Effect of Dye Laser Pulse Duration on Selective Cutaneous Vascular Injury

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The pulsed dye laser at 577 nm, a wavelength well absorbed by oxyhemoglobin, causes highly selective thermal injury to cutaneous blood vessels. Confinement of thermal damage to microvessels is, in theory, related to the laser exposure time (pulsewidth) on selective vascular injury. This study investigates the effect of 577 nm dye laser pulsewidth on selective vascular injury. Nine Caucasian, normal volunteers received 577 nm dye laser exposures at pulsewidths of 1.5–350 µs to their skin. Clinical purpura threshold exposure doses were determined in each volunteer, and biopsies of threshold and suprathreshold doses were examined in each volunteer. The laser exposure dose required to produce purpura increased as pulsewidth increased in all 9 subjects (p < 0.001). This finding corresponds to laser pulsewidths equal to or exceeding the thermal relaxation times for dermal blood vessels. Histologically, vessel damage was selectively, but qualitatively, different for short vs long pulsewidths. Pulsewidths shorter than 20 µs caused vessel wall fragmentation and hemorrhage, whereas longer pulsewidths caused no significant hemorrhage. The purpura noted clinically appears to be due to a coagulum of intraluminal denatured erythrocytes. At 24 h, there was marked vessel wall necrosis at all pulsewidths. The short pulsewidths may cause erythrocyte vaporization, rapid thermal expansion, and mechanical vessel rupture with hemorrhage. Long pulsewidths appear to cause thermal denaturation with less mechanical vessel damage. The selective, nonhemorrhagic, vascular necrosis caused by the long-pulsewidth dye laser may lead to a more desirable clinical outcome in the therapy of blood vessel disease processes. J Invest Dermatol 87:653–657, 1986

Much of laser therapy in dermatology stems from observations made by therapists and improved by trial and error. Useful treatments of capillary hemangiomas, telangiectases, and other small vessel disease processes have been developed, and rely upon thermal mechanisms of injury and repair. Scarring is the most frequent undesirable result of laser treatments, and appears to be largely related to the degree of primary thermal damage. Further improvement in treatment, with reduction in scarring and other side effects may, therefore, depend on the ability to use lasers to induce selective thermal injury of abnormal vessels or other targets in the tissue. This is possible for pigmented structures such as cutaneous vessels or melanin-containing cells, if the laser wavelength is selectively absorbed and the energy is delivered briefly enough to confine heat to the target structures during laser exposure.

This basic approach to producing highly selective thermal injury to pigmented targets has been called “selective photothermolysis” [1]. A laser wavelength must be chosen that maximizes selective absorption by the target structures and minimizes absorption by competing tissue pigments. In cutaneous microvessels, the 577 nm (yellow) absorption band of oxyhemoglobin was chosen for selective deposition of energy in vessels. Although there are stronger oxyhemoglobin absorption bands at shorter wavelengths, competing absorption by the epidermal melanin overlying the dermal vessels tends to dominate at shorter wavelengths [2]. Even at 577 nm, the longest practicable oxyhemoglobin absorption band, epidermal melanin represents a competing site for absorption and damage in dark skin types [3].

While judicious choice of wavelength ensures the selective generation of heat within targets such as vessels, the laser exposure duration (pulsewidth) largely determines the extent to which heat will remain confined to the target structures during exposure. If the energy is delivered in less time than that required for the cooling of a target vessel, there is minimal heat conduction during exposure and the vessels can become much hotter than the surrounding dermis. A pulsewidth less than or equal to the intrinsic thermal relaxation time for vessels can, therefore, result in highly selective vascular thermal damage. For superficial cutaneous microvessels, thermal relaxation times range from tens of microseconds (capillaries and very small vessels) to about one millisecond (arterioles and venules), depending on size [1,2].

A series of initial studies of the selective photothermolysis of cutaneous vessels has been previously performed, using conventional 577 nm 1-µs pulsewidth dye lasers. The exposure doses necessary for selective vascular injury are approximately those predicted by theory [1,2]. Tuning the laser wavelength beyond the 577 nm oxyhemoglobin band greatly increases the exposure necessary for damage [4]. Histologically, exquisitely selective thermal injury occurs in vessels, in contrast to the diffuse coagulation necrosis produced by other lasers [5,6]. Microvascular hemorrhage typifies the initial injury produced by these 577 nm 1-µs pulses; this is expressed clinically as purpura. The dependence of exposure thresholds for purpura upon skin temperature suggests that microvaporization largely accounts for the vessel rupture that occurs at pulsewidths of 1 µs [4,7].

These prior studies have all described the nature of selective

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photothrombolysis of vessels with 1-μs, 577 nm laser pulses. This pulsewidth is much briefer than the thermal relaxation times of even the smallest dermal vessels, and is also briefer than the thermal relaxation time of single erythrocytes. Exceedingly rapid thermal expansion, microvaporization, and mechanical damage tend to occur with such brief pulses. The question therefore arises of how the damage produced by longer pulsewidths at 577 nm may differ. One would expect progressively less efficient heating of vessels with increasing pulsewidth, because of thermal diffusion occurring from vessels during exposure. The exposure dose necessary to cause purpura should, therefore, increase with increasing pulsewidth. Selective vascular heating from longer 577 nm pulses should be more gentle, perhaps with less mechanical injury to the microvasculature. There may be important histologic differences related to longer vs shorter pulsewidths. To answer these questions, a specially designed tunable pulsewidth dye laser was developed to study the effects of 577 nm laser pulses ranging from 1.5–360 μs in pulsewidth.

MATERIALS AND METHODS

Candela Corporation MDL 250 and LDPL-1 flashlamp-pumped dye lasers were used. The MDL 250 system produces a 1.5-μs pulsewidth and the LDPL-1 system produces pulsewidths of 20, 56, 200, and 360 μs (measured at full width, half maximum). Exciton Inc. rhodamine 575 dye at 5 × 10⁻⁵ M in methanol was used, and the lasers were tuned to 577 nm. A planoconvex lens was used to deliver a 3-mm-diameter homogenous exposure site. The energy per pulse passing through the 3-mm aperture was measured with a Scientec Model 365 Energy Indicator. Energy per pulse was reproducible to within ±3%, and incident exposure doses in J/cm² were calculated as pulse energy divided by 0.071 J/cm².

Subjects

Nine, informed, consenting, healthy volunteers of skin types I and II (fair Caucasians) were included in the study. The subjects had no known systemic or cutaneous disease and no topical or systemic medication was used for at least 1 month prior to the laser study.

Methods

Each subject was allowed to equilibrate to a room temperature of 22–24°C for 20 min. A stable skin surface temperature of 33°C ± 1°C was achieved and maintained throughout the study under these conditions. The volar surface of the forearm of each subject was used to ascertain clinical threshold exposure dose, which was defined as purpura filling the complete exposure area within 10 min of laser irradiation. Two investigators evaluated each area for the threshold response in all subjects. The laser exposure dose required to produce purpura in each of the subjects was evaluated at each pulsewidth of 1.5, 20, 56, 200, and 360 μs, starting at exposures of 0.5 J/cm², and increasing in 0.25 J/cm² increments up to and beyond threshold. Threshold exposure sites were examined at 36, 72, and 120 h, and at 1, 2, and 3 weeks after exposure to observe resolution of purpura.

In addition, 6 sites of buttock skin in each of the 6 subjects were exposed to laser irradiation and subsequently biopsied, after local anesthesia with 1% lidocaine was injected intradermally near the exposure site. Biopsy sites were exposed to the threshold dose for that subject, and also to 5 and 7 J/cm². For biopsies, each of the 6 subjects was exposed to 2 of 3 pulsewidths (1.5, 20, and 360 μs) at the 3 doses. This was to allow each pulsewidth to be examined in 4 different subjects at the same time, and to maintain paired comparisons of the above pulsewidths in single individuals. Also, histologic evaluation of 4-mm punch biopsies of laser-exposed buttock skin was performed in duplicate in 2 of the 3 remaining subjects at 1.5, 20, and 360 μs pulsewidths at threshold exposure doses, at times 0 and 24 h after irradiation. All biopsies were fixed in 10% buffered formalin, processed routinely, and stained with hematoxylin and eosin.

RESULTS

Clinical responses, immediately after exposure, were qualitatively similar following pulses of various pulsewidths. Purpura was the initial response, appearing typically within a few seconds to a minute after exposure. Purpura caused by short pulsewidths, however, appeared more violaceous. Most subjects experienced a pressure or pinprick sensation as clinical threshold was reached. However, this subjective sensation did not always correlate with threshold; some subjects did not have any symptoms at threshold exposures, whereas others reported sensation at exposure doses somewhat less than those producing purpura. The time to onset and resolution of purpura, at and above threshold doses, occurred more rapidly after the 1.5-μs irradiation than after the longer, 360-μs pulsewidth.

The laser exposure required to produce threshold purpura increased as pulse duration increased in all 9 subjects (Table I). An increase of 2.44 ± 0.16 J/cm² was needed to elicit a threshold response as pulsewidth increased from 1.5–360 μs. For each increment in pulsewidth, there was an associated statistically significant mean elevation in clinical threshold dose (p < 0.001) (Fig 1).

Histologically, the alterations produced always predominantly involved blood vessels, particularly the papillary loop capillaries and the superficial horizontal vascular plexus located within 500–600 μm of the skin surface. For all pulsewidths, the most marked changes were seen at the center of the exposed sites, where vessels contained masses of fused erythrocytes with a fibrillar crystalline quality, generally conforming to the shape of the vessel lumens. The endothelium typically showed signs of degeneration, with pyknotic nuclei focally stripped off and sometimes contained within the thermally altered erythrocyte masses. Near the margins of the irradiated site, individually damaged red cells could be discrim-

### Table I. Laser Energy Required to Produce Clinical Purpura in Each Individual at the 5 Pulse Durations Studied

<table>
<thead>
<tr>
<th>Pulse Duration</th>
<th>1.5 μs (J/cm²)</th>
<th>20 μs (J/cm²)</th>
<th>56 μs (J/cm²)</th>
<th>200 μs (J/cm²)</th>
<th>360 μs (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>Skin Type</td>
<td>I</td>
<td>II</td>
<td>I/II</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1.5 μs</td>
<td>1.50</td>
<td>2.25</td>
<td>2.50</td>
<td>3.75</td>
<td>4.00</td>
</tr>
<tr>
<td>20 μs</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>3.00</td>
<td>3.50</td>
</tr>
<tr>
<td>56 μs</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
<td>3.50</td>
<td>4.00</td>
</tr>
<tr>
<td>200 μs</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>360 μs</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Mean</td>
<td>1.47</td>
<td>2.00</td>
<td>2.33</td>
<td>3.44</td>
<td>3.92</td>
</tr>
<tr>
<td>SD</td>
<td>0.08</td>
<td>0.12</td>
<td>0.18</td>
<td>0.21</td>
<td>0.20</td>
</tr>
</tbody>
</table>
minated. Some appeared to have normal erythrocyte contours and displayed hypereosinophilia, while others were ameboid with granular cytoplasm. The width and depth of damage increased with increasing energy for all pulse widths.

At suprathreshold exposures of 5 and 7 J/cm², the shortest pulsewidth, 1.5 μs, produced obliteration of many vessels with moderate to massive hemorrhage into the papillary and upper reticular dermis. The affected vessels appeared shattered with fragmented red cells scattered in the perivascular region (Fig 2). In stark contrast, irradiation with the same exposure doses but a longer pulsewidth, 360 μs, rarely resulted in hemorrhage, and vascular architecture appeared preserved (Fig 3). The predominant change was erythrocyte fusion similar to that seen at threshold doses. Endothelial cells and pericytes were hyperchromatic but were not disrupted.

At suprathreshold doses, denaturation of perivascular collagen was evident and appeared more extensive after 1.5-μs irradiation than after pulses of 360 μs. There was basophilia and swelling of the affected collagen fibers with a loss of fibrillar texture, resulting in a homogenized “ground glass” appearance (Fig 4). This change was seen primarily around the papillary loop capillaries and the superficial venular plexus.

There was no evidence of epidermal damage at any of the threshold exposures. For equal suprathreshold exposures, epidermal damage was greater for shorter pulsewidths. At 5 J/cm² (suprathreshold), cytoplasmic vacuolization and nuclear hyperchromasia occurred focally in the basal layer. At 7 J/cm², multiple basal lacunae were produced in type I subjects. In type II subjects, focal epidermal ablation occasionally occurred at the center of 7 J/cm² exposures. The epidermal separation appeared to originate superior to the basal nuclei, a site coincident with the greatest concentration of epidermal melanin (Fig 5). Epidermal separation was more prominent as pulse duration decreased.

Biopsies taken 24 h after laser exposure at threshold dose showed marked necrosis of vessel walls and intraluminal fibrin within the irradiated zone. More vessel damage was seen with 360-μs pulsewidth compared with 1.5- and 20-μs pulse durations. In no case was there evidence of residual damaged erythrocytes. A moderate-intensity perivascular lymphohistiocytic infiltrate with rare intact neutrophils and eosinophils was present in all biopsies at 24 h. Abundant fragmented nuclear debris was apparent throughout the papillary and upper reticular dermis, indicative of a prior acute inflammatory infiltrate (Fig 6). There was no evidence of
epidermal basal cell layer changes in any of the threshold dose biopsies taken 24 h after laser irradiation.

DISCUSSION

This study reveals several interesting and clinically important differences between the selective microvascular injury induced by 577 nm laser pulses of different durations. First, microvascular rupture and hemorrhage is seen with short (1 μs) pulses as compared with longer (up to 360 μs) pulses, although purpura is the immediate threshold response for all pulsewidths studied. The longer pulses apparently produce purpura by forming an intravascular coagulum, not by extravasation. Both short and long pulses induce highly selective vascular injury with subsequent vasculitis. Second, the incident exposure dose necessary for damage increases with increasing pulsewidth. Third, there are subtle histologic differences in perivascular collagen alteration with short vs long laser pulses.

These differences may be largely explained on the basis of rates of heat production and thermal transfer occurring in vessels during laser exposure. The yellow 577 nm wavelength is selectively and strongly absorbed by oxyhemoglobin and hemoglobin, making erythrocytes the primary heat source during laser exposure. The thermal relaxation time of a single erythrocyte is several microseconds, such that during the shortest (1.5 μs) pulsewidth studied, the thermal energy is still largely confined to erythrocytes at the end of this laser pulse. It is conceivable that microvaporization of erythrocytes accounts for the vessel rupture and hemorrhage seen at threshold only with the shortest (1.5 μs) pulses. This mechanism was also suggested by previous studies of variations in purpura threshold with ambient skin temperature [4,7], but remains theoretical. For example, even in the absence of an explosive phase change (vaporization), the hemorrhage seen with short pulsewidths may simply be due to rapid thermal expansion. The estimated rate of erythrocyte heating during a 1.5-μs pulse at purpura threshold exposures is about 10^7°C/s [2], which may be responsible for inducing pressure waves capable of causing vessel rupture.

The relative lack of microvessel rupture and hemorrhage at pulsewidths of 20 μs or greater is of potentially major clinical importance. There are few medical situations in which microvascular hemorrhage is beneficial. Our interest in pulsed 577 nm dye lasers lies largely in their potential for selective vascular injury in the treatment of hemangiomas, telangiectasia, angiodysplasia, and other vascular abnormalities. Hemorrhage is generally not desirable, suggesting the practical value of using pulses longer than 20 μs duration for clinical work.

The finding in this study that the exposure threshold for inducing purpura increases with increasing pulsewidths is explained by the heat transfer occurring from microvessels during laser exposure. For a range of vessel diameters between 10–40 μm, thermal relaxation times (τ) range from about 200–3000 μs, respectively. By definition, significant heat loss occurs from a selectively heated vessel during pulsewidths equal to or greater than the vessel’s thermal relaxation time. The selective heating process, therefore, becomes less efficient as pulsewidth is increased beyond the thermal relaxation time, and a greater exposure dose becomes necessary to achieve damaging temperatures, as exemplified in Fig 1. The curve in Fig 1 is in reasonable agreement with theoretical predictions, given the range of vessel sizes present [8].

Another effect of lengthening pulsewidth beyond 1 τ is that more heat is distributed to the perivascular tissue during exposure. For a given peak vessel temperature, e.g., at or near threshold exposure, one might expect longer pulsewidths to produce more perivascular thermal injury. This was not observed in this study, perhaps because the longest pulsewidth examined (360 μs) is not much longer than the average thermal relaxation time for cutaneous microvessels. It is also possible that perivascular structures are somewhat more heat stable, or that such damage may be harder to detect by routine light microscopy. The finding of subtly greater perivascular alteration with short pulsewidths at the fixed suprathreshold exposures of 5 and 7 J/cm² is consistent with attainment of greater peak vessel temperatures with short pulsewidths, as would be expected. Ultrastructural or other more sensitive means for high-resolution assay of perivascular collagen alteration may be helpful in quantitating perivascular thermal alteration vs pulsewidth for threshold exposures in future studies. In any case, the diffuse thermal coagulation necrosis typical of continuous laser exposures [5,9] is clearly absent for all of the pulsewidths examined in this study.

In summary, the selective cutaneous vascular injury caused by 577 nm laser pulses is substantially different at pulsewidths up to 360 μs as compared with 1.5-μs pulses. There is much less hemorrhage, and the purpura observed appears to be due to formation of intravascular coagulum as opposed to extravasation of erythrocytes. The exposure threshold for purpura increases at longer pulsewidths, as expected when pulsewidth exceeds thermal relaxation times for the vessels. The more gentle, and yet still highly selective vascular heating caused by the long-pulse tunable dye laser developed for this study is probably more desirable for the treatment of cutaneous vascular abnormalities. This study also suggests that microvascular hemostasis, although possible with
1-μs 577 nm pulses [10,11], is probably more effective with longer pulsewidths.

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REFERENCES