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Singlet oxygen dosimetry modeling for photodynamic therapy

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Abstract

Photodynamic therapy (PDT) is an important treatment modality for cancer and other localized diseases. In addition to PDT dose, singlet oxygen ($^1\text{O}_2$) concentration is used as an explicit PDT dosimetry quantity, because $^1\text{O}_2$ is the major cytotoxic agent in photodynamic therapy, and the reaction between $^1\text{O}_2$ and tumor tissues/cells determines the treatment efficacy. $^1\text{O}_2$ concentration can be obtained by the PDT model, which includes diffusion equation for the light transport in tissue and macroscopic kinetic equations for the generation of the singlet oxygen. This model was implemented using finite-element method (FEM) by COMSOL. In the kinetic equations, 5 photo-physiological parameters were determined explicitly to predict the generation of $^1\text{O}_2$. The singlet oxygen concentration profile was calculated iteratively by comparing the model with the measurements based on mice experiments, to obtain the apparent reacted $^1\text{O}_2$ concentration as an explicit PDT dosimetry quantity. Two photo sensitizers including Photofrin and BPD Verteporfin, were tested using this model to determine their photo-physiological parameters and the reacted $^1\text{O}_2$ concentrations.

Keywords

Singlet oxygen concentration; Photodynamic therapy; Photosensitizer

1. Introduction

Photodynamic therapy (PDT) is an important treatment modality for cancer and other localized diseases [1]. During the treatment, photosensitizers excited by light react with ground state oxygen $^3\text{O}_2$, which leads to generation of the major cyto-toxic agent - singlet oxygen $^1\text{O}_2$ - to kill the surrounding tissues and cells. Compared with other treatments, PDT has its unique advantages including local delivery, being non-ionizing, faster post-operative recovery, and better cosmetic outcome. However, one major deficiency of current PDT technology is the lack of accurate dosimetry to assess PDT efficacy. We have been focusing on developing an explicit PDT dosimetry model using apparent reacted $^1\text{O}_2$ concentration, $[^1\text{O}_2]_{\text{rx}}$, as the clinical PDT dosimetry quantity [2-4].

In this paper, an optimization algorithm was implemented based on the previous model, in which PDT spatial-dependent light distribution was modeled in COMSOL, and time-

dependent $[^1\text{O}_2]_{\text{rx}}$ was modeled in Matlab dynamically linked with COMSOL. Once the spatial light distribution was determined, the optimization algorithm compared the model with experimental necrosis radius from mice studies, and determined the optimal photophysiological parameters. PDT dosimetry prediction using $[^1\text{O}_2]_{\text{rx}}$ was then obtained using these parameters. Two photosensitizers were studied in this paper, namely Photofrin and BPD. Three groups of mice experiments were performed using Photofrin to obtain the photophysiological parameters for this photosensitizer, while one group of mice experiments was performed on BPD. Five photophysiological parameters were determined to characterize the two different photosensitizers, and thus were used to predict the necrotic distance for each experiment from BPD treatment.

2. Materials and Methods

2.1 Animal model

Experiments were carried out using C3H mice with RIF tumor as animal model. The experimental procedure was previously introduced [2]. Briefly, subcutaneous tumor implantation were performed when the rodents were 6-8 weeks old and PDT was performed roughly 10 days later, when tumor diameters were ~ 8 mm. Animals were euthanized at 24 hours after PDT and tumors were excised for determination of the radius of necrosis, by quantifying the H&E-stained sections of the treated tumor. A control animal was used for each group of study, to quantify the simultaneous necrosis.

2.2 Experimental quantities

Photofrin and BPD were used as photosensitizers in the experiments, and photophysiological parameters were extracted for these two drugs from in vivo mice studies. For each photosensitizer, a number of experiments were performed using different conditions including different light source strengths and treatment periods. For each experiment, optical properties, initial photosensitizer concentrations, and necrotic depths were also determined from measurements. These variables were then used as inputs for the fitting algorithm to determine the photophysiological parameters.

Two catheters were inserted into the tumor parallel to each other during experiments. One of them went through the center of the tumor, while the other one went through the peripheral part. During the PDT experiment, the in vivo optical properties of the sample were determined first interstitially under treatment wavelength (For Photofrin, the treatment wavelength is 632 nm, while for BPD, the treatment wavelength is 690 nm). A 2 mm light point source was inserted in the center catheter inside the tumor, and an isotropic detector was placed in the peripheral catheter to measure the light fluence rate profile of the tumor when the light source was on. This measurement was done before the PDT treatment, and the optical properties were determined from a fitting algorithm from the recorded light profiles [5].

Photosensitizer concentrations were determined by in vivo fluorescence measurements, which were implemented by a side-emitting fiber going through the center catheter using a 405 nm diode laser as excitation. The in vivo fluorescence was separated from the measured spectrum using a singular value decomposition method [6], and the photosensitizer

concentration was obtained by comparing the in vivo fluorescence with that measured in phantoms of known photosensitizer concentrations.

PDT treatments were performed interstitially after the measurements, by linear sources with designated source strengths and treatment periods. The sources strengths and treatment times are listed in table 2 and 3 for Photofrin and BPD studies, respectively. The necrotic depths were determined from excised H&E slides, which was described in detail elsewhere [2].

2.3 Macroscopic singlet oxygen model

The macroscopic singlet oxygen model has been introduced in our previous publications [2]. The basic theory was derived from Type II PDT processed with $^1\text{O}_2$ mediated bleaching mechanism. A set of differential equations can be simplified from the photochemical reaction equations as:

$$\mu_a \varphi - \nabla \cdot \left[\frac{1}{3\mu'_s} \nabla \varphi \right] = S, \quad 1$$

$$\frac{d[S_0]}{dt} + \left(\xi \sigma \frac{\phi([S_0] + \delta)[^3\text{O}_2]}{[^3\text{O}_2] + \beta} \right) [S_0] = 0, \quad 2$$

$$\frac{d[^3\text{O}_2]}{dt} + \left(\xi \frac{\phi[S_0]}{[^3\text{O}_2] + \beta} \right) [^3\text{O}_2] - g \left(1 - \frac{[^3\text{O}_2]}{[^3\text{O}_2](t=0)} \right) = 0, \quad 3$$

$$\frac{d[^1\text{O}_2]_{rx}}{dt} - \left(\xi \frac{\phi[S_0][^3\text{O}_2]}{[^3\text{O}_2] + \beta} \right) = 0, \quad 4$$

where ϕ is the light fluence rate, S is the source term, and μ_a and μ'_s are the absorption and reduced scattering coefficient of the sample, respectively, ξ represents the photochemical oxygen consumption rate per light fluence rate and photosensitizer concentration under the condition of infinite $^3\text{O}_2$ supply and prior to photobleaching. σ is the probability ratio of a $^1\text{O}_2$ molecule reacting with ground-state photosensitizer compared to the $^1\text{O}_2$ 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 $^3\text{O}_2$. The relationships between these parameters to the more fundamental photochemical rates are shown in Table 1.

The sensitivity of parameters ξ , σ , β to the $[S_0]$, $[^3\text{O}_2]$, and $[^1\text{O}_2]$ were studied [2, 7]. It is found that β is not sensitive to the model outcome and thus a fixed value (11.9 μM) was used for β in this study [8] for both Photofrin and BPD. g is the maximum oxygen perfusion rate where there is no oxygen gradient. Another quantity, the apparent singlet oxygen threshold concentration $[^1\text{O}_2]_{rx,sh}$, was introduced to characterize photosensitizer properties, for the fitting algorithm. In the fitting algorithm, spatial distribution of reacted singlet oxygen

concentration was calculated over time for each experiment to fit the measured necrotic radius and extract the model parameters (g , ξ , σ , and $[^1\text{O}_2]_{\text{rx,sh}}$). The objective function of the fitting algorithm is the maximum relative difference between measurements and calculation of the threshold singlet oxygen concentration.

3. Results and Discussions

3.1 Results for Photofrin

The experimental measurements for Photofrin PDT are shown in Table 2. In this set of studies, three groups of experiments, namely Group A, B, and C, were performed separately. The mice from different groups of studies were indexed from 1 to 12, showing the total number of mice being treated by PDT. However, only mice with PDT effect were shown in Table 2, and only the data from PDT effective mice were used for the fitting algorithm.

Fitting algorithm were performed on the data from experiments Group A, Groups AB, and Groups ABC, respectively. The fitting results are shown in Figure 1, and the extracted parameters are shown in Table 3.

Using the fitted parameters, one can predict the necrotic distance once knowing the experimental conditions, as shown in Table 2. The predicted necrotic depths are used to validate the accuracy of the fitting algorithm, and are shown in Figure 2 for Photofrin PDT experiments, in which the blue line represents the predicted necrotic depths being identical with measured necrotic depths, while the red squares represent the relationship between predicted necrotic depths based on extracted parameters for Photofrin and measured necrotic depths. Figure 2 shows a decent agreement between the parameter-predicted necrotic depths and the measured ones.

3.2 Preliminary results for BPD

The experimental measurements for BPD PDT are shown in Table 4. In our BPD PDT studies, only one group (3 mice) of PDT treatment has been performed. Therefore there are only three known data from experiments, under which condition the fitting algorithm wouldn't be reliable if four parameters are still all being fitted. Thus, σ was fixed with value of 1.5×10^{-5} , while the other parameters are being fitted. The fitting results are shown in Figure 3. Both reacted singlet oxygen concentration and predicted necrotic depths are shown.

The fitted photophysiological parameters for BPD PDT are shown in Table 5, compared with the parameters for Photofrin PDT. From the results, one can tell that ξ , the photochemical oxygen consumption rate per light fluence rate and photosensitizer concentration under the condition of infinite $^3\text{O}_2$ supply and prior to photobleaching, has a much higher value for BPD than Photofrin. However, g , the maximum oxygen perfusion rate remains similar between BPD and Photofrin. Because of limited animal experiments, the parameter σ was fixed at 1.5×10^{-5} . The fixed σ value is determined empirically, chosen to obtain a reasonable value for ξ . According to definition of ξ [2].

$$\xi = S_{\Delta} \frac{k_5}{k_3 + k_5} \varepsilon / h\gamma / \left(\frac{k_6}{k_7 [A]} + 1 \right), \quad 5$$

where $k_5/(k_3+k_5)$ is the triplet quantum yield, ε is the molar absorption coefficient. Assuming $k_6/k_7[A] \ll 1$ in Eq. 5 remain same for Photofrin and BPD, the difference for ξ is determined by the product of triplet quantum yield and molar absorption coefficient from each photosensitizer. For Photofrin, $k_5/(k_3+k_5) = 0.68$, and $\varepsilon = 33000 \text{ (M}^{-1} \text{ cm}^{-1})$ [9], while for BPD, $k_5/(k_3+k_5) = 0.8$, and $\varepsilon = 3500 \text{ (M}^{-1} \text{ cm}^{-1})$ [10]. Thus, one target for the fitting algorithm is to obtain $\xi \approx 3.8 \times 10^{-3}$ (ξ for Photofrin) $\times (33000 \times 0.68) / (3500 \times 0.8) \approx 30 \times 10^{-3}$ for BPD. From our fitting program and existing animal study data, when σ decreases from 8.1×10^{-5} to 1.5×10^{-5} , the ξ increases from 5.2×10^{-3} to 23.7×10^{-3} , and $[^1\text{O}_2]_{\text{rx,sh}}$ increases from 0.22 mM to 0.49 mM, a value similar to that for Photofrin. Further animal experiments will be performed to obtain more BPD PDT data and improve the accuracy of photophysiological parameters for BPD without fixing any parameters.

4. Conclusion

In this study, a fitting algorithm was introduced to extract photophysiological parameters for both Photofrin and BPD, based on type II PDT photochemical differential equations. In vivo animal studies have been performed to provide fitting variables including initial photosensitizer concentration, absorption coefficient, reduced scattering coefficient, source strength, treatment time, and necrotic distance. The fitting algorithm was performed using these variables, and the photophysiological parameters were extracted for Photofrin and BPD. Predicted necrotic depths were used to verify the accuracy of the fitting results. The proposed parameter, apparent singlet oxygen threshold concentration $[^1\text{O}_2]_{\text{rx,sh}}$, are proved to be consistent for different photosensitizers for type II PDT.

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Figure 1.

Fitting plots for Photofrin PDT showing reacted singlet oxygen concentrations as functions of necrotic distances. (a) Fitting plot from Group A mice data. (b) Fitting plot from Groups A and B mice data. (c) Fitting plot from Groups A, B, and C mice data.

Figure 2.

Relationship between parameter-predicted necrotic radius and measured necrotic radius. Red squares represent data from each experiment, and blue line represent the ideal condition.

Figure 3.

Fitting plots for BPD PDT showing (a) reacted singlet oxygen concentrations and (b) predicted necrotic depths as functions of necrotic depths.

Table 1
Relationship between ξ , σ , β and 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 $^1\text{O}_2$
$\delta(\mu\text{M})$	Low concentration correction
$\xi(\text{cm}^2\text{mW}^{-1}\text{s}^{-1})$	$S \frac{k_5}{k_3 + k_5} \varepsilon \left h\gamma \right \left(\frac{k_6}{k_7[A]} + 1 \right)$
$\sigma(\mu\text{M}^{-1})$	$k_1/k_7[A]$
$\beta(\mu\text{M})$	k_4/k_2

Table 2

Experimental variables and measurements for Photofrin PDT.

Group	Group A			Group B			Group C		
	M2	M3	M4	M7	M9		M10	M11	M12
Mouse index									
Initial PII concentration (μM)	1.62	3.72	1.92	9.07	9.85		1.43	3.77	2.53
μ_a (cm^{-1})	0.41	1.51	0.72	1.98	0.67		0.56	1.28	0.59
μ'_s (cm^{-1})	13.49	12.29	16.39	13.00	13.00		15.96	11.99	16.82
Source strength (mW/cm)	33.43	33.43	74.82	70.84	145.66		20.00	150.00	50.00
Treatment time (s)	1316	2632	1333	1333	667		3000	400	1000
Necrotic distance (mm)	2.62	2.61	2.45	3.75	4.74		2.58	2.65	2.06

Table 3

Photophysiological parameters from fitting results for Photofrin PDT.

Parameters	Group A	Groups AB	Groups ABC
ξ (cm ² /s/mW)	4.8×10^{-3}	3.9×10^{-3}	3.8×10^{-3}
σ (1/ μ M)	7.8×10^{-5}	11.5×10^{-5}	8.1×10^{-5}
β (μ M)	11.9	11.9	11.9
g (μ M/s)	0.58	0.56	0.68
$[^1\text{O}_2]_{\text{rx,sh}}$ (mM)	0.44 $\pm$ 0.01	0.41 $\pm$ 0.05	0.46 $\pm$ 0.10

Table 4

Experimental variables and measurements for BPD PDT.

Mouse index	M1	M2	M3
Initial BPD concentration (μM)	0.41	0.35	0.29
μ_a (cm^{-1})	0.66	0.55	0.53
μ'_s (cm^{-1})	10.44	10.78	14.28
Source strength (mW/cm)	75.00	30.00	30.00
Treatment time (s)	1800	1980	4500
Necrotic distance (mm)	2.14	1.99	1.40

Table 5

Photophysiological parameters from fitting results for BPD PDT.

Parameters	Photofrin	BPD
ξ (cm ² /s/mW)	3.8×10^{-3}	23.7×10^{-3}
σ (1/ μ M)	8.1×10^{-5}	1.5×10^{-5}
β (μ M)	11.9	11.9
g (μ M/s)	0.68	0.79
$[^1\text{O}_2]_{\text{rx,sh}}$ (mM)	0.46 $\pm$ 0.10	0.49 $\pm$ 0.09