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Determination of the low concentration correction in the macroscopic singlet oxygen model for PDT

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Abstract

The macroscopic singlet oxygen model has been used for singlet oxygen explicit dosimetry in photodynamic therapy (PDT). The photophysical parameters for commonly used sensitizers, HPPH and BPD, have been investigated in pre-clinical studies using mouse models. So far, studies have involved optimizing fitting algorithms to obtain some of the photophysical parameters (ξ , σ , g) and the threshold singlet oxygen dose ($[^1\text{O}_2]_{\text{rx,sh}}$), while other parameters such as the low concentration correction, δ , has been kept as a constant. In this study, using photobleaching measurements of mice *in vivo*, the value of δ was also optimized and fit to better describe experimental data. Furthermore, the value of the specific photobleaching ratio (σ) was also fine-tuned using the photobleaching results. Based on literature values of δ , σ for photosensitizers can be uniquely determined using the additional photobleaching measurements. This routine will further improve the macroscopic model of singlet oxygen production for use in explicit dosimetry.

Keywords

photodynamic therapy; BPD; HPPH; macroscopic model; explicit dosimetry

1. INTRODUCTION

The macroscopic explicit singlet oxygen model has been in development for use as a dosimetry tool in photodynamic therapy (PDT) as it is being used for the treatment of cancer and other localized diseases [1–3]. The singlet oxygen explicit dosimetry (SOED) model incorporates the dynamic processes and interactions of light, photosensitizer, and ground state oxygen ($^3\text{O}_2$) to calculate the singlet oxygen ($^1\text{O}_2$) that is produced in a type II PDT process [3–13]. The photochemical parameters that are involved in the SOED model have been studied extensively [2–5, 7–9, 14]. In this study, the low concentration factor (δ) was investigated in more depth.

The term for photobleaching kinetics of the ground state photosensitizer ($[S_0]$) undergoing $^1\text{O}_2$ -mediated photobleaching has a low concentration correction constant [15, 16]. In the PDT process, $^1\text{O}_2$ is generated at the site of the parent photosensitizer molecule, and due to its short diffusion distance in biological media (~ 10 – 100 nm), there is a higher probability of reacting with $[S_0]$ than with adjacent molecules. The rate of photobleaching depends only on the rate of singlet oxygen generation because the volume through which each $^1\text{O}_2$ can diffuse before reacting will contain exactly one photosensitizer molecule,

independent of the total photosensitizer concentration. The factor δ is the concentration of $[S_0]$ where the intermolecular distance is equal to the 1O_2 distance. This value has been estimated to be between 3 and 3000 μM [17]. While there have been studies to investigate the value of δ *in vitro* using cells, *in vivo* values have not been determined as extensively [18]. For further improvement on the SOED model for PDT, *in vivo* mouse studies were performed to investigate the value of δ .

2. MATERIALS AND METHODS

2.1 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.

2.2 Measurements

Optical property and fluorescence measurements were performed on mice before and after treatment with PDT. Two photosensitizers (BPD and HPPH) were used. A number of experiments were performed using different treatment conditions, such as different light source strengths and treatment times. Each measured experimental value was used as input for the SOED model to determine the best fit for the photophysical parameters of ξ (the specific oxygen consumption rate), σ (the specific photobleaching ratio), g (the macroscopic maximum oxygen supply rate), and $[^1O_2]_{rx,sh}$ (the threshold singlet oxygen concentration). For the initial fit, the parameters of β (the oxygen quenching threshold concentration) and δ (the low concentration correction) were held as constants. In this study, δ was varied to determine the validity of the values used previously.

The absorption and scattering coefficients were determined using a fitting algorithm previously described [19]. For each sensitizer, optical properties were measured at the treatment wavelength of 690 nm and 665 nm for BPD and HPPH, respectively.

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 [20]. The *in vivo* concentration of photosensitizer as obtained by comparing the *in vivo* spectrum with those measured in phantoms of known photosensitizer concentrations.

Treatment was delivered via a 1 cm cylindrically diffusing fiber located centrally in the tumor. Light delivery was done at the appropriate drug-light interval (DLI) for each sensitizer (3 hours for BPD and 24 hours for HPPH).

2.3 SOED Model

The explicit singlet oxygen model was used to calculate the amount of sensitizer that is inside the tumor over the course of PDT. The PDT process can be modeled using a set of

coupled differential equations, details of which can be found elsewhere. [2, 13, 14, 21]. The equations for the photobleaching of sensitizer is given by

$$\frac{d[S_0]}{dt} = -\sigma \xi \frac{[{}^3O_2]}{[{}^3O_2] + \beta} ([S_0] + \delta) \phi [S_0], \quad (1)$$

where ϕ is the light fluence rate, $[S_0]$ is the concentration of the ground state sensitizer, and $[{}^3O_2]$ is the concentration of ground state oxygen. Measured sensitizer concentration before and after PDT for each sensitizer was compared to the model-calculated values to determine a best-fit value of δ and σ .

The initial values of the photochemical parameters have been determined by performing tumor necrosis studies, described previously [2, 5, 7, 8, 11].

3. RESULTS

Using the singlet oxygen explicit model, sensitizer concentration calculated over the treatment time was calculated using the appropriate photochemical parameters. Values of δ and σ were varied to obtain the best fit to measured sensitizer concentration before and after PDT.

In figure 1, the value of δ was varied from 10 μM to 150 μM . The initial drug concentration (at $t = 0$) was matched between calculation and measurements. Fluorescence spectra were taken at 5 different locations inside the tumor along the catheter. There were three mice for each treatment condition group.

The value of σ was also varied, as seen in figure 2 to determine a best-fit value.

The final set of parameters is shown in table 1 and figure 3. By incorporating post-PDT sensitizer concentrations, the photochemical parameters can be optimized further. Future experiments involve measurement of the sensitizer concentration continuously throughout treatment.

Similar analysis was performed for mice treated with HPPH. Figure 4 shows optimized data as well as data calculated for a value of $\delta = 10 \mu\text{M}$.

The appropriate value of δ was found to be $\sim 33 \mu\text{M}$ for both BPD- and HPPH-mediated PDT *in vivo*. Further studies are planned to investigate the sensitizer concentration during treatment both *in vivo* and *in vitro*. This will help to further narrow the range of the photochemical parameters obtained. Compared to previous work, this adds another restraint to the optimization routine. Previously, only calculated reacted singlet oxygen was used to obtain the apparent threshold dose at the measured depth of necrosis.

4. CONCLUSION

Mice were treated with BPD- and HPPH-mediated PDT to investigate the photochemical parameters involved in the macroscopic explicit singlet oxygen model. Initial fitting of data involved certain fixed parameters, such as the low concentration factor, δ . The value of δ was

verified by looking at a range of values for δ and comparing calculated sensitizer concentration over the delivered fluence to the measured amounts of sensitizer pre- and post-PDT. Furthermore, the specific photobleaching ratio, σ , was varied to validate best-fit values. Compared to previous studies where the apparent singlet oxygen threshold ratio was used along with necrosis data, this method of optimizing measured and calculated sensitizer adds another restraint to the optimization routine. Future works include continuous measurement of sensitizer concentration during treatment to obtain more data points over time.

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Figure 1. Sensitizer concentration over total fluence (calculated from fluence rate and time) with varied δ . The lines are calculated values from the parameters described in the title of each plot, and the symbols represent measured BPD concentration post-PDT. The value of δ was set to (a) 10 μM and (b) 150 μM .

Figure 2.

Sensitizer concentration over total fluence (calculated from fluence rate and time) with varied σ . The lines are calculated values from the parameters described in the title of each plot, and the symbols represent measured BPD concentration post-PDT. The value of σ was set to (a) $8 \times 10^{-6} \mu\text{M}^{-1}$ and (b) $3.4 \times 10^{-5} \mu\text{M}^{-1}$.

Figure 3.
Optimized parameter set used to plot sensitizer concentration over delivered fluence for BPD-mediated PDT. Lines lines indicate

Figure 4.

Sensitizer concentration plotted over delivered fluence for mice treated with HPPH-mediated PDT. (a) Calculation with optimized parameters and $\delta = 33 \mu\text{M}$ and (b) calculated values with $\delta = 10 \mu\text{M}$ that do not produce a best fit.

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Table 1

Photochemical parameters for BPD and HPPH

Sensitizer	β (μM)	δ (μM)	σ (μM^{-1})	g ($\mu\text{M/s}$)	$^1\text{O}_2[\tau_{\text{KSH}}]$ (μM)
BPD (3 hr DLI)	11.9	33	1.7×10^{-5}	1.67	0.7
HPPH (24 hr DLI)	11.9	33	1.1×10^{-5}	1.54	0.6