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Comparison of PDT parameters for RIF and H460 tumor models during HPPH-mediated PDT

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Abstract

Singlet oxygen ($^1\text{O}_2$) is the major cytotoxic species producing PDT effects, but it is difficult to monitor *in vivo* due to its short life time in real biological environments. Mathematical models are then useful to calculate $^1\text{O}_2$ concentrations for PDT dosimetry. Our previously introduced macroscopic model has four PDT parameters: ξ , σ , β and g describing initial oxygen consumption rate, ratio of photobleaching to reaction between $^1\text{O}_2$ and cellular targets, ratio of triplet state (T) phosphorescence to reaction between T and oxygen ($^3\text{O}_2$), and oxygen supply rate to tissue, respectively. In addition, the model calculates a fifth parameter, threshold $^1\text{O}_2$ dose ($[^1\text{O}_2]_{\text{rx, sd}}$). These PDT parameters have been investigated for HPPH using radiation-induced fibrosarcoma (RIF) tumors in an *in-vivo* C3H mouse model. In recent studies, we additionally investigated these parameters in human non-small cell lung carcinoma (H460) tumor xenografts, also using HPPH-mediated PDT. *In-vivo* studies are performed with nude female mice with H460 tumors grown intradermally on their right shoulders. HPPH (0.25 mg/kg) is injected i.v. at 24 hours prior to light delivery. Initial *in vivo* HPPH concentration is quantified via interstitial HPPH fluorescence measurements after correction for tissue optical properties. Light is delivered by a linear source at various light doses (12–50 J/cm) with powers ranging from 12 to 150 mW per cm length. The necrosis radius is quantified using ScanScope after tumor sectioning and hematoxylin and eosin (H&E) staining. The macroscopic optimization model is used to fit the results and generate four PDT parameters. Initial results of the parameters for H460 tumors will be reported and compared with those for the RIF tumor.

Keywords

Photodynamic therapy; HPPH; H460 tumor; RIF tumor; dosimetry; singlet oxygen; macroscopic model

1. INTRODUCTION

Photodynamic therapy (PDT) is a treatment modality using light to activate a photosensitizer, which transfers energy to ground-state tissue oxygen and generates singlet-

state oxygen ($^1\text{O}_2$). Singlet oxygen is considered as the main cytotoxic species causing therapeutic effects via reacting with the surrounding biological molecules. These reactions usually take place so rapidly that direct *in-vivo* monitoring on $^1\text{O}_2$ becomes very difficult. The *in-vivo* life time of singlet oxygen is estimated to be in the range of 30 - 180 ns.¹ The surrogate way to derive information about temporal and spatial distribution of singlet oxygen is to use mathematical models.^{2,3,4,5,6}

Wang *et al*⁵ introduced a macroscopic model to determine a PDT dosimetry parameter - "reacted singlet oxygen threshold concentration" ($[\text{}^1\text{O}_2]_{\text{rx,rd}}$). In addition, the model has four other PDT parameters: ξ , σ , β and g that are defined as initial oxygen consumption rate, ratio of photobleaching to reaction between $^1\text{O}_2$ and cellular targets, ratio of triplet state (T) phosphorescence to reaction between T and oxygen ($^3\text{O}_2$), and oxygen perfusion supply rate to tissue, respectively. In Wang's previous work,⁵ all five parameters were examined for photosensitizer Photofrin using radiation-induced fibrosarcoma (RIF) tumors in an *in-vivo* C3H mouse model. Following this, some preliminary studies have been performed on a different photosensitizer, 2-(1-Hydroxyethyl)-2-Devinylpyropheophorbide-a (HPPH), using the same tumor model.⁷

Recently, further studies were conducted for HPPH-mediated PDT using both RIF and human non-small cell lung carcinoma (H460) tumor xenografts models. The first objective is to refine those PDT parameters for the RIF tumor model by investigating more treatment conditions. The second goal is to compare the parameters derived from the two *in-vivo* tumor models. This manuscript will show the comparison results.

2. THEORY AND METHOD

2.1 The macroscopic model

This section briefly describes our previously introduced macroscopic PDT model and the definition of the five parameters to be derived by fitting the *in vivo* experimental results. The more detailed description of the model and the fitting routine can be found elsewhere.⁵

The coupled time-dependent differential equations, as shown in equations (1) to (3), are solved to calculate temporal and spatial distributions of drug ($[\text{S}_0]$), $[\text{}^3\text{O}_2]$, and $[\text{}^1\text{O}_2]$ concentrations. The cumulative concentration of reacted singlet oxygen ($[\text{}^1\text{O}_2]_{\text{rx}}$) can be obtained via the integration of equation (3) over time.

$$\frac{d[\text{S}_0]}{dt} + \left(\xi \sigma \frac{\phi([\text{S}_0] + \delta) [\text{}^3\text{O}_2]}{[\text{}^3\text{O}_2] + \beta} \right) [\text{S}_0] = 0, \quad (1)$$

$$\frac{d[\text{}^3\text{O}_2]}{dt} + \xi \frac{\phi[\text{S}_0] [\text{}^3\text{O}_2] (1 + \sigma([\text{S}_0] + \delta))}{[\text{}^3\text{O}_2] + \beta} = g \left(1 - \frac{[\text{}^3\text{O}_2]}{[\text{}^3\text{O}_2]_{t=0}} \right), \quad (2)$$

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

$$\mu_a \phi - \nabla \cdot \left(\frac{1}{3\mu'_s} \nabla \phi \right) = S_L. \quad (4)$$

In these studies, light fluence rate distribution within tumor tissue is calculated for a linear source with source strength S_L using diffusion theory as shown in equation (4). The definitions of the parameters are given in table 1. The parameters (ξ , σ , g and threshold dose $[{}^1\text{O}_2]_{rx, sd}$) are obtained via fitting calculated $[{}^1\text{O}_2]_{rx}$ spatial distribution profiles to the measured necrotic radius. The optimization routine varies the PDT parameters to minimize the difference between the $[{}^1\text{O}_2]_{rx}$ at the necrotic radius for each mouse/group and the value of $[{}^1\text{O}_2]_{rx, sd}$. The parameter β was not varied for all studies because it has minimum influence on the $[{}^1\text{O}_2]_{rx}$ profiles.⁵ Hence, the value of 11.9 μM for Photofrin has been adapted in the model as no value for HPPH is reported in the literature.

2.2 *In vivo* mouse experiments for HPPH-PDT

Two cohorts of mouse experiments were performed for these studies. One was performed with C3H female mice (about 9 weeks old) with RIF tumors grown intradermally on their right shoulders. This cohort was designed to expand treatment conditions based on our previous work in order to refine the parameters. The other group was conducted using nude female mice (about 9 weeks old) with H460 tumors to investigate the dependency of the parameters on different tumor types.

For both groups, mice were given HPPH (0.25 mg/kg) via tail vein injection when the radius of tumor was about 4 mm, and treated with two-catheter interstitial PDT using a 665 nm linear light source (1 cm) after 24 hrs of incubation time. The treatment light source was placed in one of two parallel catheters inserted along the central axis of the tumor. Prior to PDT, a point source coupled to a 665 nm laser was placed in the central catheter, and an isotropic point detector was inserted in the other parallel catheter to measure light fluence rate profiles. The measured light fluence rates were then fitted using diffusion approximation to extract the spatial distribution of optical properties of tumor. The *in vivo* photosensitizer concentration was determined by measuring the *in vivo* HPPH fluorescence spectra excited by a 405 nm diode laser. The spectra were collected along the non-central catheter, and then analyzed using singular value decomposition (SVD) method. After the correction for the attenuation of the fluorescence photons due to absorption of tumor using measured optical properties of tumor, the spectra were compared with that measured in phantom of known HPPH concentration to extract the *in vivo* HPPH concentration.⁸

3. RESULTS AND DISCUSSIONS

Table 2 summarizes the treatment conditions for HPPH-mediated PDT on the RIF tumor model. Each group has 3 mice. The concentration of HPPH, μ_a , μ'_s and necrosis radii are the

average and standard deviation of the measured values for 3 mice in each group. The summary for the H460 tumor model is listed in the table 3.

Figures 1(a) and 1(c) show the calculated $[^1\text{O}_2]_{\text{rx}}$ profiles (solid color lines) fitted to the measured PDT-induced necrotic radius (symbols) for RIF and H460 tumor models, respectively. The input data for the calculations are presented in tables 2 and 3. The obtained singlet oxygen threshold dose, $[^1\text{O}_2]_{\text{rx, sd}}$, for RIF and H460 are 0.41 ± 0.25 and $0.37 \pm 0.2 \mu\text{M}$ respectively. Figures 1(b) and 1(d) show the comparison between measured and calculated necrosis radii for two tumor models, and they are mostly consistent with error bars.

Figure 2 shows the similar plots for all the individual mouse in all the groups. The range of the singlet oxygen concentration at all necrosis radii are $0.16\text{--}0.58 \mu\text{M}$ and $0.2\text{--}0.5 \mu\text{M}$ for RIF and H460 respectively.

The obtained other three PDT parameters (ξ , σ and g) are shown in table 4, and they are mostly consistent except for the g for H460 is little higher. Table 4 also summarizes average values for μ_a , μ'_s and [HPPH] of the RIF and H460 tumors in all C3H and nude mice respectively. The slightly lower threshold dose $[^1\text{O}_2]_{\text{rx, sd}}$ for H460 tumor may be due to the lower HPPH concentration in H460. H460 tumor in nude mice on average has lower optical absorption and higher scattering than RIF tumor in the gray C3H mice.

4. CONCLUSION

These studies use our macroscopic model to fit *in vivo* HPPH-mediate PDT experimental results for the RIF and H460 tumor models so that photochemical PDT parameters can be obtained. These parameters between two tumor models are similar. Singlet oxygen threshold dose are 0.41 and 0.37 mM for RIF and H460 respectively.

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Figure 1.
Fitting results for group.

Figure 2.
Fitting results individual mouse.

Table 1

Definitions of the PDT parameters.

Symbol	Definition	Units
ξ	$S_{\Delta} \left(\frac{k_5}{k_5 + k_3} \right) \frac{\sigma_c}{h\nu} \frac{k_7[A]/k_6}{k_7[A]/k_6 + 1}$	(cm ² mW ⁻¹ s ⁻¹)
σ	k_1/k_7 [A]	μM^{-1}
δ	Low drug concentration correction	μM
β	k_4/k_2	μM
[A]	Molecular substrate	M
k_1	Bimolecular rate constant for reaction of ¹ O ₂ with S ₀	M ⁻¹ s ⁻¹
k_2	Bimolecular rate constant for reaction of ³ O ₂ with T ₁	M ⁻¹ s ⁻¹
k_3	S ₁ → S ₀	s ⁻¹
k_4	T ₁ → S ₀	s ⁻¹
k_5	S ₁ → T ₁	s ⁻¹
k_6	¹ O ₂ → ³ O ₂	s ⁻¹
k_7	Bimolecular rate constant for reaction of ¹ O ₂ with A	M ⁻¹ s ⁻¹
S	Fraction of T ₁ and ³ O ₂ reactions that produce ¹ O ₂	
σ_c	Absorption cross section of S ₀	cm ⁻²

Table 2

Treatment conditions and experimental results for RIF tumor.

Group ID	[HPPH] (μ M)	LS strength (mW/cm)	Time (s)	μ_a (cm^{-1})	μ_s (cm^{-1})	Necrosis radius (mm)
2	0.40 $\pm$ 0.06	12	3600	0.63 $\pm$ 0.1	9.1 $\pm$ 0.8	2.45 $\pm$ 0.9
3	0.59 $\pm$ 0.3	30	660	0.74 $\pm$ 0.08	8.9 $\pm$ 0.2	2.68 $\pm$ 0.7
5	0.39 $\pm$ 0.1	75	996	0.79 $\pm$ 0.01	8.5 $\pm$ 0.9	3.2 $\pm$ 0.8
6	0.47 $\pm$ 0.3	30	1000	0.65 $\pm$ 0.05	9.6 $\pm$ 1	3.0 $\pm$ 0.8
7	1.6 $\pm$ 0.6	30	500	0.63 $\pm$ 0.1	9.55 $\pm$ 1	2.67 $\pm$ 1
9	0.69 $\pm$ 0.1	75	400	0.69 $\pm$ 0.1	8 $\pm$ 1	2.7 $\pm$ 0.4
10	0.32 $\pm$ 0.05	75	666	0.95 $\pm$ 0.2	10.5 $\pm$ 3	3.2 $\pm$ 0.4
11	0.46 $\pm$ 0.08	150	666	0.83 $\pm$ 0.05	11 $\pm$ 2	4.63 $\pm$ 2

Table 3

Treatment conditions and experimental results for H460 tumor.

Group ID	[HPPH] (μ M)	LS strength (mW/cm)	Time (s)	μ_a (cm^{-1})	μ_s (cm^{-1})	Necrosis radius (mm)
1	0.34 $\pm$ 0.1	30	1000	0.5 $\pm$ 0.1	10.6 $\pm$ 2	2.5 $\pm$ 1
2	0.29 $\pm$ 0.06	75	666	0.61 $\pm$ 0.1	9.5 $\pm$ 2	2.2 $\pm$ 0.8
3	0.19 $\pm$ 0.02	150	333	0.46 $\pm$ 0.1	12 $\pm$ 2	2.2 $\pm$ 1
4	0.49 $\pm$ 0.1	12	1000	0.48 $\pm$ 0.1	13.2 $\pm$ 2	2.7 $\pm$ 0.9
5	0.31 $\pm$ 0.07	30	1666	0.47 $\pm$ 0.1	10.9 $\pm$ 0.7	3.1 $\pm$ 1
7	0.21 $\pm$ 0.1	12	2500	0.61 $\pm$ 0.1	10.9 $\pm$ 0.9	2.2 $\pm$ 0.8
8	0.31 $\pm$ 0.2	100	500	0.80 $\pm$ 0.08	7.0 $\pm$ 0.7	2.6 $\pm$ 1

Table 4

PDT parameters.

Parameter	Values	
	RIF (C3H mice)	H460 (Nude mice)
$\xi(\text{cm}^2\text{mW}^{-1}\text{s}^{-1})$	$33\pm3\times10^{-3}$	$34\pm3\times10^{-3}$
$\sigma(\mu\text{M}^{-1})$	$1.05\pm0.2\times10^{-5}$	$1.02\pm0.1\times10^{-5}$
$g(\mu\text{M/s})$	1.3 ± 0.4	1.7 ± 0.6
$[^1\text{O}_2]_{\text{rx},\text{sd}}(\text{mM})$	0.41 ± 0.25	0.37 ± 0.2
$\beta(\mu\text{M})$	11.9	11.9
$\delta(\mu\text{M})$	33	33
$\mu'_s(\text{cm}^{-1})_{(\text{cm}^{-1})}$	9 ± 1	11 ± 2
$\mu_a(\text{cm}^{-1})$	0.7 ± 0.1	0.5 ± 0.1
$[\text{HPPH}](\mu\text{M})$	0.6 ± 0.4	0.3 ± 0.1