Talaporfin Sodium–Mediated Photodynamic Therapy Alone and in Combination with Pulsed Dye Laser on Cutaneous Vasculature

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TO THE EDITOR

Currently, standard treatment for port wine stain (PWS) birthmarks in the United States involves the use of lasers or intense pulsed light to photocoagulate selectively the abnormal vasculature. With photothermal therapy, PWS often become lighter, but patients must undergo many treatments (15–20 are not uncommon; Koster *et al.*, 2001). Furthermore, treatment of skin types IV–VI is difficult owing to absorption of light by overlying epidermal melanin, limiting treatment safety and efficacy.

Photodynamic therapy (PDT), an alternative treatment option, involves optical excitation of an exogenous photosensitizer and subsequent energy transfer from the photosensitizer to oxygen to create cytotoxic singlet oxygen (Gorman *et al.*, 2006). Excitation of photosensitizers localized primarily within the intravascular compartment enables targeted vascular destruction. Treatment can be effective but it is associated with prolonged photosensitivity and substantial scarring risk (Lu *et al.*, 2010).

Talaporfin sodium (TS) is a photosensitizer with proven selective vascular effects in preclinical studies, an acceptable photosensitivity period of 5–7 days, and good safety data (Kujundzic *et al.*, 2007; Bromley *et al.*, 2011; Akimoto *et al.*, 2012). Previously, we determined that the characteristic radiant exposure required for persistent vascular shutdown ($RE_{50/7}$ value) for TS-mediated PDT, using a custom-built light-emitting diode array (664 nm, full width at half maximum = 20 nm), was 85 J cm^{-2} (Moy *et al.*, 2012). On the basis of these and other published data (Smith *et al.*, 2006; Channual *et al.*, 2008; Tournas *et al.*, 2009), we hypothesized that dual phototherapy treatment with TS-mediated PDT and ensuing pulsed dye laser (PDL) therapy will achieve persistent vascular shutdown with otherwise subtherapeutic radiant exposures of PDT and PDL. To test this hypothesis, we conducted studies to determine the $RE_{50/7}$ to achieve persistent vascular shutdown with PDL irradiation and the associated $RE_{50/7}$ values for dual phototherapy. We postulate that lower radiant exposures can be used for both TS-mediated PDT and ensuing PDL, minimizing adverse effects, allowing treatment of all skin types, and potentially achieving enhanced treatment efficacy, compared with either alone.

Using a protocol approved by UC Irvine Institutional Animal Care and Use Committee, we installed dorsal window chambers (Moy *et al.*, 2011) on adult C3H mice (25–30 g, $n = 38$) anesthetized with isoflurane. For PDT, we used a custom-built light-emitting diode array centered at 664-nm excitation (full width at half maximum = 20 nm). For TS-mediated PDT, we reconstituted TS (Light Sciences Oncology; Bellevue, Washington) using sterile saline to form a solution of 25 mg ml^{-1}. We injected TS (5 mg kg^{-1}) into the bloodstream via retro-orbital injection and began PDT immediately afterward (irradiance 100 mW cm^{-2}; radiant exposure 0–260 J cm^{-2}). For PDL irradiation, we used a clinical 595-nm laser (Vbeam Perfecta, Candela Corporation, Wayland, Massachusetts; 10-mm diameter spot size, 1.5-ms pulse duration, radiant exposure 3.25–10.00 J cm^{-2}). We randomized the experiment order.

To test the hypothesis that the dual therapy protocol enables persistent vascular shutdown with lower radiant exposures of either PDT or PDL irradiation, we restricted our study to radiant exposure values of PDT (20–60 J cm^{-2}) and PDL (4–6 J cm^{-2}) that were below the associated $RE_{50/7}$ values for either treatment alone. We performed PDL irradiation within 5 s after PDT.

To monitor blood-flow dynamics, we used laser speckle imaging (Moy *et al.*, 2011). We used an experimental design based on dose–response analysis (Moy *et al.*, 2012). We performed 19 experiments to establish a dose–response curve for PDL and 19 experiments for PDT + PDL. We collected raw speckle images before and at time points during the ensuing week (Choi *et al.*, 2008). Five of the authors (BC, WJM, KMK, BSL, and JJM) independently reviewed the SFI images collected on day 7 and graded them as ''0'' (no persistent vascular shutdown) or ''1'' (persistent vascular shutdown achieved). Prism (version 5.0d, GraphPad Software, San Diego, California) was used to estimate the $RE_{50/7}$ for each study. We used an *F*-test to compare the log ($RE_{50/7}$) values determined from PDT (85 J cm^{-2} from Moy *et al.* (2012)) and PDT + PDL. Our null hypothesis was that the $RE_{50/7}$ values for the two studies do not differ in a statistically significant manner.

We observed three dose-dependent responses: (1) minimal acute change in blood flow and no persistent vascular shutdown (Figure 1a); (2) marked acute change in blood flow, followed by partial-to-full recovery of blood flow and no persistent vascular shutdown (Figure 1b); and (3) marked acute or delayed reduction in blood flow, followed by complete vascular shutdown at day 7 (Figure 1c). With application of dose–response methodology, we estimated an $RE_{50/7}$ of 7.1 J cm^{-2} required

Abbreviations: LSI, laser speckle imaging; PDL, pulsed dye laser; PDT, photodynamic therapy; PWS, port wine stain; SFI, speckle flow index; TS, talaporfin sodium

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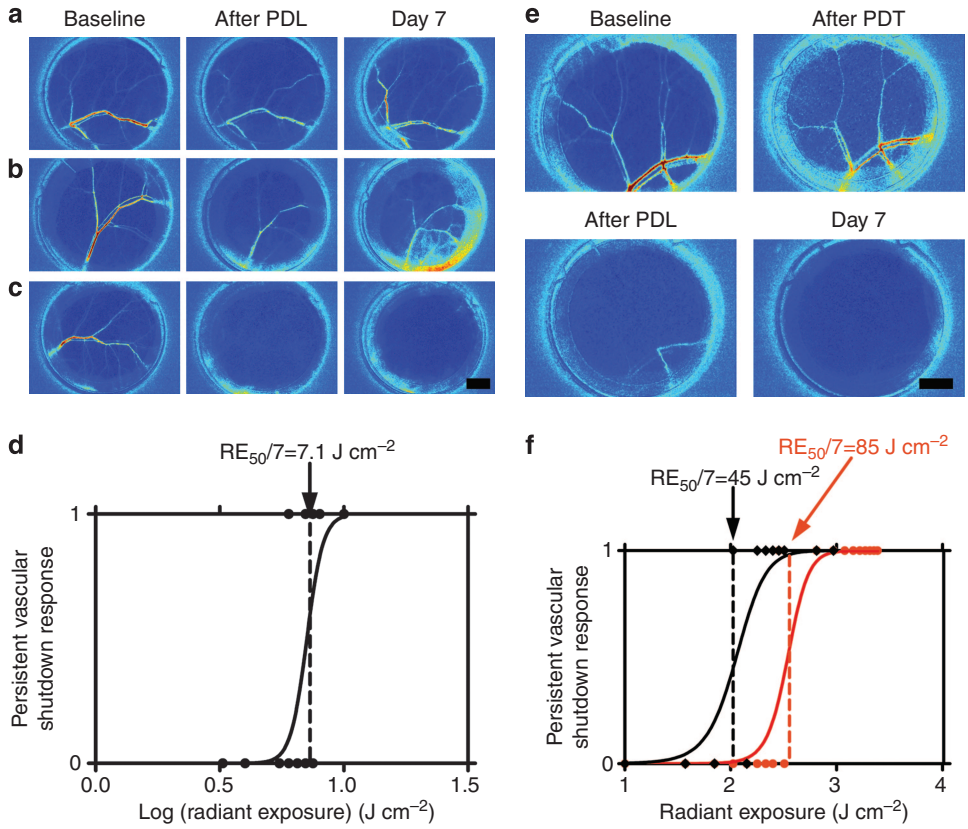

Figure 1. The combination of talaporfin sodium (TS)-mediated photodynamic therapy (PDT) and pulsed dye laser (PDL) irradiation leads to a significant reduction in the characteristic PDT radiant exposure required to achieve persistent vascular shutdown. We first determined the characteristic radiant exposures associated with persistent vascular shutdown following 595-nm PDL irradiation. We performed PDL on the epidermal side of window chambers and imaged blood flow using laser speckle imaging (LSI). We assessed persistent vascular shutdown on day 7. We assigned a "0" score if some evidence of blood flow was present, and a "1" score if flow was no longer evident. We used dose–response analysis to calculate a characteristic radiant exposure ($RE_{50/7}$) at which 50% of irradiated window chambers are expected to have vascular shutdown on day 7. (**a**–**c**) Representative LSI data associated with 595-nm PDL irradiation, in which persistent vascular shutdown was not (**a**, **b**) and was (**c**) achieved, using radiant exposures of (**a**) 4, (**b**) 6, and (**c**) 10 J cm^{-2}, respectively. (**d**) On the basis of data from 19 experiments, we identified an $RE_{50/7}$ of 7.1 J cm^{-2} for PDL irradiation. We then studied the combination of TS-mediated PDT and PDL irradiation. In this set of experiments, we used PDT (20–60 J cm^{-2}) and PDL radiant exposures (4–6 J cm^{-2}) that were below the $RE_{50/7}$ values of 85 J cm^{-2} (Moy *et al.*, 2012) and 7.1 J cm^{-2} (**d**), respectively. (**e**) Representative maps of blood flow that demonstrate persistent vascular shutdown at day 7. In this specific example, we applied PDT (60 J cm^{-2}) followed by PDL (6 J cm^{-2}). This combination resulted in marked acute vascular shutdown, which persisted through day 7. (**f**) On the basis of data from 30 experiments, we determined that the characteristic radiant exposure required to achieve persistent vascular shutdown decreased from 85 J cm^{-2} with PDT alone to 45 J cm^{-2} for the combined PDT + PDL protocol. Bar = 2 mm.

to induce persistent vascular shutdown with PDL irradiation (Figure 1d). With PDT + PDL, the characteristic PDT radiant exposure required to achieve persistent vascular shutdown, decreased from 85 to 45 J cm^{-2} (Figure 1e). This difference in PDT $RE_{50/7}$ was found to be statistically significant ($P = 0.0002$).

We evaluated the degree of synergy between PDT and PDL vascular effects with dual phototherapy (Madsen *et al.*, 2002):

$$\alpha = \frac{f_{PDT} f_{PDL}}{f_{PDT+PDL}} \quad (1)$$

where fraction of photodynamic therapy experiments (f_{PDT}) and fraction of pulsed dye laser experiments (f_{PDL}) are the fractions of single-phototherapy experiments and $f_{PDT+PDL}$ is the fraction of combined experiments, which do not induce persistent vascular shutdown. An additive (or absence of any) effect is indicated by degree of interaction (α) = 1, $\alpha > 1$ indicates a synergistic effect, and $\alpha < 1$ indicates an antagonistic effect. Our data (Table 1) suggest the synergistic nature ($\alpha = 2.7$) of PDT + PDL.

Table 1. Summary of observations of persistent vascular shutdown for experiments in which the PDT radiant exposure was 20–60 J cm^{-2} and/or the PDL radiant exposure was 4–6 J cm^{-2}

Experimental condition	Number of experiments meeting criterion	Number of occurrences of persistent vascular shutdown	f_{PDT}, f_{PDL}, or $f_{PDT+PDL}$
PDT	4	0	100%
PDL	7	1	86%
PDT + PDL	19	13	32%

Abbreviations: f_{PDL}, fraction of pulsed dye laser experiments; f_{PDT}, fraction of photodynamic therapy experiments; $f_{PDT+PDL}$, fraction of combined experiments; PDL, pulsed dye laser; PDT, photodynamic therapy.
The PDT data are taken from Moy *et al.* (2012). On the basis of these data and equation 1, the degree of interaction is 2.7, suggesting that PDT and PDL irradiation together achieve a synergistic shutdown effect.

Collectively, our results reveal that PDT + PDL reduces the PDT light dose required to achieve persistent vascular shutdown, even at low PDL radiant exposures. We hypothesize that TS-mediated PDT enhances persistent vascular shutdown achieved with ensuing PDL therapy, primarily via endothelial cell damage (Mitra and Foster, 2008); mechanistic studies currently are underway.

Dual phototherapy represents a potential new approach for more effective treatment of PWS birthmarks. We have initiated a trial approved by the Investigational Review Board to evaluate intravenously administered TS/664-nm laser light–mediated dual phototherapy for PWS treatment. Treatment has been painless and notable lesion lightening has been achieved with both PDT and PDT + PDL in a single session. Patients are photosensitive for 5–7 days post procedure, and for the first 72 hours they must remain indoors with lights dimmed. Completion of this study will determine whether lesion lightening is greater with dual phototherapy than PDL alone. It is our intent that this combined low-energy dual phototherapy will offer clinicians and patients of all skin types improved lesion lightening in fewer treatments.

CONFLICT OF INTEREST

Light Sciences Oncology provided TS for this research.

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Multiple Self-Healing Palmoplantar Carcinoma: A Familial Predisposition to Skin Cancer with Primary Palmoplantar and Conjunctival Lesions

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TO THE EDITOR

Familial keratoacanthomas (KAs) are characterized by the appearance of multiple epithelial tumors that are believed to arise from adjoining hair follicles (Schwartz, 1994). These lesions, which phenotypically and histologically resemble squamous cell carcinomas (SCCs) (Cribier *et al.*, 1999), have a fast evolution and spontaneous regression. To date, four familial forms of multiple KAs have been described: (1) multiple self-healing squamous

Abbreviations: KA, keratoacanthoma; MSPC, multiple self-healing palmoplantar carcinoma; MSSE, multiple self-healing squamous epithelioma; SCC, squamous cell carcinoma

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