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Evaluation of Vascular Effects of Photodynamic and Photothermal Therapies Using Benzoporphyrin Derivative Monoacid Ring A on a Rodent Dorsal Skinfold Model

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ABSTRACT

Background and Objectives: Pulsed dye laser (PDL) irradiation is the standard clinical treatment for vascular lesions. However, PDL treatment of port wine stain birthmarks (PWS) is variable and unpredictable. Photodynamic therapy (PDT) using benzoporphyrin derivative monoacid ring A (BPD) and yellow light may induce substantial vascular effects and potentially offer a more effective treatment. In this study, we utilize a rodent dorsal skinfold model to evaluate the vascular effects of BPD-PDT at 576 nm as compared to PDL.

Study Design/Materials and Methods: A dorsal skinfold window was created on the backs of female Sprague-Dawley rats, allowing epidermal and subdermal irradiation and subdermal imaging. One mg/kg BPD was administered intravenously via a jugular venous catheter. Study groups were: control (no BPD, no light), PDL (585 nm, τ_p 1.5 ms, 10 J/cm^2), and PDT (BPD + continuous wave irradiation (CW) at 576nm, τ_p 16 min, 96 J/cm^2). Vessels were imaged and assessed for damage using laser speckle imaging (LSI) before, immediately after, and 18 hours post-intervention.

Results: Epidermal irradiation was accomplished without blistering, scabbing or ulceration. PDL and PDT resulted in similar reductions in vascular perfusion 18 hours post-intervention (34.6% and 33.4%, respectively).

Conclusions: BPD-PDT can achieve safe and selective vascular effects and may offer an alternative therapeutic option for treatment of hypervascular skin lesions including PWS birthmarks.

Keywords: dermatology, flow dynamics, photosensitizer, speckle imaging, vascular perfusion.

1. INTRODUCTION

The current standard treatment for port wine stains (PWS) is the pulsed dye laser (PDL). The PDL achieves reasonably good results in some PWS patients due to its ability to destroy selectively cutaneous blood vessels. However, few patients (< 10%) achieve complete blanching of their PWS even after receiving multiple laser treatments. There are several reasons for PDL treatment failure. The PDL is capable of removing vessels with diameter greater than 20 μm diameter, but in many patients, small superficial vessels persist after laser irradiation (1). Also, absorption of laser energy by epidermal melanin reduces the light dosage reaching the blood vessels, decreasing the amount of heat produced in the targeted PWS vessels and leading to sub-optimal lesion blanching (2).

Photodynamic therapy (PDT) utilizes a photosensitizer and specific wavelengths of light to generate reactive oxygen species (3) which creates an opportunity for targeted lesion destruction. PDT has been used to treat a wide range of benign, pre-malignant and malignant conditions, including age-related macular degeneration (4), actinic keratoses (5) and cancers of the skin, lung and gastrointestinal tract (6). While PDL therapy uses short-pulsed, high-intensity irradiation to create photothermal injury, PDT uses a continuous wave (CW) light source to induce photochemical reactions with negligible heat generation. Milliwatt light exposure used during PDT does not cause epidermal thermal injury produced by high peak power PDL therapy. Furthermore, since PDT uses continuous low irradiance light over long exposure times (several minutes), vascular injury occurs deeper in the tissue as exposure time is increased. This contrasts sharply with conventional PDL therapy, which must achieve a sufficient "temperature jump" with a single laser

pulse. Multiple PDL pulses do not significantly increase the depth of treatment and have a limited effect on PWS blanching response, and also subject the epidermis to a higher risk of thermal injury (7). Finally, in contrast to PDL therapy, PDT destroys all vessels containing photosensitizer, irrespective of size (8).

In order for PDT to be used to treat cutaneous vascular lesions, the treatment protocols must be carefully designed. We believe BPD to be an excellent choice for PDT of cutaneous vascular lesions for the following reasons: 1) vascular predominance at early timepoints after administration (9-12); 2) proven safety and efficacy in humans (4, 13); 3) photosensitivity of relatively short duration (1-5 days for BPD depending on the dose administered) (13); and 4) presence of an absorption peak in the desired yellow wavelength range.

A preliminary study in the chick chorioallantoic membrane (CAM) model was performed to evaluate the potential of BPD-PDT for the treatment of vascular lesions. That study suggested that significant and selective vascular injury could be achieved with BPD-PDT (14).

In the current study, the safety and vascular effects of BPD-PDT were studied and compared to PDL treatment, utilizing a rodent dorsal skinfold model, a model which is more similar to human skin than the CAM.

2. MATERIALS AND METHODS

Our protocol was approved by the University of California, Irvine Institutional Animal Care and Use Committee. Eleven female Sprague-Dawley rats weighing 400 to 425 g were obtained with a jugular venous catheter in place (Zivic Laboratories, Pittsburgh, PA).

2. 1. Rodent Dorsal Skinfold Model

A rodent dorsal skinfold model has been used for investigations of light interaction with microcirculation (15-19). The dorsal skin was lifted and sutured to a C-clamp. A circular "window" section with an approximate diameter of 1 cm was cut from one side of the symmetrical skin fold, thus exposing a thin layer of skeletal muscle, blood vessels, and subcutaneous tissue of the underlying skin. An aluminum chamber was then sutured to both sides of the skin allowing observation of blood vessels from the subdermal side and intervention from either the epidermal or subdermal side. Epidermal irradiation was performed on three animals (1 PDT, 2 PDL) to determine whether treatment would result in adverse cutaneous effects such as blistering, scabbing or ulcer formation. The thickness of the rodent dorsal skin fold is approximately 2 mm. Incident yellow light is absorbed primarily by more superficial cutaneous vessels, and hence the majority of yellow light does not reach a depth of 2 mm. As such, to determine vascular treatment effect of PDL versus PDT, we irradiated from the dermal side of the flap (four and three animals, respectively).

2. 2. Experiments

2.2.1 Control

Surgery and no further intervention was performed on one animal to serve as a control.

2.2.2 PDL Irradiation

A 585 nm PDL (ScleroPlus™, Candela, Wayland, MA) with a 1.5 millisecond pulse duration was used to irradiate the test site at a fluence of 10 J/cm^2, using a 7 mm diameter spot. For epidermal irradiation studies, cryogen spray cooling was used with a 30 ms spurt duration and a 20 ms delay. No cooling was used with subdermal irradiations.

2.2.3 Photodynamic Therapy

BPD (Verteporfin®, QLT, Vancouver, BC, Canada) liposomal powder was reconstituted in water at a concentration of 1 mg/ml. This working solution was protected from light and used within four hours of preparation. One mg/kg of BPD solution was administered intravenously via a jugular venous catheter using a Hamilton syringe with a 20 gauge needle. Rats were kept in the dark post BPD injection and further manipulations were performed in subdued light. Fifteen minutes after BPD injection, CW irradiation was performed. A CW argon pumped-dye laser (Coherent, Santa Clara, CA) tuned to 576 nm, was used to irradiate the test site inside the window, at a power of100 mW/cm^2 for 16 minutes, yielding a total radiant exposure of 96 J/cm^2.

2.2.4 Laser Speckle Imaging (LSI)
Laser speckle imaging (LSI) was performed prior to treatment and then immediately and 18 hours post-treatment. LSI has been used to measure perfusion in blood vessels in the rodent dorsal skinfold model (19). Briefly, a HeNe laser (λ = 633 nm, 30 mW, Edmund Industrial Optics, Barrington, NJ) is used to irradiate the fold window. A planoconvex lens is then used to expand the laser beam to irradiate uniformly an area of approximately 2.5 cm in diameter. The speckle pattern is imaged with an 8-bit monochrome CCD camera (Model XC-70, Sony, Japan) equipped with a macro lens. The field of view is set to an area of approximately 5 X 4 mm^2. The image integration time set to 10 ms, and the lens configuration is selected to match approximately the speckle size (15 μm) with the camera pixel size. Video images acquired at 30 frames per second are transferred from the camera to a PC equipped with a frame grabber (National Instruments, Austin, TX). Custom software written in LabVIEW (Version 7, National Instruments) and MATLAB (Version 6.1, The MathWorks, Inc., Natick, MA) is used to acquire and process the images. The image processing algorithm has been previously described in detail (15, 20). Images are converted to relative flow images by applying a 7 X 7 pixel sliding window to each 640 X 480 pixel image. To quantify changes in blood flow, the average perfusion index (PI) and standard deviation is calculated for each image and plotted with a maximum PI of 700.

3. RESULTS

Clinical observation 18 hours after epidermal irradiation; 1 PDT, 2 PDL revealed no disruption (no blistering, scabbing or ulceration) in any of the animals. After subdermal irradiation, changes in vascular flow were observed but no other tissue changes were evident from visual inspection.

Figures 1, 2 and 3 show LSI images of the control, PDL and PDT groups before, immediately after, and 18 hours post subdermal irradiation. The control animal (surgery only) demonstrated a slight change (8.7% increase) in PI 18 hours post-procedure (Table 1).

PDL results are presented in Figures 2 and 4. PDL animals A, B and C had a marked reduction in perfusion immediately after laser irradiation with a further reduction in flow at 18 hours. PDL animal D demonstrated a decrease in perfusion immediately post-treatment and reperfusion at 18 hours. PDL treatment PI values for animals A, B, C, and D resulted in an average decrease in perfusion of 35.2% immediately after and 34.6% 18 hours post-intervention (Table 1).

PDT results are presented in Figures 3 and 5. PDT animals A and B demonstrated an increase in perfusion immediately after PDT, followed by a noticeable reduction in flow 18 hours later. PDT animal C had very few vessels in the window before therapy and it was unclear whether the vessels were intact. In this animal, perfusion did not decrease significantly after treatment (Figure 5). PDT PI values for animals A, B, and C showed a slight increase in average perfusion immediately after PDT (6.7%) followed by a reduction in flow of 33.4% at 18 hours (Table 1).

4. DISCUSSION

This study was designed to determine the safety and vascular effects of BPD-PDT using 576 nm light compared to PDL treatment. Several important points can be established from our data. First, we were able to perform treatment from the epidermal side without any notable disruption of the skin surface. Epidermal disruption increases the risk of scarring or dyspigmentation which would not be acceptable. It is well known that with epidermal cooling, PDL treatment at the radiant exposures used in this study are well tolerated (1, 7). However, PDT treatment frequently results in epidermal disruption (8), and so the parameters of this study (specifically the wavelength, irradiance and radiant exposure) were carefully chosen in order to attain a vascular effect without epidermal injury.

Second, BPD-PDT achieved a vascular effect which was at least equal to PDL treatment 18 hours post-intervention. It should be noted, however, that it is likely that PDT can achieve a significantly greater effect than PDL treatment. The

PDL parameters used in this study (585 nm, 7mm 10 J/cm^2) are commonly utilized in our clinic and can achieve a good clinical response in certain patients. Clinically, laser irradiation is delivered to the epidermis and melanin absorption and scattering diminishes the amount of light which ultimately reaches the vascular target. As such, the effective fluence at the level of the most superficial blood vessels in a PWS, approximately 200 μm below the skin surface, would be about 7 J/cm^2 (21). In this study, irradiation was performed from the subdermal side, directly onto the vessels. As described above, this was done because rodent skinfold thickness was approximately 2 mm and epidermal irradiation did not allow light penetration to the observable vessels. We plan further experiments with the dorsal skin fold model using a PDL subdermal radiant exposure of 7 J/cm^2 which will allow further comparison of these two therapeutic modalities.

Third, it is interesting to note that PDT resulted in an initial increase in perfusion immediately after intervention followed by a reduction in flow at 18 hours. Several mechanisms may be responsible for this initial increase in flow including a compensatory response to oxygen depletion which occurs as a consequence of the photochemical reactions induced by PDT (Figs. 3b and 3e). We have noted that evaluation of PDT effects 18 hours after treatment is essential to accurately measure reductions in vascular perfusion.

Alternative treatment options should be sought for cutaneous vascular lesions such as PWS, as current PDL treatment achieves variable and unpredictable results and many patients do not achieve adequate lesion clearing. This study demonstrates that BPD-PDT can be performed without epidermal disruption and can achieve vascular effects comparable to high radiant exposure PDL and therefore should be explored further in clinical trials.

CONCLUSIONS

BPD-PDT can achieve safe and selective vascular effects and may offer an alternative therapeutic option for treatment of hypervascular skin lesions including PWS birthmarks.

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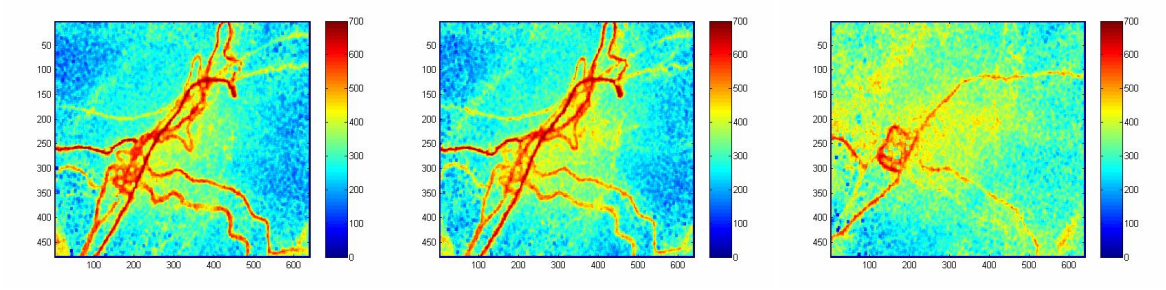
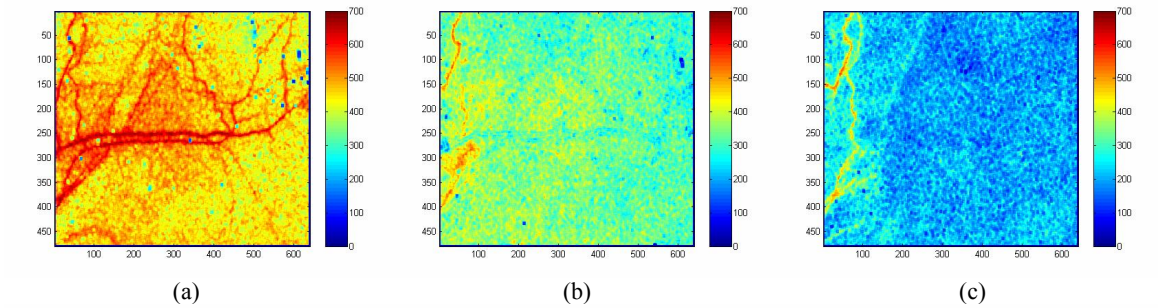
TABLE 1. Average percent change in PI calculated for each study group immediately and 18 hours after intervention.

Study Group	Percent Change in PI Immediately After	Percent Change in PI 18 Hours After
Control	0.9	8.7
PDL	-35.2	-34.6
PDT	6.7	-33.4

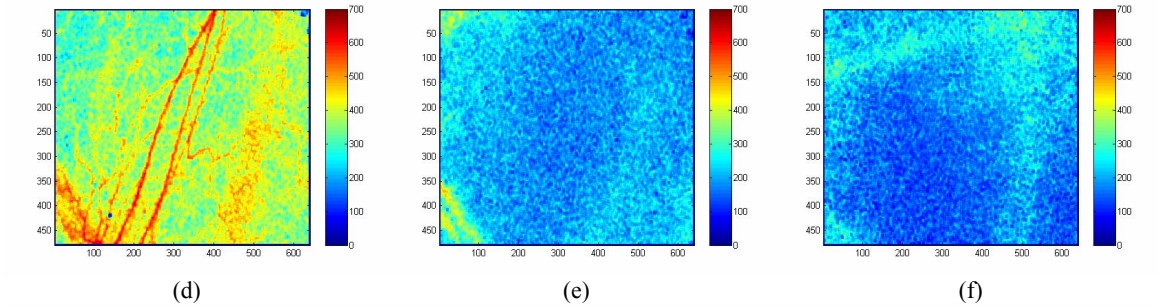


(a) (b) (c)

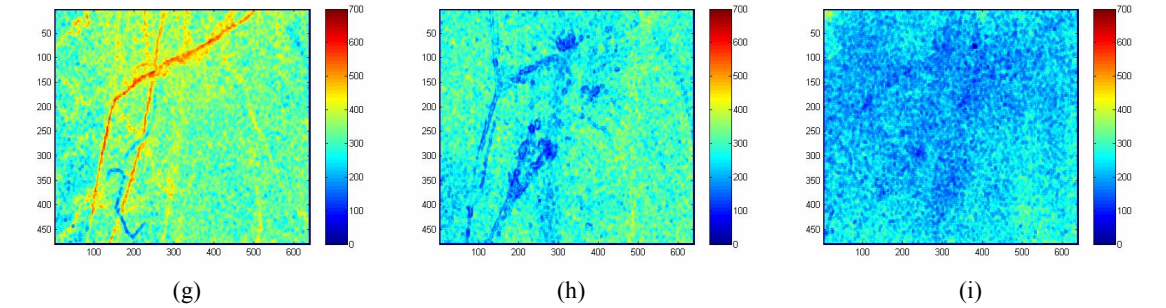
Figure 1. LSI images [5 X 4 mm^2 (640 X 480 pixels)] displaying vascular perfusion of the control group plotted over range of PI's from 0 to 700. Fig. 1a displays the perfusion image after the rodent dorsal skinfold surgical procedure, 1b is the perfusion image 16 minutes after 1a and, 1c is the perfusion image 18 hours after surgical intervention.



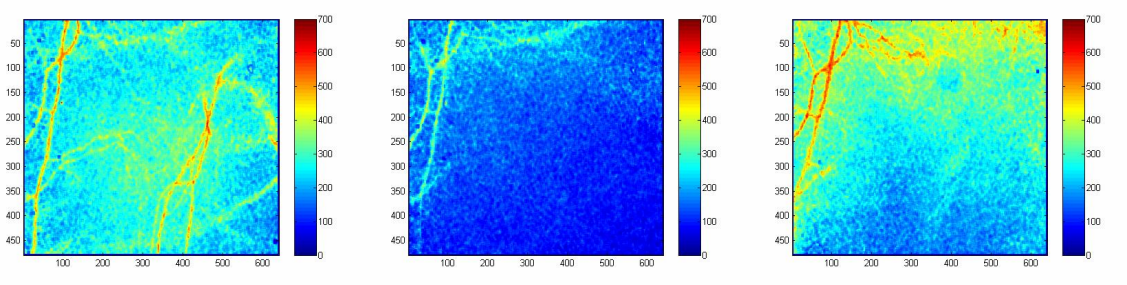
(a) (b) (c)



(d) (e) (f)

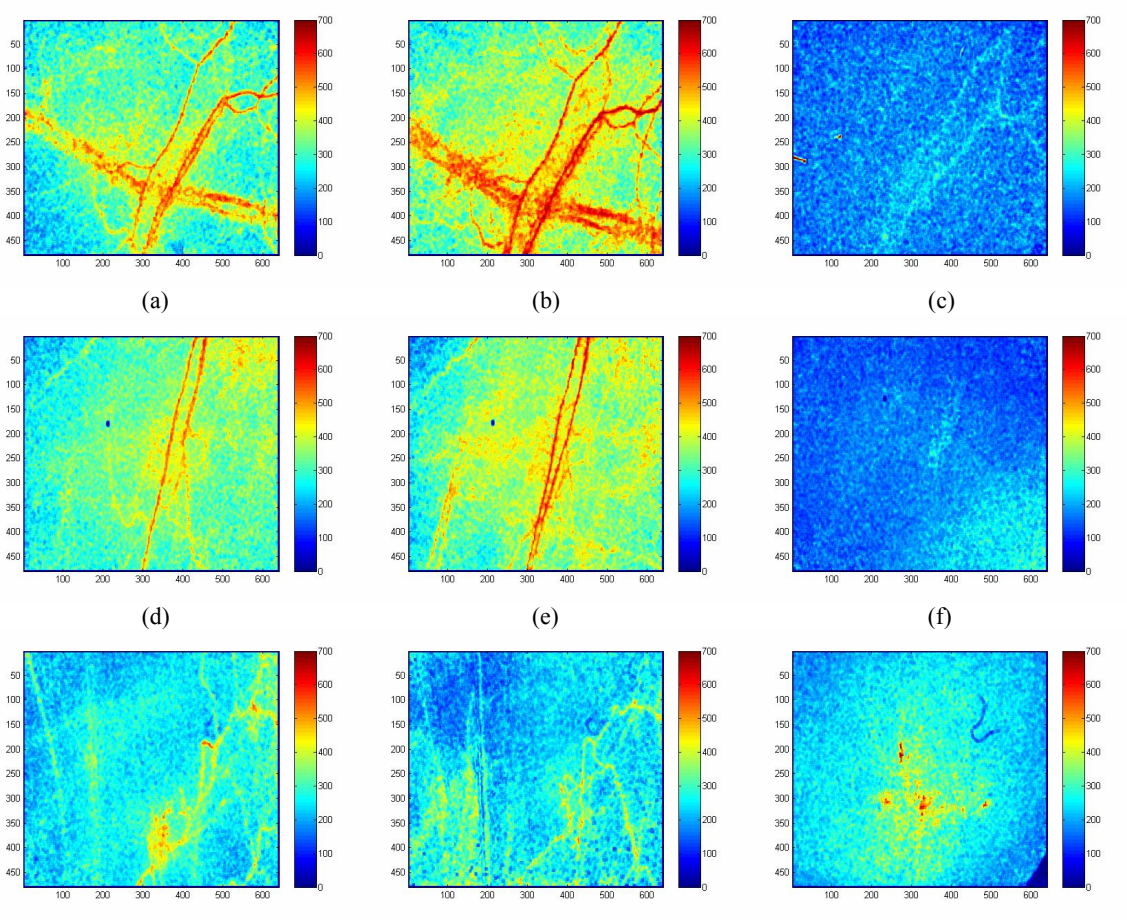


(g) (h) (i)



(j) (k) (l)

Figure 2. LSI images [5 X 4 mm^2 (640 X 480 pixels)] displaying vascular perfusion of the PDL group. Figs. 2a, 2b, 2c are the perfusion images of PDL animal A before, immediately after, and 18 hours after laser irradiation. Figs. 2d, 2e, 2f are the perfusion images of PDL animal B before, immediately after, and 18 hours after laser irradiation. Figs. 2g, 2h, 2i are the perfusion images of PDL animal C before, immediately after, and 18 hours after laser irradiation. Figs. 2j, 2k, 2l are the perfusion images of PDL animal D before, immediately after, and 18 hours after laser irradiation.



(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 3. LSI images [5 X 4 mm^2 (640 X 480 pixels)] displaying vascular perfusion of the PDT group. Figs. 3a, 3b, 3c are the perfusion images of PDT animal A before, immediately after, and 18 hours after laser irradiation. Figs. 3d, 3e, 3f are the perfusion images of PDT animal B before, immediately after, and 18 hours after laser irradiation. Figs. 3g, 3h, 3i are the perfusion images of PDT animal C before, immediately after, and 18 hours after laser irradiation.

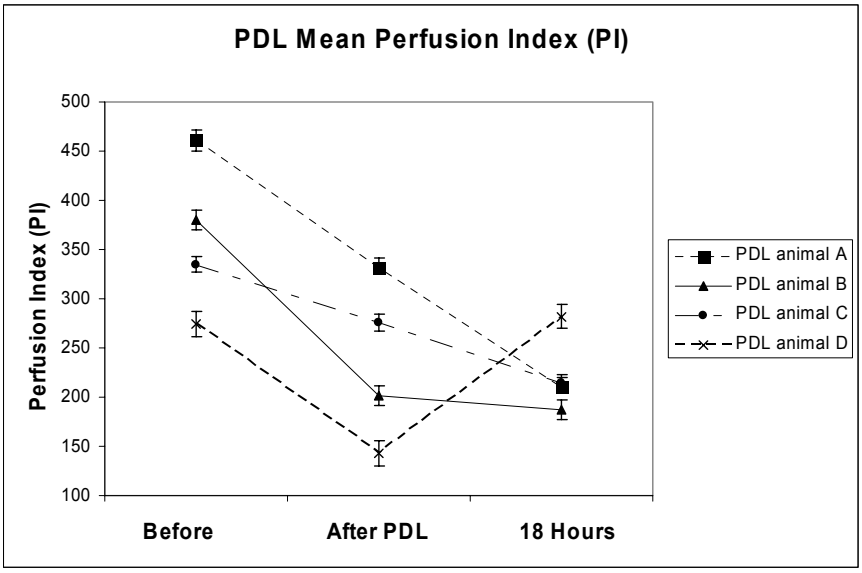


Figure 4. Perfusion indices for 4 PDL animals before, immediately after and 18 hours post PDL irradiation.

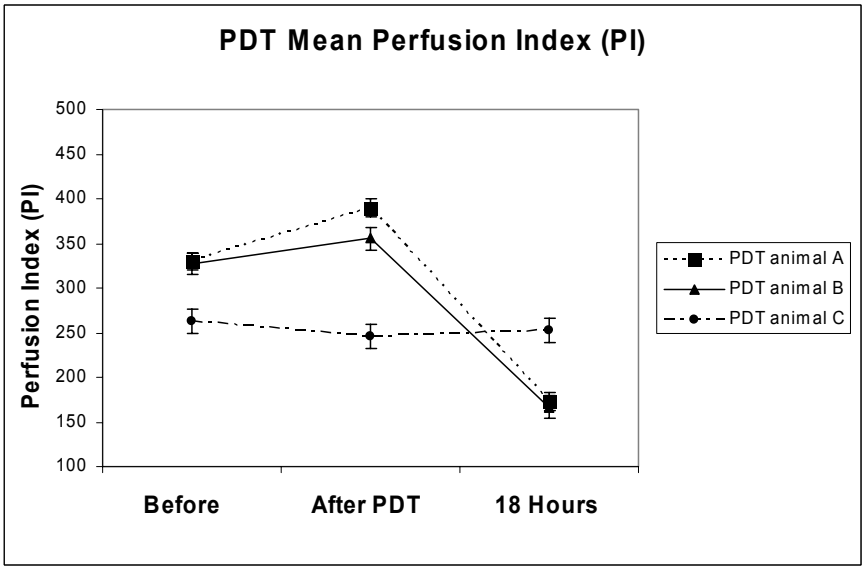


Figure 5. Perfusion indices for 3 PDT group animals before, immediately after and 18 hours post PDT.

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