Increase of Dermal Blood Volume Fraction Reduces the Threshold for Laser-Induced Purpura: Implications for Port Wine Stain Laser Treatment

Lars O. Svaasand, PhD,[1,2]* Guillermo Aguilar, PhD,[2] John A. Viator, PhD,[2] Lise L. Randeberg, MSc,[1] Sol Kimel, PhD,[2,3] and J. Stuart Nelson, MD, PhD[2]
[1]*Norwegian University of Science and Technology, NO-7491, Trondheim, Norway*
[2]*Beckman Laser Institute, University of California, Irvine, California 92612*
[3]*Technion-Israel Institute of Technology, Haifa 32000, Israel*

Background and Objectives: The average success rate in achieving total blanching of port wine stain (PWS) lesions treated with laser-induced selective photothermolysis is below 25%, even after multiple treatments. This is because smaller diameter (5–20 μm) PWS blood vessels are difficult to destroy with selective photothermolysis since the volumetric heat generated by absorption of laser light is insufficient to adequately heat the entire vessel wall. The aim of this study was to investigate a potential technique for more efficient photocoagulation of small diameter PWS blood vessels in PWS that respond poorly to selective photothermolysis.

Study Design/Materials and Methods: The blood volume fraction (BVF) in the upper dermis of the forearm of human volunteers was increased by placing an inflated blood pressure cuff on the upper arm. Applied pressures were in the range of 80–100 mm Hg for up to 5 minutes. The increased BVF was determined by matching reflectance spectra measured with that computed using a diffusion model. The impact of increased BVF on purpura formation induced by a 0.45 milliseconds pulsed dye laser (PDL) at 585 nm wavelength was investigated in normal and in PWS skin.

Results: In the presence of a 100 mm Hg pressure cuff, the BVF, as determined from the diffusion model, increased by a factor of 3 in the forearm and by 6 in the hand. Increasing BVF by a factor of 3 corresponds to an increase in blood vessel diameters by a factor of $\sqrt{3} \approx 1.7$. BVF increased at 1–3 minutes after application of the pressure cuff, remained constant at 3–5 minutes, and returned to baseline values at 3 minutes after removal of the pressure cuff. Approximately 40% less radiant exposure was needed to induce the same amount of purpura after PDL irradiation when the blood pressure cuff was used. Applying an 80 mm Hg pressure cuff reduced the required radiant exposure for purpura formation by 30%. Heating of blood vessels was calculated as a function of vessel diameter and of radiant exposure (at 585 nm and at 0.5 and 1.5 milliseconds pulse duration).

Conclusions: Enlarging the vessel lumen, for example, by obstructing venous return, can significantly reduce the "small-vessel-limitation" in PDL treatment of PWS. Dilation of PWS blood vessels enables a more efficient destruction of smaller vessels without increasing the probability of epidermal damage. Lasers Surg. Med. 34:182–188, 2004.

Key words: blood volume fraction (BVF); laser-induced purpura; port wine stains (PWS)

INTRODUCTION

Laser induced photothermolysis is currently the preferred modality for treatment of dermal vascular lesions, such as port wine stain (PWS). The optical wavelength and the pulse duration are selected to give optimal absorption in blood, allowing enough heat to diffuse into the vessel wall, but not into perivascular tissues [1], leading to thermal denaturation of the vessel wall as a result of heating to about 70°C while leaving the perivascular tissues well below this threshold damage temperature. Most clinical procedures utilize a wavelength of 585 nm and pulse duration of 0.45 milliseconds. The acceptable radiant exposure, which must be limited to avoid unwanted damage to the epidermis, is dependent on the patient skin type. Selective epidermal cooling by cryogen spray, which reduces the epidermal/basal layer temperature, allows for an increase in radiant exposure by a factor of more than 2 [2,3].

Dark purple PWS usually respond well to the first three to five treatments, with improved lesion blanching after each successive treatment. However, in many cases, complete blanching is never achieved, and the lesion color stabilizes at a red-pink. Moreover, those PWS that are

Contract grant sponsor: National Institutes of Health; Contract grant numbers: GM-62177, AR-47551, HD-42057; Contract grant sponsor: Air Force Office of Scientific Research; Contract grant sponsor: Beckman Laser Institute Endowment.
*Correspondence to: Lars O. Svaasand, PhD, Beckman Laser Institute, University of California, 1002 Health Sciences Rd. East, Irvine, CA 92612-1475. E-mail: svaasand@fysel.ntnu.no
Accepted 29 October 2003
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20005

initially red-pink generally respond poorly to laser treatment [4–6].

The lack of complete PWS blanching response arises from the difficulty in destroying smaller (5–20 μm) diameters vessels. Such vessels are difficult to destroy because the intraluminal blood volume is too small a fraction of the entire heated volume, which in case of a 0.45 milliseconds pulse is composed of both the blood and the vessel wall/perivascular tissues to a depth of about 7 μm [7]. Improved therapeutic outcome might be achieved if these smaller vessels are dilated immediately before laser exposure. Dilation can be attained pharmacologically or mechanically, for example, by obstructing venous blood return. The extent of vessel damage can be evaluated minutes after irradiation by inspection of laser-induced purpura, which is formed by microemboli vessel rupture and erythrocyte leakage, serving as an indirect measure of treatment efficacy.

The present study presents: (1) an analysis of vessel dilation by obstructing venous return by placing a blood pressure cuff on the proximal arm and, (2) a preliminary study on the impact of vessel dilation on formation of laser-induced purpura.

MATERIALS AND METHODS

Patients

After signing informed consent, four subjects with Fitzpatrick skin type II–III were enrolled in the study. One subject had a PWS on the left upper extremity; the other three subjects had normal skin. The laser-induced purpura was measured on one subject with normal skin and on one PWS patient. The PWS, which had previously received 36 treatments, was of a red-pink color.

Technical Systems

The BVF evaluated by measuring reflectance spectra in the 450–830 nm wavelength region, using a Hewlett-Packard (Palo Alto, CA) 8452A spectrometer equipped with a 25-mm aperture Labsphere RSA-HP-84 integrating sphere.

Laser irradiation was performed with a Candela (Wayland, MA) C-Beam^{TM} laser emitting at 585 nm wavelength and 0.45 milliseconds pulse duration. The laser was used with a 7-mm diameter spot, and without or with tetrafluoroethane cryogen spray skin cooling (30 milliseconds spurt duration, 30 milliseconds spurt-pulse delay) before laser exposure. The blood pressure cuff was an Omron (Vernon Hills, IL) digital blood pressure monitor HEM-412C.

Study Protocol

Reflectance spectra were measured on shaved regions of the volar aspect of the forearm and hand of all subjects before, during, and after application of 80 or 100 mm Hg cuff pressure to the proximal arm. Evaluation of laser-induced purpura was performed with the same cuff pressure settings and monitored up to 4 weeks after treatment.

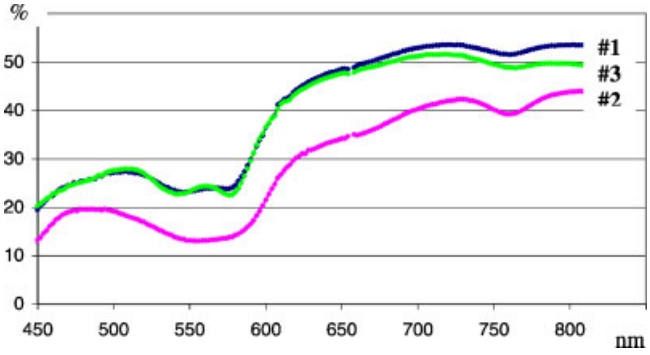

Fig. 1. Reflectance spectra (in %) for the hand of Subject 2. **Curve #1**: Before cuff pressure. **Curve #2**: Cuff pressure 100 mm Hg for 5 minutes. **Curve #3**: 3 minutes after pressure removal. Systolic/diastolic pressure 139/89 mm Hg.

RESULTS

Blood Volume Fraction (BVF)

Results from cuff pressure on the upper extremity of one subject are shown in Figure 1. The upper curve (#1) gives the reflectance spectrum of the hand before cuff pressure. Applying a pressure of 100 mm Hg obstructed venous return but allowed arterial blood flow in the systolic phase. Obstruction resulted in progressive erythema, which was manifested by a reduction in the reflection coefficient. Reflectivity decreased rapidly over the first minute after cuff placement, and reached a saturation value 3–4 minutes later. The lower curve in Figure 1 (#2) indicates reflectivity after 5 minutes of cuff pressure. The reduction in reflectivity over the entire spectrum is consistent with an increased BVF, as predicted by the diffusion model [8]. Moreover, the disappearance of two characteristic oxyhemoglobin peaks at 542 and 577 nm, together with an increase in the deoxyhemoglobin peak at 760 nm, indicates formation of the latter [9]. After cuff pressure was released, oxygenation and BVF rapidly returned to baseline values. This is demonstrated in the middle curve (#3), which shows reflectivity 3 minutes after cuff pressure was released. The BVF is now close to baseline values, but a slightly higher blood oxygenation was noted, probably due to post-obstruction compensatory arterial vasodilation.

The change in BVF in the upper dermis was determined from the reflectance spectrum in two steps. The first step involved determining skin parameters such as epidermal/dermal absorption, scattering coefficients, and blood volume by fitting the measured spectrum and reflectance values to those predicted by an analytical model based on diffusion theory [8]. This fit was made by measuring spectra before cuff pressure. Thereafter, the BVF value was adjusted to fit the change in reflectance at 586 nm with cuff pressure. This wavelength, which corresponds to an isosbestic point in the blood absorption spectrum, was selected because the measured spectra revealed oxygen depletion during cuff pressure. The measurements indicate a three- to sixfold increase in the dermal BVF after

TABLE 1. Measured Changes in Reflectivity at 586 nm Wavelength of Normal Skin With a Cuff Pressure of 100 mm Hg, Together With Calculated Corresponding Changes in BVF of Upper Dermis

Subject age (year)	Systolic/diastolic pressure (mm Hg)	Hand reflectivity before/after cuff pressure	Forearm reflectivity before/after cuff pressure	Hand increased BVF	Forearm increased BVF
33	120/75	0.30/0.16	0.31/0.23	8	3
65	139/89	0.27/0.15	0.27/0.20	4	3
37	129/79	0.23/0.14	—	4	—

3–5 minutes of 100 mm Hg pressure depending on the subject and anatomic location. The results for the three subjects are summarized in Table 1.

The corresponding increase in BVF in the forearm, when using a sub-diastolic cuff pressure of 80 mm Hg, was in the range of 2.5 (data not shown).

Purpura Measurements (Normal Skin)

Without cuff pressure, no purpura was observed for radiant exposures $\leq$3.0 J/cm^2. The degree of purpura intensified with increasing radiant exposure and, for exposures $>$3.5 J/cm^2, purpura was quite marked and immediately observable. The threshold for purpura was determined by increasing the radiant exposure from the sub-threshold value of 3 J/cm^2 and up to 7 J/cm^2, in increments of 0.5 J/cm^2. The threshold radiant exposure in absence of cuff pressure was thus found to be in the range of 3–3.5 J/cm^2. Purpura intensity increased with higher light dosage, and all test sites healed without acute blistering/scabbing, skin textural change or dyspigmentation. With 100 mm Hg cuff pressure applied for 3–5 minutes, the purpura induced by 2.0 J/cm^2 was approximately the same as for 3.5 J/cm^2 without cuff pressure. Unfortunately, the threshold value for purpura with cuff pressure could not be determined, as the laser did not allow selection of light dosage below 2 J/cm^2.

The influence of the duration of cuff pressure was examined by exposing the skin after onset of 100 mm Hg cuff pressure at 1 minute intervals to a radiant exposure kept constant at 3 J/cm^2. The observed purpura increased with duration of cuff pressure up to about 3 minutes and remained relatively constant thereafter. After 5 minutes, the cuff pressure was removed and the skin exposed to a final laser pulse, 3 minutes later. No purpura was observed for the latter case. These results are consistent with the temporal dynamics of the spectroscopic measurements; the reflectance at 586 nm decreased during the first 1–3 minutes after application of cuff pressure and returned to normal about 3 minutes after pressure was removed.

The influence of higher radiant exposure on purpura formation was studied by maintaining the cuff pressure at 100 mm Hg and increasing the light dosage in increments of 0.5 J/cm^2 from 2 to 4 J/cm^2. Purpura increased with radiant exposure and was quite notable for 4 J/cm^2; again all test sites healed without acute blistering/scabbing, skin textural change, or dyspigmentation.

The amount of purpura obtained with and without cuff pressure was assessed by visual inspection up to 14 days after treatment. The purpura at 4 and 7 J/cm^2 without pressure were subjectively judged to be about the same as with pressure at, respectively, 2.5 and 4 J/cm^2. Thus, the same amount of purpura was achieved with a 40% lower radiant exposure compared to that without pressure.

Similar measurements were carried out on one subject at the reduced cuff pressure of 80 mm Hg. The results indicated a reduction in radiant exposure of about 30% produced similar purpura to that without pressure. Typical results are displayed in Figure 2, which shows the response

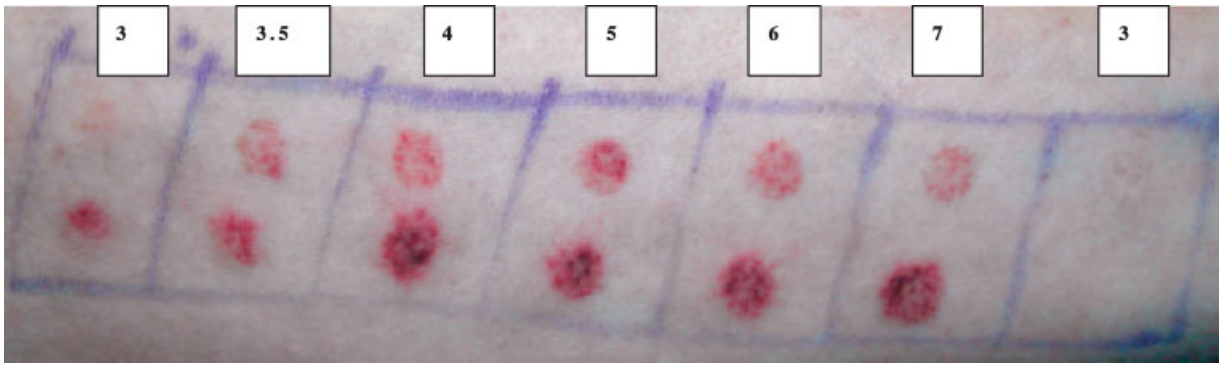

Fig. 2. Development of purpura in normal forearm, 1 day after laser exposure. First six columns, radiant exposure increased from 3 to 7 J/cm^2 (as shown in **Row 1**); last column (on right side) repeat the initial exposure of 3 J/cm^2. **Row 2**: No pressure. **Row 3**: First six columns, 3 minutes after application of 80 mm Hg cuff pressure; last column 3 minutes after release of pressure.

1 day after exposure to 3–7 J/cm^2 with and without 80 mmHg cuff pressure.

Purpura Measurements (PWS Skin)

Laser-induced purpura was measured on the forearm of a PWS subject. This patient had been treated 36 times over the last 7 years for an extensive lesion involving most of the left upper extremity. The PWS, originally of deep purple color, had blanched completely in several regions, whereas in other regions a red-pink coloration remained. Measurements were done on an area where the PWS had a red-pink color. Purpura was measured for a radiant exposure of 8 J/cm^2 without pressure, and for the same exposure 2–3 minutes after application of 100 mm Hg cuff pressure. A third area was exposed to 6 J/cm^2 with the same pressure as above. Cryogen spray cooling (30 milliseconds spurt duration, 30 milliseconds delay) before laser exposure was applied to all test sites.

The development of purpura 15 minutes after laser exposure is shown in Figure 3. Purpura in the region exposed to 8 J/cm^2 with100 mm Hg was significantly more prominent as compared to that in the region exposed to the same radiant exposure without pressure. Purpura in the region exposed to 6 J/cm^2 with pressure was also much more marked than for the 8 J/cm^2 exposures without cuff pressure. After 1 week, purpura in all regions had faded, but purpura in the region exposed without cuff pressure resolved more quickly than for regions exposed with cuff pressure (Fig. 4).

A previously treated PWS region on the dorsal aspect of the hand was exposed to 2, 4, 6, and 8 J/cm^2 with and without a 100 mm Hg pressure cuff. In the absence of pressure, there was no purpura at 2 J/cm^2, and minimal purpura at 4 J/cm^2. Thus, the threshold for purpura was estimated to be close to 4 J/cm^2. With cuff pressure, there was no purpura at 2 J/cm^2, but marked purpura with 4 J/cm^2. Furthermore, the amount of purpura for 4 J/cm^2 with pressure was somewhat less than the purpura obtained for 6 J/cm^2 without pressure (data not shown). Therefore, although the difference between radiant exposures with and without pressure was less pronounced on the dorsal hand than for the volar forearm (Figs. 3 and 4), it can be concluded that the 100 mm Hg pressure reduced the threshold radiant exposure for purpura by approximately 30%.

DISCUSSION

Modeling

The relation among vessel heating, radiant exposure, and vessel diameter is illustrated in Figure 5. The

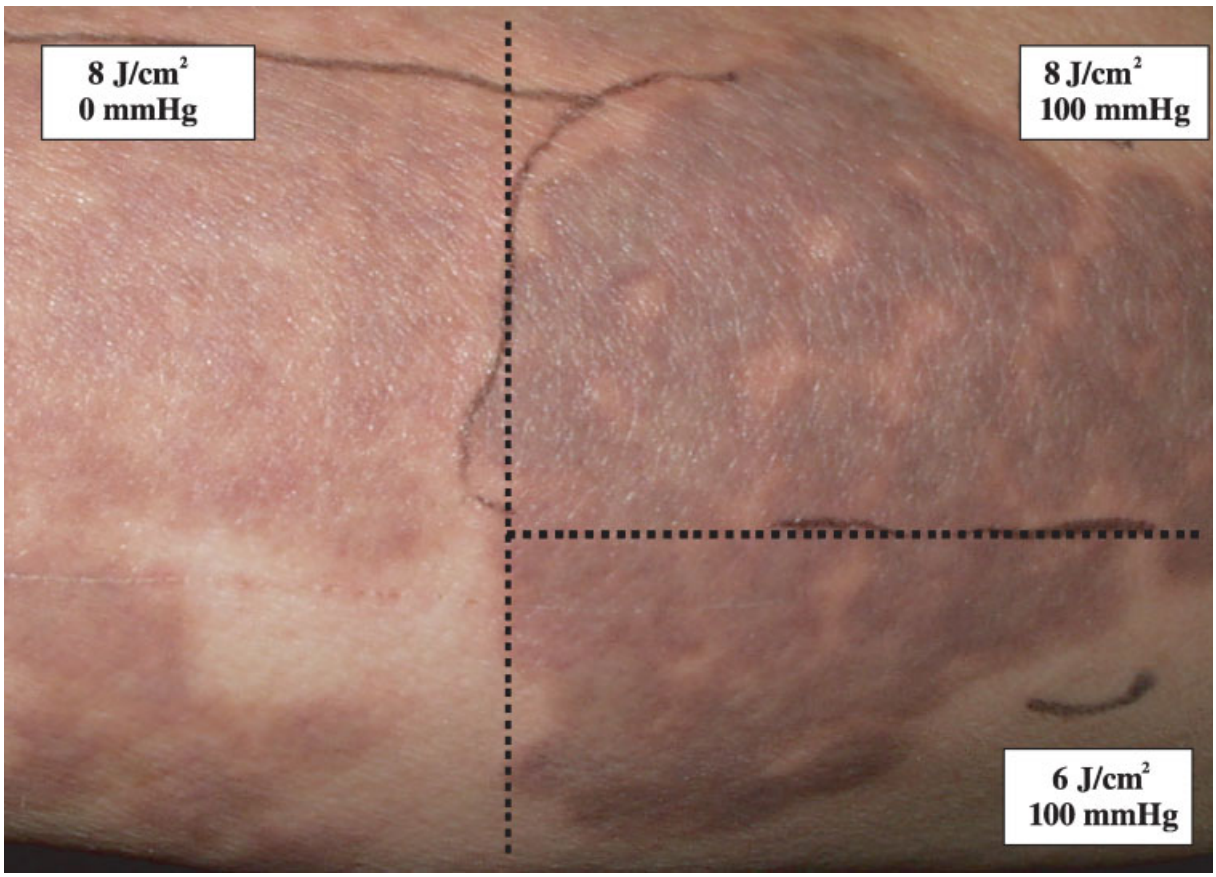

Fig. 3. Development of purpura in previously treated PWS on the forearm, 15 minutes after laser exposure.

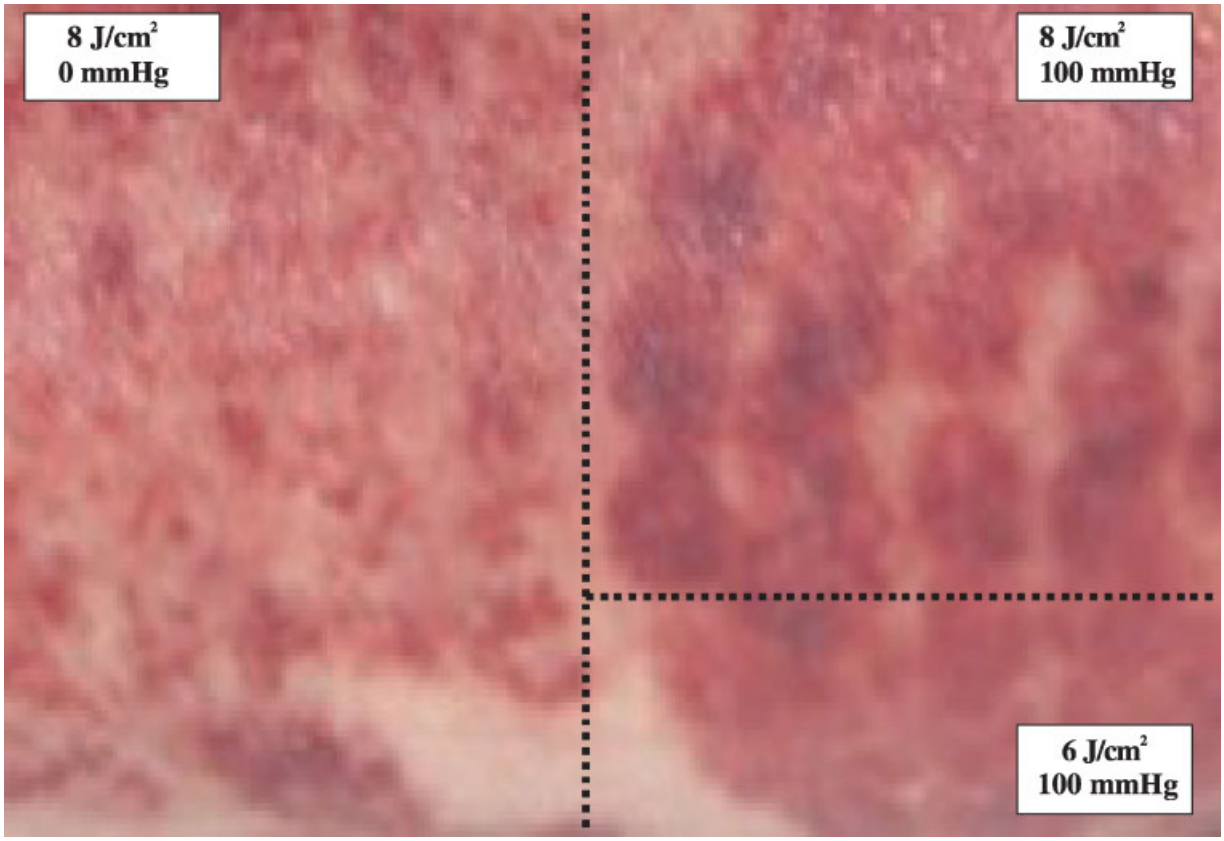

Fig. 4. Development of purpura in previously treated PWS on the forearm, 1 week after laser exposure.

mathematical expression for this relation is given by Equations A2 and A3 in the Appendix. The left panel displays the required fluence (in situ) for a 35°C temperature rise in blood vessels during exposure to a 0.5 milliseconds laser pulse. The right panel gives the corresponding values for a 1.5 milliseconds pulse. A temperature rise of 35°C will raise the temperature of the vessel wall up to 70°C, which is adequate for thermal denaturation of tissue on a millisecond time scale. The fluence is plotted as a function of vessel diameter: Curve A gives the fluence required for denaturation of normal vessels, whereas Curves B and C correspond, respectively, to increases in vessel diameter by factors of 1.7 and 2.5. Under the assumption that the blood volume in all vessels expands at the same rate for an increase in dermal BVF, these values correspond to increases in dermal BVF of $1.7^2 \approx 3$ and $2.5^2 \approx 6$.

The left panel in Figure 5 demonstrates that heating a 5-μm diameter vessel up to 70°C (i.e., 35°C above ambient temperature) requires 3.4 J/cm^2 for a 0.5 milliseconds pulse at 585 nm (Curve A). For a three- to sixfold increase in BVF, the corresponding fluences, in case of dilated vessels, are 2.4 (Curve B) and 2.0 J/cm^2 (Curve C), respectively. Thus, a three- to sixfold increase in the BVF reduces the required radiant exposure for denaturation of a 5-μm diameter vessel by 30–40%. Conversely, the Figure 5 shows that the temperature rise in 5-μm vessels is expected to increase by about 40% when the BVF is increased by a factor of 3, and by 70% for a sixfold increase. Furthermore, note that according to Figure 5 (Equations A2 and A3), the vessel diameter corresponding to the minimum fluence required for denaturation is reduced from 50 to 30 μm and 20 μm when the BVF increases three and six times, respectively.

The results for a 1.5 milliseconds pulse are shown in the right panel of Figure 5. The fluence required for heating a 5-μm diameter vessel to 70°C is 5.4 J/cm^2, and values for BVF increases of three and six times are 3.4 and 2.8 J/cm^2, respectively. Thus, the required fluence, and thereby the radiant exposure, for a three- to sixfold BVF increase are 40–50% lower than for non-dilated vessels. It should also be noted that the required radiant exposure for a 1.5 milliseconds pulse is higher than for a 0.5 milliseconds pulse for all examples under study. The reason is that more heat is conducted into the perivascular tissues during a 1.5 milliseconds pulse as compared to a 0.5 milliseconds pulse. The vessel diameter corresponding to the lowest fluence for reaching 70°C is reduced from 60 to 35 μm and 25 μm when the BVF increases by factors of 3 and 6, respectively.

Clinical Results

The diffuse reflection from human skin is composed of backscattered light from the upper 0.5–0.7 mm of the dermis. Thus, reflectance measurements, which

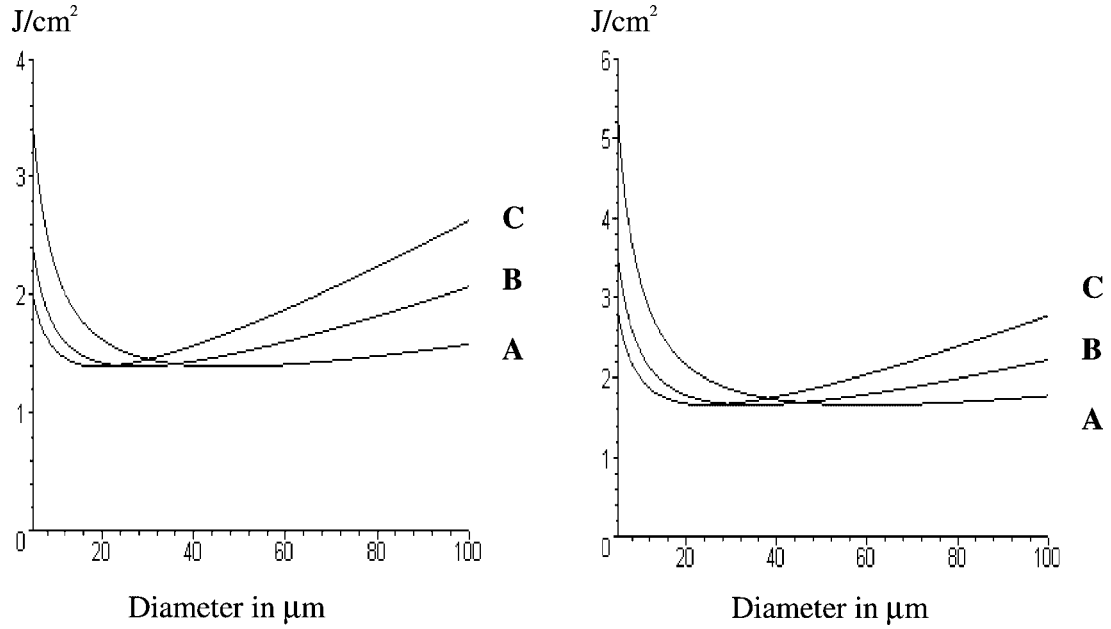

Fig. 5. Fluence in J/cm^2 (in situ) required for a 35°C temperature rise in blood vessels exposed to a laser pulse at 585 nm wavelength versus initial vessel diameter ranging from 5 to 100 μm. Pulse durations 0.5 milliseconds (**left panel**) and 1.5 miliseconds (**right panel**). Thermal diffusivity, conductivity, and optical absorption coefficient of blood are $\chi = 1.2 \times 10^{-7}$ m^2/seconds, $\kappa = 0.4$ W/mK, and μ_a=15,000 m^{-1}, respectively [9,10]. **Curve A**: Normal blood vessels; **Curve B**: corresponds to Curve A, when vessel diameters are expanded by $\sqrt{3} \approx 1.7$, corresponding to a threefold increased BVF; **Curve C**: corresponds to Curve A, when vessel diameters are expanded by $\sqrt{6} \approx 2.5$ corresponding to a sixfold increased BVF.

demonstrated that 100 mm Hg cuff pressure induced a BVF of three times the normal value in the forearm and 4–6 in the hand, should be considered as average values for the papillary and upper reticular dermis. Assuming that the BVF increase is the same for all vessels, the increase in vessel diameter is estimated to be 1.7 times the initial value in the forearm and 2–2.5 in the hand.

It is important to note that in these experiments, the highest values were measured for the hand, which had to be pressed against the spectrometer aperture to obtain good optical contact. Skin compression might have contributed to blood depletion thus introducing an artifact because the baseline value for the BVF before cuff placement might be too low. This phenomenon was of minor importance for in the forearm since good optical contact was obtained with minimum pressure.

The threshold radiant exposure for laser-induced purpura was reduced with increased BVF, and a comparable degree of purpura was formed in the forearm with 40% less energy with a 100 mm Hg cuff pressure on the proximal arm applied for 2–3 minutes. This result is believed to be due to more efficient targeting of smaller blood vessels as they expand during cuff pressure; vessel diameter corresponding to the minimum required radiant exposure is reduced from 50 to 30 μm for a 0.5 milliseconds pulse. Thus, vessel expansion enables a more efficient destruction of smaller vessels without affecting the threshold for epidermal damage.

It should be noted that the decrease in purpura threshold is independent of epidermal pigmentation. For example, if the probability for epidermal damage in a patient with skin type IV limits the acceptable radiant exposure to 5 J/cm^2, the efficacy of this dose with cuff pressure will be equivalent to a dose of 8.5 J/cm^2, with the probability for epidermal damage unchanged.

CONCLUSIONS

This study reveals that purpura, which is used as an indicator of PWS response to laser-induced photothermolysis, can be obtained with significantly reduced radiant exposures when the targeted vessels are dilated. A cuff on the proximal arm with pressure just above the diastolic value can increase the BVF in the upper dermis of the forearm by a factor of 3, and thereby enlarge vessel diameters by a factor of 1.7. Such dilation is of importance when targeting smaller (<20 μm) diameter PWS vessels. Destroying these vessels often requires such high radiant exposures that epidermal heating can result in dyspigmentation and scarring. A cuff pressure in the range of 100 mm Hg applied for 2–3 minutes resulted in the same amount of purpura as without pressure, with 40% less radiant exposure from a 0.45 milliseconds laser pulse at 585 nm. Since the required radiant exposure is reduced by 40%, the efficacy of the applied light dose is increased by almost 70%.

The principle of dilating targeted vessels can be applied to PWS at any locations of the body. Increasing the BVF of, for example, facial PWS, can be done pharmacologically, by treating the patient while in the Trendelenberg position, or

by applying suction to the treated area immediately before or during laser exposure. Measurements of blood volume fractions during application of a suction cup demonstrated that a very slight under-pressure of about 100 mm Hg for less than 1 minute resulted in BVF increase by at least a factor of 3. Further on, the same enhanced purpura as obtained by the cuff pressure was obtained by using a suction cup on the forearm during laser exposure. A detailed study of this suction technique is being carried out, and will be reported on shortly.

ACKNOWLEDGMENTS

The authors acknowledge support from the National Institutes of Health (GM-62177 and AR-47551 to J.S.N. and HD-42057 to G.A.), Air Force Office of Scientific Research, and the Beckman Laser Institute Endowment. The collaboration with Woody McCauley is most appreciated.

APPENDIX

Heating of blood by laser irradiation without thermal loss to adjacent tissues can be expressed by

$$T = \frac{\mu_a \phi}{\rho C} t = \frac{\mu_a \phi}{\frac{\kappa}{\chi}} t \tag{A1}$$

where T is the temperature rise and t is the time after onset of irradiation. The coefficients ρ, C, μ_a, ϕ represent, respectively, density, specific heat per unit mass, optical absorption coefficient, and fluence rate. The specific heat per unit volume can also be expressed as $\rho C = \kappa/\chi$, where κ denotes the thermal conductivity and χ the thermal diffusivity.

When blood in a vessel of diameter D is heated by irradiation, part of the thermal energy will diffuse into the optically non-absorbing vessel wall. The temperature rise immediately after a pulse of duration τ_p can be expressed as [10],

$$T = \frac{\mu_a \psi D^2}{16 \tau_p \kappa} \left\{ \ln\left(\frac{D^2 (D - 4\sqrt{\chi \tau_p})}{(D^2 - 16 \chi \tau_p)(D + 4\sqrt{\chi \tau_p})} \right) + \frac{8\sqrt{\chi \tau_p}}{D} \right\} \tag{A2}$$

where $\psi = \phi \tau_p$ is the pulse fluence.

This expression applies when light is absorbed uniformly across the lumen. When the optical penetration depth in blood, $\delta = 1/\mu_a$, that is, the reciprocal absorption coefficient, becomes comparable to D light depletion must be taken into account. Depletion can be accounted for by introducing an effective absorption coefficient corresponding to averaging over the lumen. Light absorption per unit volume of blood can then be expressed by $\mu_{a,eff}\psi$, where $\mu_{a,eff}$ is an effective absorption coefficient,

$$\mu_{a,eff} = 2 \frac{I(1, \frac{D}{\delta}) - L(1, \frac{D}{\delta})}{D} \tag{A3}$$

where $I(1,x)$ is the first order modified Bessel-I function and $L(1,x)$ is the first order modified Struve function. When depletion is negligible, that is, $\delta \gg D$, this expression reduces to $\mu_{a,eff} \Rightarrow \mu_a$ and when depletion is dominant, it becomes $\mu_{a,eff} \Rightarrow 4/\pi D$.

REFERENCES

1. Anderson RR, Parish JA. Selective photothermolysis. Precise microsurgery by selective absorption of pulsed radiation. Science 1983;226:524–527.
2. Nelson JS, Milner TE, Anvari B, Tanenbaum BS, Kimel S, Svaasand LO, Jacques SL. Dynamic epidermal cooling during pulsed laser treatment of port-wine stain. A new methodology with preliminary clinical evaluation. Arch Dermatol 1995; 131:695–700.
3. Nelson JS, Majaron B, Kelly KM. Active skin cooling in conjunction with laser dermatologic surgery. Semin Cutan Med Surg 2000;19:253–266.
4. Van der Horst CMAM, Koster PHL, deBorgie CAJM, Bossuyt PMM, van Gemert MJC. Effect of timing of treatment of port-wine stains with the flash-lamp-pumped pulsed dye laser. N Engl J Med 1998;338:1028–1033.
5. Morelli JG, Weston WL, Huff JC, Yohn JJ. Initial lesion size as a predictive factor in determining the response of port-wine stains in children treated with the pulsed dye laser. Arch Pediatr Adolesc Med 1995;149:1142–1144.
6. Lanigan SW. Port-wine stains unresponsive to pulsed dye laser: Explanations and solutions. Br J Dermatol 1998;139: 173–177.
7. Svaasand LO, Fiskerstrand EJ, Kopstad G, Norvang LT, Svaasand EK, Nelson JS, Berns MW. Therapeutic response during pulsed laser treatment of port-wine stains; dependence on vessel diameter and depth in dermis. Lasers Med Sci 1995;10:235–243.
8. Svaasand LO, Norvang LT, Fiskerstrand EJ, Stopps EKS, Berns MW, Nelson JS. Tissue parameters determining the visual appearance of normal skin and port-wine stains. Lasers Med Sci 1995;10:55–65.
9. http://omlc.ogi.edu/spectra/hemoglobin/summary.html/
10. Duck FA. Physical properties of tissue. London: Academic Press; 1990.
11. Kimel S, Svaasand LO, Hammer-Wilson MJ, Nelson JS. Influence of wavelength on vascular response to laser photothermolysis of blood vessels: Implications of port wine stain laser therapy. Lasers Surg Med 2003;33:288–295.