Thermal Relaxation of Port-Wine Stain Vessels Probed *In Vivo*: The Need for 1–10-Millisecond Laser Pulse Treatment

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Although thermal relaxation times of cutaneous port-wine stain microvessels have been calculated and used to formulate laser selective photothermolysis, they have never been measured. A scheme to do so was devised by measuring the skin response to pairs of 585-nm dye laser pulses (250–360 microseconds each) as a function of the time interval between the two pulses, in five volunteers with port-wine stains. After a pump pulse delivering 80% of the fluence necessary for causing purpura, the fluence of a second probe pulse necessary to cause purpura was determined and was found to increase with the interval between the two pulses, in a manner consistent with thermal diffusion theory. Biopsy specimens were obtained from four of the five subjects to examine the nature and extent of vessel damage and to measure the port-wine stain vessel diameters. Using diffusion theory, the thermal relaxation time was calculated based on the measured vessel diameters. These calculated values are consistent with the increase in radiant exposure (fluence) of the probe pulse necessary to induce purpura for longer time delays. Two simple models for thermal relaxation of port-wine stain vessels are presented and compared with the data. The data and histologic assessment of the vessel injury strongly suggest that pulse durations for ideal laser treatment are in the 1–10-millisecond region and depend on vessel diameter. No dermatologic lasers presently used for port-wine stain treatment operate in this pulse width domain. ***J Invest Dermatol 105:709–714, 1995***

The treatment of microvascular malformations such as port-wine stains (PWS), telangiectases, and benign proliferative vascular lesions such as hemangiomas is one of the oldest applications of lasers in dermatology. Currently the treatment of PWS is based on the process of "selective photothermolysis" [1], which induces selective thermal damage of abnormal blood vessels and minimizes the risk of scarring [2]. Simple models of optics [3] and heat transfer [4] have been useful in developing laser treatments.

Three basic elements are necessary to achieve selective photothermolysis: 1) a wavelength that is preferentially absorbed by the desired targeted structure, 2) an exposure duration less than or equal to the thermal relaxation time of the target, and 3) sufficient radiant exposure (fluence; delivered energy per unit area) to reach a damaging temperature in the targeted structure. For selective photothermolysis of cutaneous microvessels, the 577-nm (yellow) absorption band of oxyhemoglobin was originally chosen because of high selectivity for absorption by oxyhemoglobin [4]. Subsequently, 585 nm was shown to offer similar vascular selectivity with greater depth of penetration [5] and is now generally used clinically [6]. It is likely that longer wavelengths or other absorption bands of hemoglobin will offer better optical penetration, which may be better for treating larger vessels. Pulsed dye lasers operating at 585 nm, with approximately 0.4-millisecond pulse duration and 6–8 J/cm^2, are now widely used for selective photothermolysis of PWS. Although this technique is generally effective and well tolerated, PWS clearing is inefficient, requiring typically six or more treatments [7]. For unknown reasons, PWS are frequently resistant to treatment and in rare cases are completely unresponsive [8].

Regardless of how judiciously one chooses wavelength, poorly confined damage results if the exposure duration is too long [9]. During long laser exposures, energy transfer from the vessels to the surrounding dermis *via* diffusion will eventually cause nonspecific thermal damage, even though specific pigments are the sites of optical absorption.

However, if energy is delivered to an object faster than it can diffuse away, a high temperature gradient will exist between the target and the surrounding tissue. Spatial confinement of thermal damage therefore occurs when the laser exposure duration (pulse width) is approximately equal to or less than the thermal relaxation time (τ) of the target. Unfortunately, the use of very short pulses for PWS treatment appears to have some disadvantages. Dye laser pulses less than about 20 microseconds in duration cause vessel rupture with hemorrhage, probably from violent vaporization of erythrocytes [10]. Allowing some thermal diffusion into the vessel wall during the laser exposure may also increase the effectiveness of treatment by locally extending the zone of perivascular damage. Thus the best approach is probably to use exposure durations that are equal to or slightly greater than the target vessels' thermal relaxation time, depending on the extent of peri-target thermal damage desired. Therefore, an accurate determination of the thermal relaxation time is important for better treatment of PWS.

Manuscript received November 30, 1994; final revision received June 17, 1995; accepted for publication July 12, 1995.

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Abbreviations: PWS, port-wine stain; THF, threshold fluence.

0022-202X/95/$09.50 • SSDI0022-202X(95)00339-M •

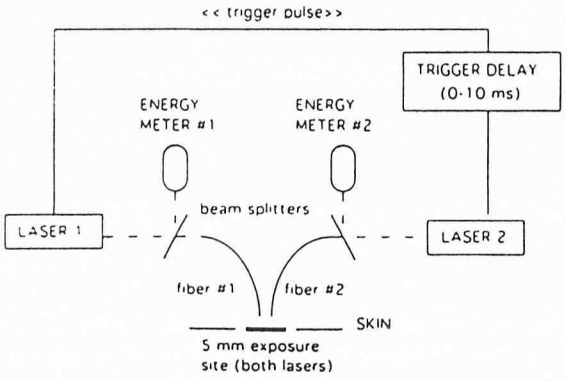

Figure 1. Apparatus for dual-pulse experiment. A sub-threshold pulse from laser 1 thermally excites PWS vessels, followed 0–10 ms later by a second laser pulse used to probe the thermal relaxation behavior.

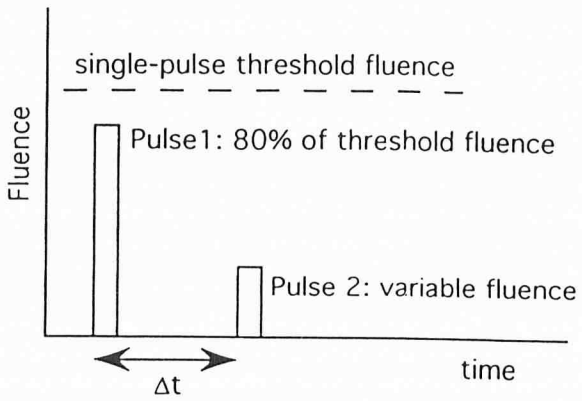

Figure 2. In this dual pulse study, a first pulse bearing 80% of the fluence necessary to cause purpura is used to thermally excite the PWS vessels. A second pulse is delivered at different time-delays, Δt, to probe the fluence required to induce purpura as the PWS vessels cool by thermal diffusion.

Thermal relaxation or "cooling" times of vessels have been estimated from theoretic models, but not measured. In theory, the thermal relaxation time for cooling by heat conduction is directly proportional to the square of the target size and related to the target shape. Thermal relaxation is also important for the efficiency with which selective damage occurs in a given vessel. Choosing laser pulse widths equal to or less than the corresponding thermal relaxation time of the PWS vessels will produce the most spatial confinement of the laser energy. For example, long exposure times should be capable of damaging larger vessels while sparing smaller vessels, because the smaller vessels have a much shorter thermal relaxation time. According to theory, the optimum pulse duration for most PWS vessels (30–150 μm in diameter) should lie between 1 and 10 milliseconds [4,11]. In this study, we probed the thermal relaxation of PWS vessels *in vivo* by determining the fluence necessary to elicit a skin response to two 585-nm laser pulses with variable time separation. The measured thermal relaxation of PWS vessels *in vivo* was then compared with theory.

MATERIALS AND METHODS

Two flashlamp-pumped tunable dye lasers were used (Candela Inc., Wayland, MA; models LPDL-1 and SPT-1P). Each produced pulses of 250–360 microseconds, measured at full-width, half-maximum. The lasers were tuned to 585 nm. The output of each laser was focused with a planoconvex lens into a 1-mm silica fiber optic, which terminated at two corresponding handpieces **(Fig 1)**. Each handpiece produced a uniform 5-mm–diameter exposure spot, which was a real image of the multimode fiber face projected onto the skin exposure site. The handpieces were positioned at $\pm 15°$ from a normal to the skin and produced a single 5-mm–diameter exposure area. Laser pulse energies were measured using pulse energy meters (Scientech, model 365). Meters 1 and 2 measured the pulse energy of lasers 1 and 2, respectively, via beam splitters. Meter 3 measured the energy at the exposure site. By establishing a ratio between the sampled energy and the energy delivered to the exposure site for each laser, we determined the actual energy delivered to the skin by each laser. Fluences in J/cm^2 were calculated as pulse energy divided by the exposure surface area. The fluences delivered ranged from 2.4 J/cm^2 for the single pulse to 7.3 J/cm^2 total for the dual pulses. A trigger delay generator in one of the lasers was used to set the onset of the second pulse relative to the first. Two silicon photodiodes (PIN 100; EG & G, Salem, MA), one at each fiber input, were connected to a digital oscilloscope (LeCroy, model 9400) to confirm the pulse widths and delay times.

Subjects Subjects were five healthy, consenting volunteers between the ages of 20 and 50 years. All subjects had extensive PWS on their lower extremities. The skin types [12] were I, II, III, and V, representing a range of skin pigmentation.

Exposures The study was set up to determine the threshold fluence for purpura, which corresponds to intravascular coagulation with or without extravasation [10]. This end point was defined as the minimum fluence needed to produce nonblanchable purpura that filled the entire exposure area in three of four given pulses within 3 min after the exposure. The fluence required to produce purpura consistently in each of the subjects was determined for a series of single pulses, and then for dual pulses at different time delays. The time delays studied (peak to peak delay) were 0.1, 1, 3, and 10 milliseconds. The maximum pulse delay jitter was ± 0.1 millisecond.

The purpura threshold fluence (THF) was first determined for each laser alone on PWS skin. For the dual-pulse exposures, the fluence of the first pulse was set at 80% of the single-pulse threshold, and the fluence of the second pulse necessary to cause purpura was then determined for each of the different time delays **(Fig 2)**.

Five separate 3-mm punch biopsy specimens were taken from each of four subjects with PWS after infiltration with 1% xylocaine without epinephrine. These specimens were from sites exposed to the threshold dose for a single pulse and to the threshold doses for the dual pulses at 0.1, 1, 3, and 10 milliseconds' delay. Samples were obtained within 1 h of laser irradiation, fixed in 10% formalin, processed routinely in paraffin, and stained with hematoxylin and eosin.

Morphometric evaluation was performed using a digitizing measurement system (Sigma Scan; Jandel Scientific, Sausalito, CA) coupled by a camera lucida to a light microscope (Labophot; Nikon, Japan). For each subject, the mean PWS vessel diameter was calculated from the average circumference of the internal elastic lamina of all PWS vessels present in the subject's biopsy specimens.

RESULTS

The mean single-pulse purpura threshold exposure is consistent with that previously reported at this pulse duration [10] and was 3.3 $\pm$ 0.6 J/cm^2 (mean $\pm$ SD) for PWS.

The fluence of the second pulse required to produce purpura, after a first pulse delivering 0.8 of the single-pulse threshold, increased as the pulse interval increased in all five subjects **(Table I)**. An increase of 2.2 $\pm$ 1.0 J/cm^2 (mean $\pm$ SD) was needed to elicit a threshold response as the time delay increased from 0 (single pulse) to 10 milliseconds **(Fig 3)**. For each pulse delay, there was an associated statistically significant increase in threshold dose compared with no delay ($p < 0.05$, paired t test).

Normalized to each subject's single-pulse threshold, **Fig 4** shows the mean total fluence delivered in two pulses necessary for purpura versus pulse delay time. This normalization compensates for individual variations in response due to skin pigmentation, hematocrit, etc. Because the fluence of the first pulse was always set to be 0.8 times the single-pulse threshold fluence, the normalized fluence of the second pulse varied between 0.2 (total normalized fluence 1.0) at time delay 0, and 1.0 (total normalized fluence 1.8) as the vessels cooled completely after the first pulse. The curve in **Fig 4** is therefore asymptotic to 1.8.

Histology The biopsy specimens taken from subjects with PWS at the sites of the purpura threshold fluences showed selective alterations involving only the blood vessels, particularly the super-

Table I. Purpura Threshold Fluences (THF) for Single and Dual Laser Pulses at Different Time Delays

Subject	Skin Type	Vessel Size[a] (μm)	THF for Purpura[b] (J/cm^2)				
			Single Pulse[c]	0.1-Millisecond Delay[d]	1-Millisecond Delay[d]	3-Millisecond Delay[d]	10-Millisecond Delay[d]
A	II	58	3.3	3.6	4.2	4.3	5.0
B	III	39	3.0	3.8	4.1	4.7	4.7
C	II	29	3.7	4.1	5.5	6.4	5.9
D	II	24	4.0	5.1	6.5	6.6	7.3
E	V		2.4	2.9	3.6	4.3	4.5
Mean		37.5	3.3	3.9	4.8	5.3	5.5
SD		15.0	0.6	0.8	1.2	1.1	1.1

[a] Mean PWS vessel diameter calculated from the average circumference of all PWS vessels present in the subject's biopsy specimen.
[b] The fluence required to produce purpura increased as the pulse interval between the two pulses increased. For each pulse delay, there was a statistically significant increase in threshold dose compared with no delay ($p < 0.05$, paired t test).
[c] Mean single-pulse THF for each laser alone.
[d] Mean total THF given as the sum of the two pulses.

ficial vascular plexus. For all PWS, the most prominent changes were seen at the center of the exposed sites. The vessels contained masses of fused erythrocytes, generally conforming to the shape of the vessel lumen. Occasionally the endothelial cells were hyperchromatic. There was no evidence of epidermal damage at any of the threshold exposures in any subject **(Fig 5)**. The mean PWS vessel diameters varied, and were (in μm): 24 ± 9.7, 29 ± 15.5, 39 ± 13.8, and 58 ± 14.5 **(Table I)**.

Data Analysis and Model of Thermal Relaxation The data can be presented as a thermal relaxation function, $f(t)$ versus time, as follows. The vessel temperature rise after the two pulses is

$$\Delta = \Delta_1 f(t) + \Delta_2, \quad (1)$$

where Δ = vessel temperature rise above the ambient skin temperature; Δ_1 = vessel temperature rise caused by the first pulse; Δ_2 = vessel temperature rise caused by the second pulse; and $f(t)$ = unknown vessel temperature relaxation function.

At the purpura threshold in the dual-pulse experiment,

$$\Delta = \Delta_p = \Delta_1 f(t) + \Delta_2, \quad (2)$$

where Δ_p is the vessel temperature rise necessary for threshold purpura. Solving for $f(t)$ at the purpura threshold, we have

$$f(t) = \frac{\Delta_p - \Delta_2}{\Delta_1}. \quad (3)$$

The vessel temperature rises produced by each pulse are proportional to the delivered fluence [4]. Therefore, $\Delta_1 = kF_1$, $\Delta_2 = kF_2$, and $\Delta_p = kF_p$, where k is a proportionality constant and F_1, F_2, and F_p are the fluences of the first pulse, the second pulse, and the single-pulse threshold fluence for purpura, respectively. In our experiment, F_1 was set such that $F_1 = 0.8F_p$.

Substituting fluences into equation 3 gives us

$$f(t) = \frac{kF_p - kF_2}{k(0.8F_p)} = \frac{F_p - F_2}{0.8F_p} = 1.25\left(1 - \frac{F_2}{F_p}\right). \quad (4)$$

Figure 6 shows $f(t)$ as determined from the experimental data versus the time separation between the two pulses (Δt). The data are widely scattered because of the different vessel diameters (and hence different thermal relaxation functions) of each subject. Simple exponential fits are shown in the figure to illustrate the

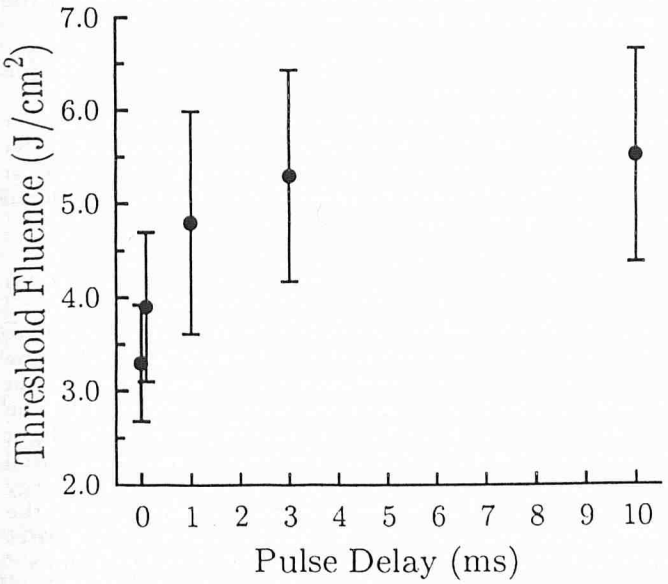

Figure 3. The THF for purpura increases as the pulse-interval between pulses increases. At time delay 0, the plotted fluence is a single pulse THF and represents the mean of the single pulse threshold fluence for each laser pulse. At 0.1-millisecond, 1-millisecond, 3-millisecond, and 10-millisecond delays, the plotted fluences are the summation of the first laser pulse and of the second laser pulse and represent the mean of the total fluence from the two pulses. (*Error bars*, mean ± SD.)

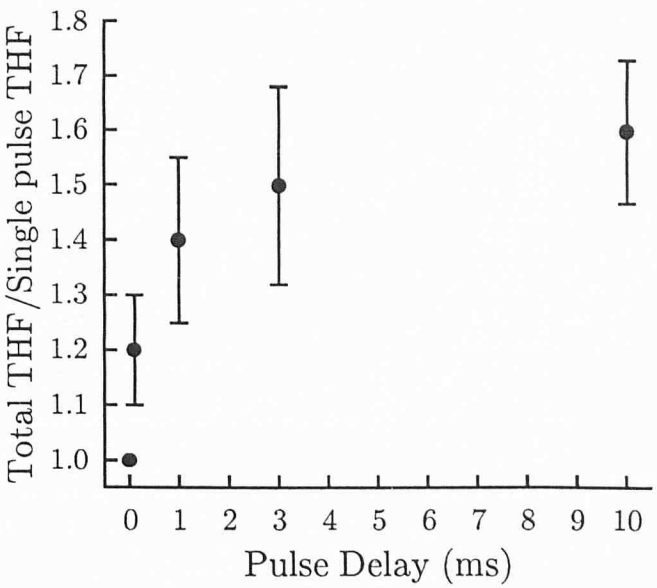

Figure 4. Threshold fluences (sum of 2 pulses) normalized to each subject's single-pulse threshold fluence versus delay time between pulses. (*Error bars*, mean ± SD.)

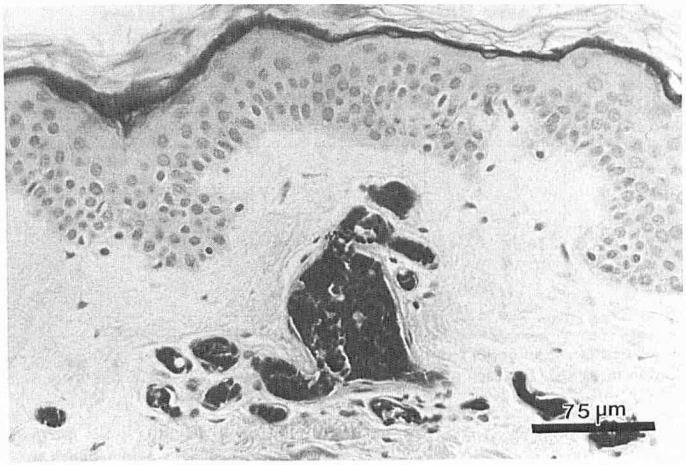

Figure 5. Demonstration of absence of epidermal damage and an intravascular coagulum in a PWS vessel exposed to the threshold fluence for purpura.

tendency of smaller-vessel lesions to cool more rapidly, but do not fit the data points as well as models for vessel cooling.

Several mathematical models of varying complexity have been proposed to describe the thermal relaxation of PWS vessels [4,13–17]. Two relatively simple models are presented here, both of which are consistent with the data. The first model assumes that a temperature distribution within the vessel at the end of a laser pulse is described by a Gaussian distribution **(Fig 7)**. At this time, the width of the distribution is set to $2\sigma = d$, where d is the vessel diameter and σ is the standard deviation of the distribution. The gaussian distribution is used because its solution is relatively simple and because the temperature distribution after heat deposition at any small site tends toward a gaussian profile as it relaxes over time. In deriving this model, we will use T' to represent the normalized thermal evolution of a line source starting at a fictitious time t'. We will then relate T' to the actual temperature T and t' to the actual time t. In cylindric coordinates with $r = 0$ representing the center of the vessel, the normalized gaussian distribution is

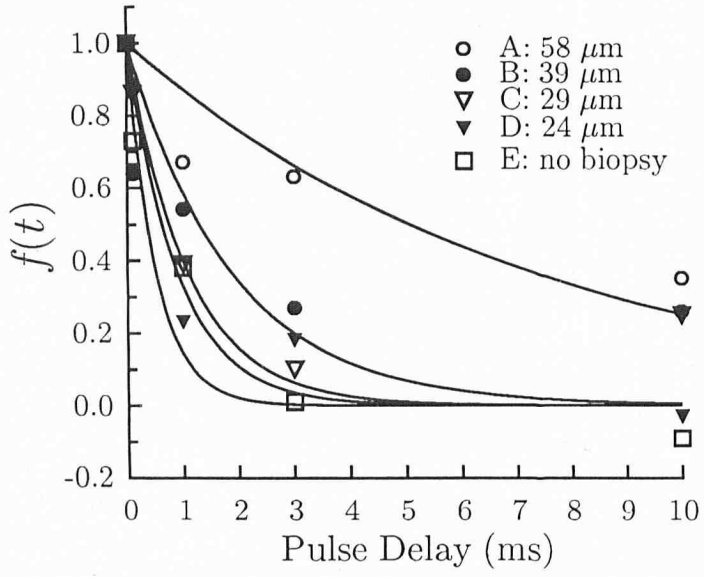

Figure 6. Thermal relaxation of each subjects' PWS vessels as deduced from data according to Eq. (4). The simple fits illustrate the tendency of smaller vessels to cool more rapidly.

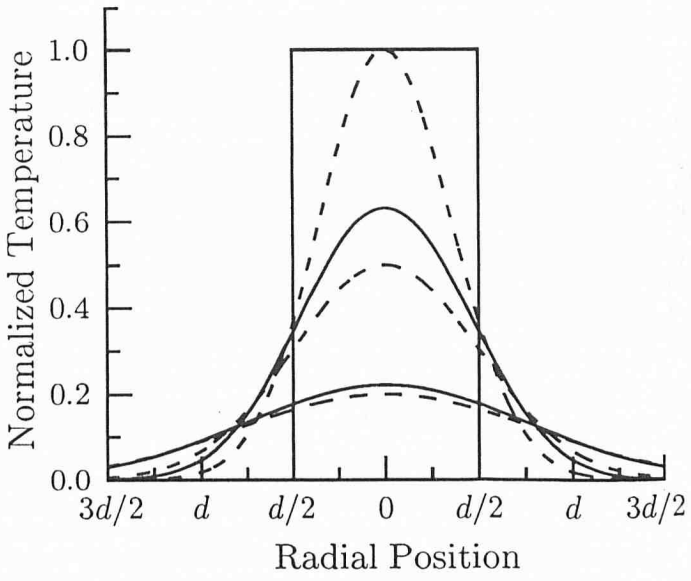

Figure 7. Temperature distribution around a vessel according to the Green's function solution (*solid curve*) and the Gaussian model (*dotted curve*), during thermal relaxation at times 0, $d^2/16\kappa$, and $d^2/4\kappa$ after a short laser pulse. Position is given in terms of d, the vessel diameter.

$$T'(r) = \frac{1}{2\pi\sigma^2}\exp(-r^2/2\sigma^2), \tag{5}$$

where r is the radius. The thermal diffusion equation in cylindric coordinates is

$$\frac{\partial T}{\partial t} = \kappa\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right)\right], \tag{6}$$

where κ is the thermal diffusivity (1.3×10^{-3} cm^2/second). To satisfy the thermal diffusion equation, the standard deviation of the Gaussian distribution is set to

$$\sigma = (2\kappa t')^{1/2}, \tag{7}$$

where t' represents time.

In our model, real time t is defined by $t = 0$ at the end of the laser pulse. At $t = 0$, the width of the temperature distribution, 2σ, is set equal to d, the vessel diameter. According to Eq. (7), this occurs at $t' = t + d^2/8\kappa$; therefore, substituting into Eq. (5), the normalized temperature distribution is

$$T'(r,t) = \frac{1}{4\pi\kappa(t + d^2/8\kappa)}\exp\left[\frac{-r^2}{4\kappa(t + d^2/8\kappa)}\right]. \tag{8}$$

Equation 8 is normalized such that the integral over all r, i.e., the "area" under the gaussian curve, is 1. It therefore describes the shape of the temperature distribution after a short laser pulse, but without the appropriate magnitude, which is determined by the amount of energy deposited. For wavelengths at which light penetrates the full diameter of the blood vessel, energy is deposited nearly uniformly across each vessel's lumen. The total energy deposited in a vessel under these conditions is proportional to the cross-sectional area of the vessel. In this experiment, 585-nm pulses were used. The optical penetration depth into blood is given by

$$\delta = 1/\mu_a,$$

where μ_a is the optical absorption coefficient of blood. At 585 nm, $1/\mu_a \approx 55$ μm in human blood [18]. The largest mean PWS vessel diameter measured (subject A) was 58 μm, which is approximately equal to the penetration depth in blood. Therefore, energy deposition was approximately uniform across most of the PWS vessels in this study.

The magnitude of the temperature distribution T' in Eq. (8) is made appropriate by setting the actual temperature T at the vessel center at time $t = 0$, immediately after the laser pulse, to a value of

$$T(r = 0,\ t = 0) = E\mu_a/\rho c,$$

where E is the local fluence, μ_a is the absorption coefficient of human blood at 585 nm, ρ is density (g/cm^3), and c is specific heat (J/g °C) [4]. According to equation 8, the normalized temperature is given by $T'(r = 0,\ t = 0) = 2/\pi d^2$. Therefore,

$$T(r = 0,\ t = 0) = (\pi d^2 E\mu_a/2\rho c)T'(r = 0,\ t = 0),$$

with the term in brackets being the normalization factor for T'.

When substituted into equation 8, the final expression for the axial vessel temperature becomes

$$T(r = 0) \approx \frac{E\mu_a}{[\rho c(8\kappa t/d^2 + 1)]}. \quad (9)$$

Thus, according to this model, the thermal relaxation function $f(t)$ obtained from our data would be expected to be

$$f(t) = \left[\frac{1}{1 + (8\kappa t/d^2)}\right], \quad (10)$$

which varies from 1 to 0 as time goes from 0 to ∞.

The second model, which uses Green's functions [19], is more realistic because it treats the vessel as uniformly heated at the end of a laser pulse. This approach assumes that at time 0, there is an elevated temperature within the vessel that varies as a function of radial position within the vessel, with the surroundings at a lower uniform temperature. The subsequent evolution of this temperature distribution is solved for and satisfies the thermal diffusion equation 6 above. The temporal and spatial temperature profile generated in an infinite cylinder whose initial temperature profile is given by a function $g(r')$ is [19]

$$T(r,t) = \frac{1}{2\kappa t}\int_0^\infty \exp[-(r^2 + r'^2)/4\kappa t]I_0\left(\frac{rr'}{2\kappa t}\right)g(r')r'\,dr'. \quad (11)$$

To calculate the temperature profile, we need to specify $g(r')$, which is the initial temperature distribution of the vessel. In our case we consider the vessel to be uniformly heated. Thus we assign a normalized temperature of 1 for all radial positions within the vessel, i.e., $0 < r' < (d/2)$, and a normalized temperature of 0 for all positions outside the vessel, i.e., $r' > (d/2)$. Thus

$$T(r,t) = \frac{1}{2\kappa t}\int_0^{d/2} \exp[-(r^2 + r'^2)/4\kappa t]I_0\left(\frac{rr'}{2\kappa t}\right)r'\,dr'. \quad (12)$$

For the center of the vessel ($r = 0$), the solution is

$$f(t) = \frac{T(r = 0,\ t)}{T(r = 0,\ t = 0)} = 1 - \exp(-d^2/16\kappa t). \quad (13)$$

The thermal relaxation of the central vessel temperature in the Gaussian model Eq. (10) and Green's function model for a uniformly heated cylinder Eq. (13) is given by

$$f(t) = \frac{1}{1 + (8\kappa t/d^2)} \text{ (Gaussian model)}$$

and

$$f(t) = 1 - \exp(-d^2/16\kappa t) \text{ (Green's function solution)},$$

where κ is thermal diffusivity, d is vessel diameter, and t is time. Note in **Fig 7** that despite different initial temperature distributions, the two models converge at later times.

When the experimentally determined $f(t)$ values are plotted against $\kappa\Delta t/d^2$, the data of all the subjects are no longer scattered and follow the trend predicted by the two models **(Fig 8)**.

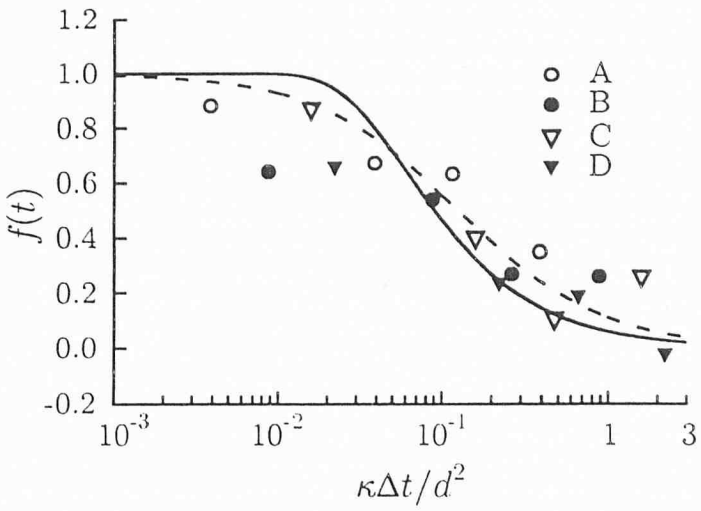

Figure 8. The experimentally determined thermal relaxation function, $f(t)$, compared with the Green function's solution (*solid curve*) and the Gaussian model (*dotted curve*).

DISCUSSION

The thermal relaxation of laser-excited PWS vessels was measured in human skin over the critical 0–10-millisecond region and confirms the validity of the thermal diffusion theory underlying selective photothermolysis. The experimental scheme of using two pulses with a time delay and noting purpura thresholds as a reporter response for vessel temperature works well and results in data that agree quantitatively with heat transfer models.

Most important, this study demonstrates the extreme dependence of thermal relaxation on vessel size among different PWS lesions and suggests that 1–10-millisecond laser pulses should be used to treat most PWS. This exposure time domain is not achieved by any of the lasers currently used to treat PWS. Because PWS vessel size is correlated with patient age [20], the data further suggest that laser exposure duration (pulse width) might ideally be tailored to patient age and the particular vessel size. PWS vessel size could potentially be measured by vital microscopy and the laser pulse duration set at about $d^2/8\kappa$ before treatment. For exposure durations on the order of 1–10 milliseconds, it is possible for thermal damage to be specific for the larger PWS vessels (30–150 μm) while sparing the capillaries. This is possible because the smaller size of the capillaries allows the deposited energy to diffuse into interstitial connective tissue during the exposure time. Furthermore, the highly selective vascular heating created by the longer pulses should be more gentle by potentially reducing the mechanical stresses generated by this process, which often lead to hemorrhage. Given the expense associated with multiple laser treatments for PWS [21] and the fact that some PWS fail to respond to treatment [7,8], the potential for improvement using 1–10-millisecond pulses should be investigated clinically when this technology becomes available.

In this study, purpura was used only as a convenient end point to probe vessel temperature; it is not the desired end point of PWS treatment. Histologically, the purpura induced by both single- and dual-pulse exposures was related to an intravascular coagulum rather than to hemorrhage. In all subjects, vessel injury was highly selective, with no epidermal injury seen in any of the biopsy specimens. It should be noted, however, that the specimens were obtained at threshold fluences for purpura and that epidermal injury and more extensive vascular injury occur at the higher fluences typically used for treatment [22,23].

An important finding is that simple thermal diffusion models appear to be quantitatively correct for selective photothermolysis of PWS vessels, within experimental error. These errors include the precision of threshold fluence determinations, pulse energy measurement, and tissue shrinkage before histologic measurements of vessel diameter. The mathematical models are grossly simplified by ignoring axial heat flow, local changes in thermal and optical

properties, and details of the optical absorption distribution in blood vessels, which both scatter and absorb light. The model first used to propose selective photothermolysis was based on a Gaussian temperature distribution [4], which fits the data of this study well, as seen in **Fig 7**.

A similar dual-pulse study probing thermal relaxation in other applications should be considered. For example, the principle of selective photothermolysis is now used to treat pigmented lesions [24–29] and tattoos [30,31]. In these applications, the targets are much smaller than PWS vessels, and sub-microsecond pulses are necessary to achieve thermal confinement. However, thermal relaxation may occur by additional processes for targets as small as melanosomes or tattoo ink granules (about 0.5 μm) heated to temperatures thought to be in the range of 300–1000°C. Radiational cooling may play a greater role for these small targets, for example [32]. The pulse-duration dependence of melanosome rupture [33] suggests that thermal relaxation occurs in about 1 microsecond. The pulse-duration dependence for tattoo treatment is essentially unknown. Given that photomechanical damage modes (e.g., acoustic waves, shock waves, cavitation, fracture) are in part linked to thermal confinement, dual-pulse studies might enlighten the dynamics of these other thermally driven effects of pulsed lasers in skin.

This work was supported in part by ONR contract number N00014-91-C-0084. We wish to thank Norm Michaud for his excellent technical assistance and Dr. Farzin Adili for his advice and helpful discussions.

REFERENCES

1. Anderson RR, Parrish JA: Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. *Science* 220:524–527, 1983
2. Garden JM, Polla LL, Tan OT: The treatment of portwine stains by the pulsed dye laser. *Arch Dermatol* 124:889–896, 1988
3. Anderson RR, Parrish JA: The optics of human skin. *J Invest Dermatol* 77:13–19, 1981
4. Anderson RR, Parrish JA: Microvasculature can be selectively damaged using dye lasers: a basic theory and experimental evidence in human skin. *Lasers Surg Med* 1:263–276, 1981
5. Tan OT, Murray S, Kurban AK: Action spectrum of vascular specific injury using pulsed irradiation. *J Invest Dermatol* 92:868–871, 1989
6. Tan OT, Morrison P, Kurban AK: 585 nm for the treatment of portwine stains. *Plast Reconstr Surg* 86:1112–1117, 1990
7. Garden JM, Polla LL, Tan OT: The treatment of portwine stains by the pulsed dye laser: analysis of pulse duration and long-term therapy. *Arch Dermatol* 124:889–896, 1988
8. Fitzpatrick RE, Lowe NJ, Goldman MP, Borden H, Behr KL, Ruiz-Esparza J: Flashlamp-pumped pulsed dye laser treatment for portwine stains. *J Dermatol Surg Oncol* 20:743–748, 1994
9. Greenwald J, Rosen S, Anderson RR, Harrist T, MacFarland F, Noe J, Parrish JA: Comparative histological studies of the tunable dye (577 nm) laser and argon laser: the specific vascular effects of the dye laser. *J Invest Dermatol* 77:305–310, 1981
10. Garden JM, Tan OT, Kershman R, Boll J, Furumoto H, Anderson RR, Parrish JA: Effect of dye laser pulse duration on selective cutaneous vascular injury. *J Invest Dermatol* 87:653–657, 1986
11. Van Gemert JMC, Welch AJ, Amin AP: Is there an optimal laser treatment for portwine stains? *Lasers Surg Med* 6:76–83, 1986
12. Fitzpatrick TB: Soleil et peau. *J Med Esthet* 2:33–34, 1975
13. Mainster MA, White TJ, Tips JH, Wilson PW: Transient thermal behavior in biological systems. *Bull Math Biophys* 32:303–314, 1970
14. Welch AJ, Wissler EH, Priebe LA: Significance of blood flow in calculations of temperature in laser-irradiated tissue. *IEEE Trans Biomed Eng* 27:164–166, 1980
15. Cummins L, Warrenberg M: Thermal effects of laser radiation in biological tissue. *Biophys J* 42:99–102, 1983
16. Birngruber R: Thermal modeling in biological tissues. In: Hillenkamp F, Pratesi R, Sacchi CA (eds.). *Lasers in Biology and Medicine*. Plenum Press, New York, 1979, pp 77–97
17. Van Gemert MJC, Hulsbergen-Henning JP: A model approach to laser coagulation of dermal vascular lesions. *Arch Dermatol Res* 270:429–439, 1981
18. Van Gemert MJC, Pickering JW, Welch AJ: Modeling laser treatment of portwine stains. In: Tan OT (ed.). *Management and Treatment of Benign Cutaneous Vascular Lesions*. Lea and Febiger, Philadelphia, 1992, pp 24–47
19. Carslaw HS, Jaeger JC: *Conduction of Heat in Solids*. Oxford University Press, Oxford, 1959
20. Barsky SH, Rosen S, Geer DE, Noe JM: The nature and evolution of portwine stains: a computer-assisted study. *J Invest Dermatol* 74:154–157, 1980
21. Goldman MP, Fitzpatrick RE: Treatment of cutaneous vascular lesions. In: Goldman MP, Fitzpatrick RE (eds.). *Cutaneous Laser Surgery, the Art and Science of Selective Photothermolysis*. Mosby, St. Louis, 1994, pp 32–50
22. Tong AKF, Tan OT, Boll J, Parrish JA, Murphy GF: Ultrastructure: effects of melanin pigment on target specificity using a pulsed dye laser (577 nm). *J Invest Dermatol* 88:747–752, 1987
23. Tan OT, Kerschmann R, Parrish JA: The effect of epidermal pigmentation on selective vascular effects of pulsed dye lasers. *Lasers Surg Med* 4:365–374, 1986
24. Polla LL, Margolis RJ, Dover JS, Whitaker D, Murphy GF, Jacques SL, Anderson RR: Melanosomes are a primary target of Q-switched ruby laser irradiation in guinea pig skin. *J Invest Dermatol* 89:281–286, 1987
25. Murphy GF, Shepard RS, Paul BS, Menkes A, Anderson RR, Parrish JA: Organelle-specific injury to melanin-containing cells in human skin by pulsed laser irradiation. *Lab Invest* 49:680–685, 1983
26. Anderson RR, Margolis RJ, Watenabe S, Flotte T, Hruza GJ, Dover JS: Selective photothermolysis of cutaneous pigmentation by Q-switched Nd-YAG laser pulses at 1064, 532 and 355 nm. *J Invest Dermatol* 93:28–32, 1989
27. Dover JS, Margolis RJ, Polla LL, Watanabe S, Hruza GJ, Parrish JA, Anderson RR: Pigmented guinea pig skin irradiated with Q-switched ruby laser pulses: morphologic and histologic findings. *Arch Dermatol* 125:43–49, 1989
28. Ara G, Anderson RR, Mandel KG, Ottesen M, Oseroff AR: Irradiation of pigmented melanoma cells with high intensity pulsed radiation generates acoustic waves and kills cells. *Lasers Surg Med* 10:52–59, 1990
29. Margolis RJ, Dover JS, Polla LL, Watanabe S, Shea CR, Hruza GJ, Parrish JA, Anderson RR: Visible action spectrum for melanin-specific selective photothermolysis. *Laser Surg Med* 9:389–397, 1989
30. Taylor CR, Gange RW, Dover JS, Flotte TJ, Gonzales E, Michaud N, Anderson RR: Treatment of tattoos by Q-switched ruby laser: a dose response study. *Arch Dermatol* 126:893–899, 1990
31. Taylor CR, Anderson RR, Gange RW, Michaud NA, Flotte TJ: Light and electron microscopic analysis of tattoos treated by Q-switched ruby laser. *J Invest Dermatol* 97:131–136, 1991
32. Vitkin IA, Wilson BC, Anderson RR, Prahl SA: Pulsed photothermal radiometry in optically transparent media containing discrete optical absorbers. *Phys Med Biol* 39:1721–1744, 1994
33. Watanabe S, Anderson RR, Brorson S, Dalickas G, Fujimoto JG, Flotte TJ: Comparative studies of femtosecond to microsecond laser pulses on selective pigmented cell injury in skin. *Photochem Photobiol* 53:757–762, 1991