has been reported.[13] Aminolevulinic acid induces follicular synthesis of protoporphyrin IX,[14] a potent photosensitizer. A 40% decrease in hair regrowth was found 6 months after a single treatment. Application of a carbon-particle suspension followed by removal from the skin surface and exposure to Q-switched Nd:YAG laser pulses has also been reported.[15] In this approach, carbon particles reportedly enter the follicle and provide local light absorption. The data suggest a growth delay at 3 months, but control sites and further follow-up have not been described.

We aimed to selectively destroy hair follicles in a large field and therefore chose a laser and optical delivery system consistent with the theory of selective photothermolysis. In the visible-near infrared region, melanin is the logical natural chromophore of choice for targeting hair follicles. Previous studies have shown deep selective damage to isolated pigmented cells in follicles in animals after Q-switched ruby laser exposures,[16] thereby causing leukotrichia. However, the submicrosecond pulses of Q-switched lasers are probably not ideal for follicle destruction because of insufficient heat transfer during the pulse to other nonpigmented cells of the follicle. In general with longer pulses, local heat transfer during the laser pulse heats a larger volume and requires greater fluence to achieve damaging temperatures. Therefore we used the normal (long-pulse) ruby laser to broaden the zone of thermal damage within and around the follicle. In the ex vivo dog skin experiment, we determined that NMRL fluences of approximately 40 to 70 J/cm^2 were required for selective follicular damage, which also appears to be the case for the subjects in this study. The 694 nm ruby laser wavelength lies in an "optical window"[10] of the spectrum, with the desired combination of selective absorption by melanins and deep penetration into the dermis. At this wavelength, approximately 15% of the incident light penetrates the entire dermis (unpublished observation). However, other red and near-infrared wavelengths should also work, to approximately 1100 nm based on eumelanin absorption spectra.[10] Hair also contains significant quantities of pheomelanin, which exhibits a different absorption spectrum in the red and near-infrared region.[17]

Skin anatomy and tissue optics are such that it is difficult to achieve the light levels needed for selective photothermolysis of the deep portions of hair follicles. The epidermis contains competing eumelanin through which light must pass to reach the follicle. Ideally follicles should be damaged without much epidermal injury. Therefore we designed and built a delivery device intended to optimize deep light delivery and to limit epidermal injury. The factors used to optimize light delivery were (1) a high-refractive-index external medium, (2) a convergent entry beam at the skin, (3) a large exposure spot diameter, and (4) forceful compression of the skin to eliminate blood and deform the dermis enough to reduce the distance between the surface and hair papillae. In addition, the device was cooled to extract more heat from the epidermis on contact. However, the influence of each of these factors has not yet been quantified.

In theory, the optimal pulse duration for selective photothermolysis is less than or approximately equal to the thermal relaxation time of the target structure. Thermal relaxation time (t_r) is given by the equation $t_r = d^2/g\kappa$, where d is a target dimension, κ is thermal diffusivity (about 2×10^{-3} cm^2/sec), and g is a geometric factor.[18] Crudely, the thermal relaxation time in seconds is approximately equal to the square of the target diameter in millimeters.[19] We estimate the thermal relaxation time to be about 40 to 100 msec for follicles 200 to 300 μm in diameter. Ideally the laser pulse duration would lie between the thermal relaxation times for epidermis (about 3 to 10 msec) and the target follicles. In this setting, it would be possible to extract heat *during* the laser pulse by conduction from the epidermis, while thermal confinement is maintained in the hair follicles. It is likely, therefore, that a pulse duration of approximately 10 to 50 msec could damage hair follicles with less epidermal injury than the pulses used in this study. In this study, the pulse duration was 270 μsec, well below the estimated thermal relaxation time for both follicles and epidermis.

We expected that the pigmented hair shaft would function as a chromophore. This hypothesis was tested by comparing response in areas shaven versus epilated before laser exposures. The presence of a hair shaft during laser exposure was not essential to induce a significant growth delay, which occurred at all fluences in both shaven and epilated sites. Presumably, there is ample melanin in the follicular epithelium and papillae alone to act as a chromophore for light absorption in the follicle. At 6 months, however, there was significant hair loss only in the shaven sites treated at the highest fluence, suggesting that the presence of the pigmented hair shaft enhances selective photothermolysis of hair follicles. We also expected to see more of a relation between fluence and response with respect to hair regrowth. However, the hair regrowth data within and between subjects does not show a significant fluence-response relation. The data in this study therefore suggest a low damage threshold for induction of growth delay and a higher damage threshold for permanent hair loss.

The mechanisms of follicular injury in this study are thermal, but the relative importance of thermal denaturation versus vaporization and mechanical damage remain uncertain. Biopsy specimens obtained immediately after laser exposure showed selective but heterogeneous follicular damage, consisting of thermal coagulation and asymmetric focal ruptures of the follicular epithelium. The heterogeneous distribution of thermal coagulation may result from local variations in follicular melanin concentration. The presence of focal ruptures suggests that vaporization (steam formation) occurred and therefore that the temperature exceeded 100° C in some areas of the follicle. In a follicle occluded by the contact delivery device, it is likely that any vaporization would lead to dissections along the follicle.

Questions remain unanswered regarding the location of the key follicular target(s) and the possible influence of the hair growth cycle on laser-induced hair removal. It had been assumed that hair stem cells are found in the matrix area of the hair bulb. However, recent evidence in mice suggests that follicular stem cells are located in a bulge, near the attachment of the arrector pili muscle in the outer root sheath. A new model of hair cycling has been proposed,[20] in which during late telogen the dermal papillae comes in close proximity to the bulge cells, which are then stimulated to proliferate and form an active new hair matrix (anagen stage). The cells grow downward in association with the dermal papillae and become hair matrix cells during early anagen. In anagen, the matrix cells proliferate for a fixed duration that determines maximum hair length. During catagen, the hair matrix cells regress, followed by retraction of the papillae to an area adjacent to the bulge, where it remains until the cycle is renewed. If the bulge hypothesis is true in human beings, then the bulge or the hair papillae, or both, are important targets for permanent hair follicle destruction.

Because the depth of the dermal papillae is dependent on the stage of the hair cycle, it is possible that cycle stage influences susceptibility to laser injury. During telogen, papillae are more superficially located and near the bulge. In contrast, during anagen, the papillae are deeper and further away from incident laser light. Therefore the papillae of telogen follicles should be more susceptible to photothermal injury. However, melanogenesis and the susceptibility of bulge cells may cause different cycle-dependent effects. Bulge cells during late telogen are rapidly dividing, whereas throughout the rest of the hair cycle they are quiescent; this may affect their susceptibility to injury. A hypothesis that cannot be addressed with the present data is that the regrowing hairs in this study represent those in the least sensitive phase of their cycle at the time of laser exposure. If true, this suggests that multiple treatments given at intervals consistent with follicular cycling would be more effective.

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