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Parameter determination for BPD mediated vascularPDT

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Abstract

The cell killing mechanism of benzoporphyrin derivative monoacid ring A (BPD) is known to be predominantly apoptotic or vascular, depending on the drug-light interval (DLI). With a 3 hour DLI, necrosis develops secondary to tumor cell damage, while with a 15 minute DLI, necrosis results from treatment-created vascular damage. The purpose of this study is to examine if the different mechanisms of cell death will affect the photochemical parameters for the macroscopic singlet oxygen model. Using the RIF model of murine fibrosarcoma, we determined the four photochemical parameters (ξ , σ , β , γ) and the threshold singlet oxygen dose for BPD-mediated PDT through evaluation of the extent of tumor necrosis as a function of PDT fluence rate and total fluence. Mice were treated with a linear source at fluence rates from 12–150 mW/cm and total fluences from 24–135 J/cm. BPD was administered at 1mg/kg with a 15 minute DLI, followed by light delivery at 690nm. Tumors were excised at 24 hours after PDT and necrosis was analyzed via H&E staining. The in-vivo BPD drug concentration is determined to be in the range of 0.05–0.30 μ M. The determination of these parameters specific for BPD and the 15 minute DLI provides necessary data for predicting treatment outcome in clinical BPD-mediated PDT. Photochemical parameters will be compared between 1mg/kg DLI 3 hours and 1mg/kg DLI 15 minutes.

INTRODUCTION

Photodynamic therapy (PDT) is a cancer treatment modality for cancer and other localized diseases. PDT incorporates light, photosensitizer, and oxygen to create activated singlet oxygen ($^1\text{O}_2$) to kill cells. PDT is uniquely advantageous compared to other treatment modalities as it can be locally delivered, it is non-ionizing, it has fast post-operative recovery, and it has better cosmetic outcome. However, assessing PDT efficacy is difficult due to the lack of accurate dosimetric methods. We have been developing an explicit singlet oxygen dosimetry model to determine the PDT outcome. [1, 2, 3, 4] The four major photochemical parameters in a macroscopic singlet oxygen model have been investigated and determined for the photosensitizer benzoporphyrin derivative monoacid A (BPD). The photochemical parameters are believed to be photosensitizer-dependent, and furthermore, they are drug-light interval (DLI) dependent. We have compared the fitted parameters for two different DLIs for BPD in this study.

In this study, we looked at vascular-mediated PDT. Typical treatment with BPD involves a 3 hour DLI, where by this time, the drug has systemically extravasated into the tumor interstitial and cellular components. Vascular-targeted PDT can be achieved using a short (15 minute) drug light interval [5]. By inducing vascular shutdown, nutrient supply and removal of metabolic waste is halted. This is beneficial as targeting tumor vasculature is easier to access, more efficient in cancer cell killing, and has a lower likelihood of developing drug resistance.

MATERIALS AND METHODS

Tumor Model

Radioactively induced fibrosarcoma (RIF) cells were cultured and injected at 1×10^7 cells/ml in the right shoulders of 6–8 week old female C3H mice (NCI-Frederick, Frederick, MD). Animals were under the care of the University of Pennsylvania Laboratory Animal Resources. All studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. The fur of the tumor region was clipped prior to cell inoculation, and the treatment area was depilated with Nair at least 24 hours before measurements.

Measurements

Measurements were done before PDT treatment and afterwards. Two catheters were inserted into the tumor to measure optical properties and fluorescence. BPD was used as the photosensitizer. A number of experiments were performed using different conditions, such as different light source strengths and treatment times. For each experiment, optical properties, initial photosensitizer concentrations, and necrotic depths were determined from measurements. These experimental values were then used as inputs for the fitting algorithm to determine the photophysiological parameters.

The light profiles were recorded with a 2 mm point source and isotropic detector. The light source was the same as the treatment wavelength (690nm for BPD). From these profiles, the absorption and scattering coefficients were determined using a fitting algorithm previously described [6]

Fluorescence spectra were measured using a side-firing fiber connected to a 405 nm light source. The spectra were analyzed with a singular value decomposition (SVD) fitting method to separate out autofluorescence and photosensitizer [7]. The in vivo concentration of photosensitizer as obtained by comparing the in vivo spectrum with those measured in phantoms of known photosensitizer concentrations.

To determine the necrotic radius around the linear source, treated tumors were excised from euthanized animals 24 hours after PDT and fixed in formalin. The tumors were sectioned and stained with hematoxylin and eosin (H&E). Sectioning was performed at the Pathology Core Labs of the Children's Hospital of Philadelphia (CHOP). Sections were made in a direction that is perpendicular to the treatment catheter insertion axis. Slides were read and the area of necrosis was quantified using a ScanScope (Model CS, Aperio Technologies, Inc., Vista, CA, USA). From the area, necrotic radius was determined using the equation A

$= \pi r^2$. Average radius of necrosis was determined for each mouse from the multiple sections, and standard deviation was determined from mice treated under the same PDT conditions.

Macroscopic Singlet Oxygen Model

The macroscopic singlet oxygen model used was previously described [2]. The theory is derived from a type II PDT mechanism. The photochemical reactions can be simplified to four coupled differential equations

$$\mu_a \phi - \nabla \cdot \left(\frac{1}{3\mu_s'} \nabla \phi \right) = S \quad (1)$$

$$\frac{d[S_0]}{dt} + \left(\xi \sigma \frac{\phi([S_0] + \delta)[^3O_2]}{[^3O_2]} [S_0] \right) = 0 \quad (2)$$

$$\frac{d[^3O_2]}{dt} + \left(\xi \frac{\phi[S_0]}{[^3O_2] + \beta} \right) [^3O_2] - g \left(1 - \frac{[^3O_2]}{[^3O_2]_{(t=0)}} \right) = 0 \quad (3)$$

$$\frac{d[^1O_2]_{rx}}{dt} - \left(\xi \frac{\phi[S_0][^3O_2]}{[^3O_2] + \beta} \right) = 0 \quad (4)$$

Here, ϕ is the light fluence rate, S is the source term, and μ_a and μ_s' are the absorption and scattering coefficients, respectively. ξ represents the photochemical oxygen consumption rate per light fluence rate and the photosensitizer concentration under the condition of infinite 3O_2 supply prior to photobleaching. σ is the probability ratio of a 1O_2 molecule reacting with a cellular target. β represents the ratio of the monomolecular decay rate of the triplet state photosensitizer to the bimolecular rate of the triplet photosensitizer quenching by 3O_2 . The parameters are fully described in Table 1. Error margins for the fitted parameters were determined by propagating the systemic and random error from the experiment through the fitting process. Maximum and minimum possible values using the data set were found for each parameter.

For a given value of light fluence rate, ϕ , spatially resolved light fluence rate profiles can be constructed using equation (1), which will then be used in the calculation of the PDT kinetics equations (Eqs. (2)–(4)). For this study, a 1 cm linear source was used as the treatment source. From the simulation results, we can see from Figure 3 that the light fluence rate distribution within a 4 mm radial distance with respect to the center of the linear source does not show significant difference for the case of experimentally varying optical properties.

RESULTS

The experimental measurements from this study are shown below in Table 2. Only the mice with viable experimental data were used for the fitting algorithm. Each treatment condition had at least 3 mice treated, and properties were analyzed for deviation and error.

The fitted parameters for vascular mediated BPD PDT are shown in Table 3 compared to the parameters for BPD PDT with a 3 hour DLI [10]. These fitted values are within range to the previously published data [1, 2, 3]. The low concentration correction (δ) was fixed to be 33 μM , in accordance to data published by Dysard et al. [8]. β was also fixed to be 11.9 μM [9]. Figure 2 shows calculated reacted singlet oxygen profiles for each experimental condition. Model-predicted necrotic radii are compared to the experimentally-measured necrotic radii. All of the data points were within the expected error margins. Table 3 summarizes the fitted parameter results from this set of experiments.

Variations of absorption and scattering coefficients were investigated by plotting the linear source fluence profiles. Figure 3 shows that for distances away from the source that are of interest to us, the variation between mice in optical properties does not vary the linear source profile beyond the standard deviation (in shaded grey).

To verify that the photosensitizer concentration is accurately determined, a comparison of initial photosensitizer concentration measured with the side-firing fiber to *ex vivo* studies was done. After 15 minutes from BPD injection, optical property and fluorescence measurements were made. Immediately afterwards, tumors were excised. These tumors were then analyzed with a spectrofluorometer to see the interstitial photosensitizer concentration. The results are summarized in Table 4 below.

Interstitial Measurement (μM)	<i>ex vivo</i> Results (μM)
0.075	0.11
0.14	0.21
0.088	0.086

CONCLUSION

The photochemical parameters for the macroscopic singlet oxygen model were determined for vascular-mediated BPD PDT using a 15 minute drug-light interval. The preliminary results show that $g = 1.66 \pm 0.9 \mu\text{M/s}$, $\xi = (33.8 \pm 10) \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$, $\sigma = (1.83 \pm 0.9) \times 10^{-5} \mu\text{M}^{-1}$, and threshold $[^1\text{O}_2] = 0.075 \pm 0.05 \text{ mM}$. The threshold singlet oxygen dose was found to be smaller for the 15 minute DLI compared to that of the 3 hour DLI, agreeing with the result that the PDT effect is greater with similar treatment conditions and less interstitial photosensitizer. Vascular damage is known to be highly effective, thus the threshold dose of singlet oxygen is less for vascular-mediated PDT.

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Figure 1.

Experimental set-up. (a) Interstitial treatment was delivered using a 1cm linear source. A second catheter was used to insert an isotropic detector to collect light fluence information and fluorescence spectra. (b) A standard set-up of interstitial PDT treatment in a mouse tumor

Figure 2.

(a) Fitting results of necrotic radius using computed $[^1\text{O}_2]_{\text{rx}}$ profiles. The horizontal error bars represent the systemic error of experimentally-measured necrotic radii for animals in addition to the random error propagated throughout the experiment. (b) Model-predicted necrotic radii plotted against the experimentally-measured necrotic radii

Figure 3.
Linear source profile as a function of distance for varying absorption and scattering coefficients

Table 1Relationship between ξ , σ , β and the photochemical parameters

Symbol	Definition
k_0	Photon absorption rate of photosensitizer per photosensitizer concentration
k_1	Bimolecular rate for $^1\text{O}_2$ reaction with ground-state photosensitizer
k_2	Bimolecular rate of triplet photosensitizer quenching by $^3\text{O}_2$
k_3	Decay rate of first excited singlet state photosensitizer to ground state photosensitizer
k_4	Rate of monomolecular decay of the photosensitizer triplet state
k_5	Decay rate of first excited state photosensitizer to triplet state photosensitizer
k_6	$^1\text{O}_2$ to $^3\text{O}_2$ decay rate
k_7	Bimolecular rate of reaction of $^1\text{O}_2$ with biological substrate [A]
S	Fraction of triplet-state photosensitizer; $^3\text{O}_2$ reactions to produce $^1\text{O}_2$
δ (μM)	Low concentration correction
ξ ($\text{cm}^2\text{mW}^{-1}\text{s}^{-1}$)	$S\Delta \frac{k_5}{k_3+k_4} \frac{\varepsilon}{h\nu} \left(\frac{k_6}{k_7[A]} + 1 \right)$
σ (μM^{-1})	$k_1/k_7[A]$
(μM)	k_4/k_2

Table 2

Experimental variables and measurements from BPD PDT study

μ_a (cm^{-1})	μ_s' (cm^{-1})	Initial [BPD] (μM)	Source Strength (mW/cm)	Treatment Time (s)	Necrosis (mm)
0.64	12.31	0.10 ± 0.06	12	2000	4.46 ± 2.04
0.72	11.27	0.17 ± 0.07	12	3000	4.87 ± 0.97
0.96	7.30	0.28 ± 0.05	12	6000	4.91 ± 0.80
0.75	6.83	0.12 ± 0.12	20	2000	3.79 ± 1.05
0.94	9.13	0.14 ± 0.06	30	1300	4.14 ± 1.24
0.80	9.95	0.17 ± 0.05	75	1800	5.08 ± 1.42
0.83	9.53	0.18 ± 0.05	150	180	4.65 ± 1.60

Table 3

Preliminary photophysiological parameters from fitting results

	15 min DLI	3 hr DLI
g ($\mu\text{M/s}$)	1.6581 ± 0.9	1.32 ± 0.7
ξ ($\text{cm}^2 \text{mW}^{-1} \text{s}^{-1}$)	$(33.8 \pm 10) \times 10^{-3}$	$(25.3 \pm 11) \times 10^{-3}$
σ (μM^{-1})	$(1.83 \pm 0.9) \times 10^{-5}$	$(1.839 \pm 0.8) \times 10^{-5}$
Threshold [$^1\text{O}_2$] (mM)	0.075 ± 0.05	0.44 ± 0.13
β (μM)	11.9	11.9
δ (μM)	33	33