Selective Photothermolysis of Cutaneous Pigmentation by Q-switched Nd: YAG Laser Pulses at 1064, 532, and 355 nm

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Exposure of skin to nanosecond-domain laser pulses affects the pigmentary system by a process called selective photothermolysis, in which melanosomes and pigmented cells are preferentially altered. Due to the broad absorption spectrum of melanin, this effect may occur with wavelengths that penetrate to vastly different depths within tissue, potentially producing different biologic responses. The effects of single near-ultraviolet (355 nm), visible (532 nm), and near infrared (1064 nm) pulses of 10–12 nsec duration were determined in guinea pig skin using gross, histologic, and electron microscopic observations. Threshold response in pigmented skin was a transient immediate ash-white discoloration, requiring 0.11, 0.20, and 1.0 J/cm², at 355, 532, and 1064 nm, respectively. At each wavelength, melanosomes were ruptured within keratinocytes and melanocytes, with cytoplasmic and nuclear alterations. Delayed epidermal depigmentation occurred, followed by gradual repigmentation. Deep follicular cells were altered only at 532 and 1064 nm, which produced permanent leukotrachia. The action spectrum for threshold response was consistent with mechanisms implied by selective photothermolysis. These data may be useful for consideration of treatment for cutaneous pigmentation abnormalities or unwanted follicular pigmentation, or both. J Invest Dermatol 93:28–32, 1989

Pulses of light that are sufficiently brief and are preferentially absorbed by pigmented structures in tissue can cause selective heating and thermal damage to the pigmented structures. This form of thermal injury, known as selective photothermolysis, can be localized on a microscopic scale, and is essentially unique to pulsed laser sources [1]. Different wavelengths can be used to preferentially damage different pigmented structures. For example, 577 nm yellow laser pulses are strongly absorbed by hemoglobins, and result primarily in microvascular injury [1–5]. In contrast, selective injury to melanin and pigmented cells occurs after 351 nm (15 nsec duration) ultraviolet laser pulses (1.6), or 694 nm (40 nsec duration) red pulses from a Q-switched ruby laser (7.8). For these wavelengths, absorption by melanin dominates over that by blood (9), and the site of primary injury appears to be melanosomes. A short pulse duration (about 1 μsec) is necessary because of rapid thermal relaxation by melanosomes [1].

Previous studies of selective photothermolysis of cutaneous melanosomes have been performed over a limited wavelength range, despite the broad absorption spectrum of melamins [9,10]. In particular, deeper structures should be affected by near-infrared radiation pulses [9]. This study was performed to examine these hypotheses, using 12 nsec Q-switched Nd-YAG (neodymium-yttrium-aluminum-garnet) laser pulses at 355, 532, and 1064 nm in pigmented and albino guinea pig skin.

MATERIALS AND METHODS
A Quanta-Ray DCR-IIA Q-switched Nd-YAG laser with amplifier and harmonic generation optics was used. This produced 1064 nm (12 nsec), 532 nm (10 nsec), and 355 nm (10 nsec) pulses. A suitably uniform (± 20%) 2.5-mm exposure field was produced by passing the beam through a diffuser made by etching a standard microscope slide in 50% aqueous hydrofluoric acid for 30 sec. A 20-cm focal length planoconvex lens was used to converge the 1 cm beam to 3 mm diameter, at which position a 2.5-mm round aperture was used, against which the animals were held. Pulse energy was measured through the aperture with a Scientek 352 laser energy meter, calibrated to ± 10% accuracy.

The backs of two albino Hartley and two black English long-haired guinea pigs were wax epilated 2 d before exposures. Animals were anesthetized with intramuscular ketamine, atropine, and xylazine. For each wavelength and animal, a response threshold was first determined, defined by the minimal radiant exposure per pulse that caused an immediate ash-white macule completely filling the 2.5-mm circular exposure area in all of five identical exposure sites. This response occurred only in pigmented animals, and was always definable within a ±0%–10% range of exposure at each wavelength. Adjacent sites were then exposed to pulses at 0.25, 0.5, 1.0, 2.0, and 3.0 (1064 nm) or 4.0 (355 and 532 nm) times the threshold exposure for each laser wavelength. Albino and pigmented animals were exposed to the same exposures. For each exposure dose, seven adjacent sites separated by 5 to 10 mm were identically exposed, to allow serial biopsies and observation. Sites received only one pulse.

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Trephine biopsies (2 mm) were performed after local anesthesia with intradermal 2% xylocaine. Paraffin-embedded sections were stained with hematoxylin-eosin and Fontana–Masson silver stain. To visualize melanocytes, biopsies were bisected and incubated with 3,4-dihydroxyphenylalanine as described [11], then embedded and sectioned routinely. Biopsies were also obtained at 1, 8, and 38 d after exposures, from separate identically exposed sites. Immediate biopsies were also obtained for transmission electron microscopy. These were immersed in Karnovsky’s II solution, then minced into 1 mm³ cubes. After 5 h, these were rinsed in 0.1 M sodium cacodylate buffer, postfixed with 2% osmium tetroxide for 2 h, rinsed twice with 0.1 M sodium cacodylate buffer, dehydrated in graded ethanol solutions, and embedded in Epon. Sections from a Porter Blum MT2-B ultramicrotome were stained with uranyl acetate and lead citrate; a Zeiss EM109 electron microscope was used.

Ellipses (1 × 2 cm) were excised from epilated black guinea pigs under general anesthesia, and kept at 37°C for 12 h, with the dermis side in 10⁴-⁴⁵ M trypsin. The epidermis was gently removed, inspected for gross damage, a portion processed for histology, and the remainder placed on a quartz microscope slide that served to hold the tissue at the entrance port of a Beckman 5270 integrating-sphere spectrophotometer. A quartz slide was also placed at the reference beam entrance. Diffuse transmittance was measured from 250 to 1200 nm. Three measurements were obtained with equivalent results after repositioning of each sample.

RESULTS

Gross Responses In pigmented animals, the threshold response at all wavelengths was a uniform ash-white macule that gradually faded within 30 min to a subtle edematous plaque. The radiant exposure (T) necessary for this response was wavelength-dependent as follows: 0.11 J/cm² at 355 nm, 0.20 J/cm² at 532 nm, and 1.0 J/cm² at 1064 nm. Exposures greater than T produced more intense and longer-lasting whitening. At 532 nm, exposure to 4T (0.80 J/cm²) produced a purplish macule, seen after fading of the immediate ash-white discoloration. Purpura was not observed at other wavelengths in the black animals. In albino animals, there was no response to 3T at 1064 nm, the highest available exposure. At 532 nm and 355 nm, exposures equal to or greater than T led to purpura appearing within seconds. Transient whitening was not observed in albino skin.

During the week after threshold and suprathreshold exposures in pigmented skin, fine exfoliation was followed by gradual, transient hypopigmentation to varying degrees. At 355 nm, mild hypopigmentation occurred after 2T or 4T. At 532 nm, mild hypopigmentation occurred after 1T, and marked hypopigmentation after 2T and 4T. At 1064 nm, 2T and 3T caused frank depigmentation. Repigmentation was evident within 4 wk, and was most rapid for the 355-nm exposures. Subthreshold exposures did not develop hypopigmentation.

Permanent leukotrichia (white regrowing hair) developed within 2 wk of exposures greater than or equal to T, at 532 and 1064 nm. Even the highest exposure at 355 nm failed to induce leukotrichia. When leukotrichia was present, regrowing hairs were uniformly white, suggesting a sustained failure of follicular pigmentation beginning abruptly after laser exposure. Leukotrichia lasted over 6 mo.

Electron Microscopic Observations The findings are summarized in Table I. In pigmented epidermis, most melanosomes within keratinocytes and melanocytes were altered by exposures of T or higher at all wavelengths. Melanosomes appeared ruptured (Fig 1), similar to those reported after 351-nm excimer laser [1,6] or 694-nm Q-switched ruby laser exposures [7]. At exposures of 3T (1064 nm) or 4T (532 and 355 nm), few recognizable melanosome remained throughout the epidermis. The unmelanized melanosome in albino animals were unchanged by laser exposures.

The depth to which melanosomes were altered within follicular epithelium was wavelength-dependent. Exposures of T at 532 and 355 nm produced alterations throughout the epidermis but without extension into follicles. In contrast, exposures of T at 1064 nm altered melanosomes to a depth of 0.2 mm. Deep (>1 mm) melanosome alterations were produced by 3T at 1064 nm and 4T at 532 nm, but not at 355 nm.

Additional changes in pigmented keratinocytes included large vacuolization and condensation of both cytoplasmic and nuclear elements. Subtle cytoplasmic changes were seen in albino skin at high exposures. In contrast, nuclear alterations (Fig 1), seen only in pigmented epidermis after threshold or greater exposures, were clearly related to the presence of melanin pigment. Bizarre-shaped, vacuolated, and electron-dense erythrocytes were noted within superficial vessels of the dermis after suprathreshold exposures at 532 and 355 nm (Fig 2). At 1064 nm, a wavelength relatively poorly absorbed by hemoglobin [9], erythrocytes appeared normal.

Light Microscopic Observations

Immediate Exposures less than T failed to produce consistent immediate histologic changes. At all three wavelengths, threshold and greater exposures produced epidermal damage in pigmented skin only. The most striking feature was “ring cells” [7], i.e., obvious vacuolated, usually basal cells with peripheral condensation of pigment. Fontana–Masson staining showed decreased prominence of epidermal melanocyte dendrites, but the degree of staining was not notably affected. At higher exposures, there was focal intrabasal separation of the epidermis. At 1064 and 532 nm only, ring cells were clearly present within follicular epithelium (Fig 3).

Other immediate changes included coagulation of erythrocytes within superficial vessels after threshold or greater exposures at 532 nm and, to a much lesser extent 355 nm, in both albino and pigmented skin. This appeared as homogeneous, hypereosinophilic staining within vessels. The dermis appeared otherwise unaltered.

Twenty-four Hours After Exposure Exposures of T at all three wavelengths showed subtle but consistent alterations. Scattered pigmented dyskeratotic epidermal cells were noted. There was an increased density of pigment in keratinocytes and prominence of melanocyte dendrites, especially with 1064-nm exposures.

Twenty-four h after threshold (T) exposures, there was a subepidermal vesicle in the 532- and 355-nm-irradiated specimens, with dyskeratotic pigmented epidermal cells and evidence of reepithelialization at all three wavelengths. The regenerative epithelium contained occasional, prominent melanocytes and scattered pigment granules, although less than in control epidermis.

Twenty-four h after suprathreshold exposures, at all three wavelengths, there was a subepidermal vesicle with full thickness necrosis of the pigmented roof, and reepithelialization along the dermoepidermal junction. There was a striking difference, however, between wavelengths in the pigmentation of this regenerative epithelium. For exposures of 2T, at 1064 nm there was virtually no pigment, and no discernible melanocytes within the regenerating epidermis. At 532 nm, there was a marked reduction in pigmentation. At 355 nm, pigmentation and melanocytes in the regenerating epidermis were similar to those of control epidermis. For exposures of 4T at 532 nm and 3T at 1064 nm, there was no pigment or recognizable melanocytes in regenerating epithelium. In contrast, pigmentation was easily seen for the 355-nm exposures of 4T. Within follicular epithelium, focal dyskeratotic pigmented cells and residual ring cells were apparent for exposures of 4T at 1064 and 532 nm. In contrast, the follicular epithelium appeared normal for exposures of 4T at 355 nm. At 24 h after all suprathreshold exposures, there was a mild degree of mixed acute and chronic inflammatory cell infiltrate in the upper dermis, with no evidence of residual erythrocyte agglutination or vasculitis.

Eight Days After Exposure Subthreshold exposures at all wavelengths produced increased pigmentation and prominence of dendritic melanocytes over unirradiated controls (Fig 4). This was most pronounced after 355 nm, T exposures. The epidermis was not hyperplastic.

Eight d after 1T at 1064 nm, there was complete depigmentation of the epidermis. There were prominent dendritic melanocytes,
Table 1. Ultrastructural Changes of Guinea Pig Skin

<table>
<thead>
<tr>
<th>Wave</th>
<th>Radiant Exposure (J/cm²)</th>
<th>Nucleus</th>
<th>Cytoplasm</th>
<th>Hair Follicle Alteration</th>
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<td></td>
<td>Internal Disruption</td>
<td>Condensation</td>
<td>Vacuolation</td>
<td>Condensation</td>
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<tr>
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<td>Disruption</td>
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<td>1064</td>
<td>3.0</td>
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<td>+</td>
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<td>1.0*</td>
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<td>532</td>
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<td>0.028</td>
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+, absent; ±, scarcely present; +, present; ++, predominantly.
* Clinical thresholds; depth (mm) of the deepest alteration from the surface.

Figure 1. Electron micrograph of pigmented guinea pig basal keratinocytes, taken immediately after exposure of 3T at 1064 nm. Melanosomes appear fractured (arrows); there is peripheral clumping of nuclear chromatin (N); desmosomes and basement membrane (*) appear intact. Bar: 1 μm.

Figure 2. Electron micrograph of blood vessel from pigmented guinea pig skin taken immediately after exposure of 4T at 532 nm. Bizarre, electron-dense, vacuolated erythrocytes (AE) are seen within an otherwise intact vessel. Normal erythrocytes (NE), endothelial cells (E), pericytes (P), and collagen (C) are seen. Bar: 1 μm.
however, within the lower portions of the follicular epithelium and hair bulb. In contrast, after 1T at 532 and 355 nm, regenerated epidermis contained pigment and melanocytes similar to unirradiated skin.

At 8 d after suprathreshold exposures at 1064 and 532 nm, there was complete depigmentation of the regenerated epidermis (Fig 5), and sparse follicular pigmentation. In contrast, the 355-nm exposures showed epidermal pigmentation and melanocytes similar to unirradiated skin. There was rare incontinent pigment in deep dermis after suprathreshold, 1064 nm exposures only. There was no evidence of fibrosis.

Thirty-eight Days After Exposure Sites exposed to 2T or 3T at 1064 nm, and to 4T at 532 nm, developed leukotrichia. There was a complete absence of DOPA-positive cells or Fontana–Masson staining in the follicles of depigmented hairs.

Pigmented Epidermis Transmittance The transmittance of pigmented guinea pig epidermis increased gradually between 300 and 1200 nm, consistent with prior observations [9,12]. These results are presented as part of Figure 6. Histologically, the samples consisted of stratum corneum plus 2–3-cell layers of stratum spinosum.

**DISCUSSION**

This study shows that melanosomes, when pigmented, are a major site for initiation of cutaneous damage caused by single submicrosecond laser pulses in the near ultraviolet, visible, and near infrared

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**Figure 3.** Histology of pigmented skin taken immediately after suprathreshold exposures at the three laser wavelengths; hematoxylin-eosin stain. A: Unexposed control skin. B: Exposure of 3T at 1064 nm, showing “ring cells” (arrow) in epidermis and follicles. C: Exposure of 4T at 532 nm, showing similar changes. D: Exposure of 4T at 355 nm, showing ring cells in epidermis only.

**Figure 4.** Increased prominence of melanocyte dendrites and melanin pigment in basal keratinocytes, seen by Fontana–Masson stain 8 d after subthreshold exposures. A: Unirradiated control. B: Eight days after exposure of 4T at 1064 nm. A similar stimulation was seen after subthreshold exposures at 532 and 355 nm.

**Figure 5.** Depigmentation of epidermis 8 d after suprathreshold exposures at 1064 and 532 nm; Fontana–Masson stain for melanin. A: Unexposed control showing epidermal pigmentation. B: Exposure of 3T at 1064 nm, showing complete depigmentation.
portion of the spectrum. At widely separated wavelengths, melanosome alterations were qualitatively similar but differed in depth distribution. These findings are consistent with the phenomenon of selective photothermolysis [1,7,8]. The threshold response is immediate, transient epidermal whitening, and correlates well with melanosome rupture (Table 1). Although immediate whitening may be directly linked to a laser-induced phase change in melanosomes, this study does not test such mechanisms.

The threshold exposure dose is wavelength-dependent in a manner grossly consistent with absorption spectra of melamins, i.e., greater exposure is required at longer wavelengths. When plotted as an uncorrected action spectrum, however, there is poor quantitative agreement with spectra for DOPA – melanin or eumelans. In essence, there is less sensitivity to the shorter wavelengths than might be expected. This may be due to several factors. First, optical transmission by the overlying stratum corneum and epidermis would modify any action spectrum based on primary events occurring in the lower epidermis. This appears to be the case in this study. After correction for epidermal transmission (Fig 6), the action spectrum closely approximates absorption spectra of melamins. Note that the action spectrum shown is not quantum-corrected, because selective photothermolysis is driven by absorbed energy rather than absorbed quanta, per se. Second, at wavelengths for which single melanosomes are nearly opaque, the physical cross section limits absorption of incident energy [1]. This effect also skews the action spectrum in the manner observed. Finally, quantitative absorption spectra of single, isolated melanosomes have not been performed, and native melanosomes may differ from isolated melanosomes.

The nuclear changes seen immediately after exposure (Fig 1) require the presence of pigmented melanosomes. It is plausible that nuclear fragmentation is caused by local high pressure waves emanating from rupturing melanosomes: similar fragmentation has been described after 193-nm excimer laser ablation of skin [13], thought to be due to such pressure waves. It is also plausible, however, that some other mechanism secondary to melanosome rupture, such as release of reactive compounds, may be involved.

Surprisingly, melanogenesis is apparently stimulated by sub-threshold exposures at all three wavelengths. It remains unknown whether this stimulation is due to primary or secondary effects on melanocytes. It is also unknown whether such stimulation occurs in human skin.

The more penetrating, longer-wavelength pulses caused selective damage to pigmented cells at greater depths. Accordingly, permanent leukotrichia due to follicular depigmentation resulted only from 1064 and 532 nm, with the most effective wavelength relative to threshold exposures being 1064 nm. It is unclear why leukotrichia was permanent, in contrast with the transient epidermal depigmentation. This has also been reported after freeze injury [14]. At 532 and 355 nm, wavelengths absorbed by hemoglobin, limited microvascular injury was also noted. The dominant cutaneous responses, however, were related to pigmented cell injury. At all wavelengths and exposures, neither scarring nor alterations in skin texture were observed.

This study may be useful for considering treatment of pigmentation abnormalities or unwanted follicular pigmentation in human skin, or for stimulation of pigmentation. Selective photothermolysis with nanosecond-domain laser pulses produces major alterations of the cutaneous pigmentary system. Skin responses are strikingly different after such injury from different spectral regions.

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REFERENCES

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