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In-vivo outcome study of HPPH mediated PDT using singlet oxygen explicit dosimetry (SOED)

Rozhin Penjweini^a, Michele M. Kim^a, and Timothy C. Zhu^{*,a}

^aDepartment of Radiation Oncology, University of Pennsylvania, Philadelphia, PA 19104

Abstract

Type II photodynamic therapy (PDT) is based on the use of photochemical reactions mediated through an interaction between a tumor-selective photosensitizer, photoexcitation with a specific wavelength of light, and production of reactive singlet oxygen. However, the medical application of this technique has been limited due to inaccurate PDT dosimetric methods. The goal of this study is to examine the relationship between outcome (in terms of tumor growth rate) and calculated reacted singlet oxygen concentration ($[^1O_2]_{rx}$) after HPPH-mediated PDT to compare with other PDT dose metrics, such as PDT dose or total light fluence. Mice with radiation-induced fibrosarcoma (RIF) tumors were treated with different light fluence and fluence rate conditions. Explicit measurements of photosensitizer drug concentration and tissue optical properties via fluorescence and absorption measurement with a contact probe before and after PDT were taken to then quantify total light fluence, PDT dose, and $[^1O_2]_{rx}$ based on a macroscopic model of singlet oxygen. In addition, photobleaching of photosensitizer were measured during PDT as a second check of the model. Changes in tumor volume were tracked following treatment and compared to the three calculated dose metrics. The correlations between total light fluence, PDT dose, reacted $[^1O_2]_{rx}$ and tumor growth demonstrate that $[^1O_2]_{rx}$ serves as a better dosimetric quantity for predicting treatment outcome and a clinically relevant tumor growth endpoint.

Keywords

photodynamic therapy; singlet oxygen; HPPH photosensitizer; *in-vivo* mice study

1. INTRODUCTION

Photodynamic therapy (PDT) has received much attention in recent years due to their use to detect and treat proliferate disorders, including cancer.¹⁻³ PDT requires administration of a photosensitizing drug (PS) that localizes in tumor tissue and is subsequently excited by exposure to a specific wavelength of light.¹⁻³ PS transfers energy to ground-state tissue oxygen (3O_2) and generates singlet-state oxygen (1O_2), which is the main cytotoxic species causing therapeutic effect via reacting with the surrounding biological molecules.⁴ Although PDT targets malignant cancer cells, and destroy them in a way that causes significantly less harm to the body than are caused by current treatments like chemotherapy, there are still

*Corresponding Author: tzhu@mail.med.upenn.edu; phone 1 215 662 4043; fax 1 215 615 5600.

obstacles preventing the widespread clinical use of PDT. The lack of a PDT dosimetry quantity that correlates with PDT efficacy is prominent among these limitations.^{5–7}

Under well-oxygenated conditions, PDT dose, i.e., the light dose absorbed by PS is the most well defined biophysical dosimetry quantity and a good predictor of treatment outcome in explicit PDT dosimetry. The quantity of this method is proportional to the time integral of the product of local PS concentration and light fluence rate. However, theoretical and experimental mice studies have shown that in tumors, which are frequently poorly oxygenated to begin with, high fluence rate PDT modality can create even more severe hypoxia during illumination and result in less effective treatment.⁴ To overcome this problem and characterize the PDT treatment outcome, it is suggested to account for 2O_1 production during PDT based on its luminescence signals at 1270 nm.^{8–10} However, the rapid reactions of 2O_1 with biological environment as well as the weak and short lifetime luminescence signals (in the range of 30–180 ns) are major obstacles to use this method for the clinical application, especially in interstitial applications.

A macroscopic model with four PDT parameters of initial oxygen consumption rate (ξ), ratio of photobleaching to reaction between 2O_1 and cellular targets (σ), ratio of triplet state (T) phosphorescence to reaction between T and 3O_2 (β) and oxygen perfusion rate to tissue (g) has been introduced in 2010 to determine the reacted singlet oxygen threshold concentration ($[^1O_2]_{rx,sh}$) in Photofrin-mediated PDT; $[^1O_2]_{rx}$ is defined as the 1O_2 effectively leading cell death.⁴ The sensitivity of the model parameters to calculated $[^2O_1]_{rx,sh}$ profiles has been also explored for interstitial condition in female C3H mice, which showed practical and accurate calculations of the local 1O_2 .

The aim of this study is to use the macroscopic model to calculate the local 1O_2 concentration in *in-vivo* HPPH-mediated PDT. The idea is that cumulative $[^1O_2]_{rx}$ concentration correlates better with PDT outcomes than either light or PDT dose because $[^1O_2]_{rx}$ concentration accounts for PDT-induced 3O_2 consumption and PS photobleaching. A series of *in-vivo* mice PDT including a range of total fluence (source strengths of 50, 75 and 150 mW/cm² and exposure times of 900, 1333, 1667, 1800 and 2700s) is used to span the different growth rate parameters. Then, the correlations between total light fluence, reacted $[^1O_2]_{rx}$ and tumor growth are evaluated to obtain the best dosimetric quantity for predicting treatment outcome and a clinically relevant tumor growth endpoint.

2. THEORY AND METHOD

2.1 The macroscopic 1O_2 model

The intention of this section is to use an empirical macroscopic model to calculate $[^1O_2]_{rx}$ for different excitation conditions, which relates directly to the 3-dimensional distribution of PS, light fluence rate (ϕ), and mean tissue oxygenation distribution.⁴ In the macroscopic model, spatial distribution of ϕ in tumor is calculated based on diffusion approximation:

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

The PDT process can be expressed as a set of coupled differential equations incorporating five PS-specific reaction-rates parameters (ξ , β , σ , δ , g) and $^1\text{O}_2$ threshold dose, $[^1\text{O}_2]_{\text{rx,sh}}$. Temporal and spatial distributions of PS (S_0), $^3\text{O}_2$ and cumulative $[^1\text{O}_2]_{\text{rx}}$ concentrations are obtained via solving the time-dependent differential equations:

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

$$\frac{d[^3\text{O}_2]}{dt} + \left(\xi \frac{\varphi[S_0](1 + \sigma([S_0] + \delta))}{[^3\text{O}_2] + \beta} \right) [^3\text{O}_2] = \Gamma_s \quad (3)$$

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

The parameters μ_a and μ'_s represent absorption and scattering coefficients, respectively. S describes the light source and the symbol Γ_s denotes the rates at which ground-state oxygen is supplied to the surrounding tissue. δ is the low concentration correction parameter. The parameters used in this study is listed in Table 1, which is based on previous tumor necrosis study^{12–13} but is refitted with the values of ξ increased by a factor of $\ln(10) = 2.303$ due to the new knowledge that the extinction coefficient for HPPH is actually expressed in base of 10 instead of in base of e .

2.2 Establishment of the small animal tumor models for *in-vivo* studies

As shown in Fig. 1A, radiation-induced fibrosarcoma (RIF) tumors were propagated on the shoulder of female C3H mice (9–11 weeks old) by the intradermal injection of a suspension of 3×10^5 in vitro-maintained cells. When the tumors volume reached $\sim 20\text{--}50 \text{ mm}^3$ (about 7–10 days after cell injection), the mice received 0.25 mg/kg HPPH PS by tail vein injection. Then, PDT has been performed following HPPH drug-light intervals of 24 h.

2.3 *In-vivo* tumor growth study

PDT has been performed by surface illumination of the RIF tumor using an experimental setup as well as a diode laser emitting an 8-Watt maximum power and 665nm beam as shown in Figure 1B. The light was delivered via a 140 μm -diameter optical fiber and the laser beam was collimated through a coupling lens on the end of the fiber to a 1cm diameter beam spot over the tumor surface. A range of total fluence including different source strengths of 50, 75 and 150 mW/cm^2 and exposure times of 900, 1333, 1667, 1800 and 2700s (total PDT doses of 100, 135 and 250 J/cm^2) have been used to span the different growth rate parameters. Tumor-bearing mice with no PS and excitation were considered as controls. To assess the treatment response, width (a) and length (b) of the treated mice and controls have been measured daily with slid calipers until tumor volume (V) reaches about 1000 mm^3 . The tumor volume was calculated using the formula $V = \pi \times a^2 \times b / 6$. The tumor growth factor (k) were obtained by fitting our V to an exponential growth equation ($V = A e^{(1/k)d}$); d presents the number of the days after the PDT treatment and A is the amplitude.

2.4 Explicit $^1\text{O}_2$ dosimetry in subcutaneous tumors

The aim of this section is the practical and accurate calculation of the local $^1\text{O}_2$ concentration in tissue, *in-vivo*. Explicit measurement of ϕ , tissue optical properties (μ_a and μ'_s) as well as the PS and tumor-average hemoglobin oxygen saturation (SO_2) concentrations are required to rigorously quantify $[^1\text{O}_2]_{\text{rx}}$ in tumor. HPPH-PDT treatments with different concentrations of PSs have been performed previously by another study using interstitial catheters in RIF tumors.¹² For sequential measurements of the PS fluorescence emission spectrum along the catheter axis before and after PDT treatment, a side-cut fiber that serves as both an excitation source connected to a 405 nm diode laser and a photodetector has been used. In order to determine the drug concentration in the tumor, the raw spectrum has been fitted using the singular value decomposition (SVD) algorithm, and has been compared with the results obtained in the same manner from a set of phantoms with known drug concentration. The effects of the light absorption and scattering by tissue on the measured PS fluorescence signals has been taken into account by applying correction factors obtained from the phantom studies.^{12–13} Absorption (diffuse reflectance-based) spectroscopy has also been used to determine tumor SO_2 before and after PDT. An isotropic fiber-optic detector measured the fluence-rate profiles at a certain distance (normally 3 mm) from the centurial catheter throughout treatment. The spatially-resolved optical properties have been extracted by fitting these data to a diffusion theory model as explained in section 1.1. A range of ϕ and different modes of light delivery was used to calculate the tissue optical properties, initial tumor PS concentration and spatial distribution of ϕ as shown in Table 1 and Figure 2A.^{12–13} The initial values were taken from the previous *in-vivo* experimental and theoretical studies.^{4,12–13}

2.5 Statistical analyses

Each tumor growth study was independently carried out two times. The tumor growth factor of any two group is expressed as mean $\pm$ standard error of the measurement. To assess the consistency of the measurements, the tumor volumes in each of the two groups were compared by using Kruskal-Wallis tests; 12–14 volume values (number of the days post treatment) were considered for each group. Wilcoxon tests were also used to evaluate whether the growth rate of the tumors in each two groups of samples, control and treated mice, are significantly different from each other. Analyses were carried out using SPSS 14.0 software. Statistical significance was defined at $p < 0.05$ level (95% confidence level).

3. RESULTS AND DISCUSSION

To study treatment responses vs. total PDT dose (fluence $\times$ exposure time), the excitation of HPPH PS was performed by illumination (at 665 nm) of the RIF tumor surface as shown in Fig. 1B. The treatment conditions included source strengths of 50, 75 and 150 mW/cm² and light delivery at 100, 135 and 250 J/cm². Tumor regrowth assay post PDT treatment was used to quantify the treatment effects of PDT.⁷ The amounts of $[^1\text{O}_2]_{\text{rx}}$ has also been calculated by using the macroscopic model incorporating the information of the HPPH concentration as well as the ϕ and mean tissue oxygenation distributions. The detailed descriptions of the model and the fitting routine can be found elsewhere.^{4,12–13} Table 2 presents the calculated mean and sum $^1\text{O}_2$ as well as the tumor growth factor k for each of

our test conditions compared to untreated animals (control). The correlations between the tumor growth and reacted $[^1O_2]_{rx}$ at the same PDT dose has also been shown in Fig.3 for the total fluence of 135 J/cm^2 (fluence rates of 50, 75 and 150 mW/cm^2).

Based on our statistical analyses presented in Table 3, all treatments showed effective control of the tumor growth, but source strength of 75 mW/cm^2 and exposure time of 1333 s (100 J/cm^2 total PDT dose) had no significant control of the tumor growth as compared with non-treated mice. Among all treatment conditions, source strength of 50 mW/cm^2 and exposure time of 2700 s (135 J/cm^2 total PDT dose) had the most significant control of the tumor growth. The calculated singlet oxygen concentration also showed a mutual agreement with the results obtained for the tumor regrowth study. Moreover, our results show the different tumor responses for the same fluence but different fluence rates. This indicates that PDT dose, i.e., the light dose absorbed by PS is not an accurate and a good predictor of PDT treatment outcome. The correlations between total light fluence, PDT dose, reacted $[^1O_2]_{rx}$ and tumor growth demonstrate that $[^1O_2]_{rx}$ serves as a better dosimetric quantity for predicting treatment outcome and a clinically relevant tumor growth endpoint.

4. CONCLUSION

By simplifying and combining the energy transfer processes in PDT, a set of governing equations are produced, which describes the creation of $[^1O_2]_{rx}$. These equations are dependent on various parameters such as the light source strength, source geometry, drug-light interval, tissue optical properties and photochemical parameters. The relationship between this metric and the tumor response (tumor growth) was determined in this study. The predictive power of $[^1O_2]_{rx}$ metric and PDT dose was also evaluated through comparison to the tumor growth. Although total fluence or PDT dose is commonly used as a dose metric, the results of this study showed that treatment conditions with different fluence rates and exposure times but the same total PDT dose have different PDT outcomes and cannot be a reliable metric for the evaluation of the PDT. However, $[^1O_2]_{rx}$ derived from the type II PDT energy transfer mechanisms is more accurate and a good metric to show the PDT outcomes.

Although the results have shown a good estimation of the PDT treatment outcomes, our study needs more comprehensive mice studies for different PS concentration, drug-light interval and various excitation light doses.

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References

1. Dougherty TJ, et al. Photodynamic therapy. J NCI. 1998; 90(12):889–905.
2. Penjweini R, Loew H-G, Breit P, Kratky KW. Optimizing the antitumor selectivity of PVP-Hypericin re A549 cancer cells and HLF normal cells through pulsed blue light. Photodiagnosis and Photodynamic Therapy. 2013; 10:591–599. [PubMed: 24284116]

3. Penjweini R, Loew H-G, Eisenbauer M, Kratky KW. Modifying excitation light dose of novel photosensitizer PVP-Hypericin for photodynamic diagnosis and therapy. *J Photochem Photobiol B: Bio.* 2013; 120:120–129.
4. Wang KKH, et al. Explicit dosimetry for photodynamic therapy: macroscopic singlet oxygen modeling. *J Biophoton.* 2010; 3(5–6):304–318.
5. Wang KK-H, Mitra S, Foster TH. Photodynamic dose does not correlate with long-term tumor response to mTHPC-PDT performed at several drug-light intervals. *Med Phys.* 2008; 35(8):3518–3526. [PubMed: 18777912]
6. Sharikova AV, Finlay JC, Liang X, Zhu TC. PDT dose dosimetry for pleural photodynamic therapy. *Proc SPIE.* 2013; 8568:856817.
7. Zhou X, et al. Pretreatment photosensitizer dosimetry reduces variation in tumor response. *Int J Radiat Oncol Biol Phys.* 2006; 64:1211–1220. [PubMed: 16504761]
8. Niedre MJ, et al. In-vitro tests of the validity of singlet oxygen luminescence measurements as a dose metric in photodynamic therapy. *Can Res.* 2003; 63:7986–7994.
9. Niedre MJ, et al. Singlet oxygen luminescence as an in vivo photodynamic therapy dose metric: validation in normal mouse skin with topic amino-levulinic acid. *Bri J Can.* 2005; 92:298–304.
10. Jarvi MT, et al. Singlet oxygen luminescence dosimetry (SOLD) for photodynamic therapy: current status, challenges and future prospects. *Photochem Photobiol.* 2006; 82:1198–1210. [PubMed: 16808593]
11. Niedre MJ, Patterson MS, Wilson BC. Direct near-infrared luminescence detection of singlet oxygen generated by photodynamic therapy in cells in vitro and tissues in vivo. *Photochem Photobiol.* 2002; 75:382–391. [PubMed: 12003128]
12. Liu B, Kim MM, Gallagher-Colombo SM, Busch TM, Zhu TC. Comparison of PDT parameters for RIF and H460 tumor models during HPPH-mediated PDT. *Proc SPIE.* 2014; 8931:89311C–1.
13. Liu B, Kim MM, Zhu TC. A theoretical comparison of macroscopic and microscopic modeling of singlet oxygen during Photofrin and HPPH mediated-PDT. *Proc SPIE.* 2013; 8568:856808–1.

Figure 1.

A) Radiation-induced fibrosarcoma (RIF) tumors propagated on the shoulder of female C3H mice. B) Experimental setup for the investigation of the optical properties of the RIF tumors, grown over the shoulder of the mice. This setup was also used for PDT treatment.

Figure 2.

Spatial distribution of A) light fluence rate due to the optical properties, B) singlet oxygen in tumor. The presented fluence rates are normalized to their value in air. A range of the optical properties (μ_a , and μ_s) and total fluence (including different fluence rates in mW/cm² and exposure times in s) were used in the calculations.

Figure 3.

A) Tumor growth study post HPPH-PDT treatment. Symbols are data and lines are fits using exponential growth equation. B) Calculated singlet oxygen for the same excitation conditions and the same total PDT dose.

Table 1HPPH optical properties and PDT photochemical parameters.¹²⁻¹³

Parameters	Definitions	Values
$\mu_a(\text{cm}^{-1})$	Optical absorption coefficient	$0.86 \pm 0.05 \text{ cm}^{-1}$
$\mu'_s(\text{cm}^{-1})$	Optical scattering coefficient	$7.95 \pm 0.46 \text{ cm}^{-1}$
g	Maximum oxygen supply rate	$1.65 \text{ }\mu\text{M/s}$
$\xi(\text{cm}^2 \text{ mW}^{-1} \text{ s}^{-1})$	Initial oxygen consumption rate	$(72.6 \pm 29) \times 10^{-3} \text{ cm}^2/\text{s/mW}$
$\alpha(\mu\text{M}^{-1})$	Ratio of photobleaching to reaction between $^2\text{O}_1$ and cellular targets	$(1.09 \pm 0.3) \times 10^{-6} \mu\text{M}^{-1}$
$\beta(\mu\text{M})$	Low concentration correction	33
$\beta(\mu\text{M})$	Ratio of phosphorescence to reaction between T and $^3\text{O}_2$	$11.9 \text{ }\mu\text{M}$
PS (μM)	Photosensitizer interstitial concentration	$0.14 \pm 0.08 \text{ }\mu\text{M}$
$[^1\text{O}_2]_{\text{rx,th}}$	Reacted $^1\text{O}_2$ threshold concentration	$0.66 \pm 0.24 \text{ mM}$

Table 2

Calculated values for the amounts of the singlet oxygen generation and tumor growth factors for each PDT treatment condition.

Source strength (mW/cm ²)	Exposure time (s)	Fluence (J/cm ²)	Mean ² O ₁ (μM)	Sum ² O ₁ (μM)	k (day)
50	2700	135	0.57	16.96	3.29 ± 0.16
75	1800	135	0.23	6.87	2.67 ± 0.10
150	1667	250	0.19	5.76	2.63 ± 0.42
150	900	135	0.18	5.46	2.50 ± 0.06
75	1333	100	0.16	4.56	2.50 ± 0.12
Control		0	0	0	2.27 ± 0.26

Table 3

Kruskal-Wallis and Wilcoxon statistical analyses

Groups for the analyses	<i>P</i> ^b	Pairs for the comparisons	<i>p</i>
75 mW/cm ² , 1333 s, 100 J/cm ²	0.92	75 mW/cm ² , 1333 s, 100 J/cm ² & Control	0.10
75 mW/cm ² , 1800 s, 135 J/cm ²	0.92	75 mW/cm ² , 1800 s, 135 J/cm ² & Control	0.00
50 mW/cm ² , 2700 s, 135 J/cm ²	0.66	50 mW/cm ² , 2700 s, 135 J/cm ² & Control	0.00
150 mW/cm ² , 1667 s, 250 J/cm ²	0.14	150 mW/cm ² , 1667 s, 250 J/cm ² & Control	0.00
150 mW/cm ² , 900 s, 135 J/cm ²	0.84	150 mW/cm ² , 900 s, 135 J/cm ² & Control	0.00
Control ^a	0.51		

^aThe mice with no PS and no treatment.^b*p* values obtained by Kruskal-Wallis and Wilcoxon tests are generated by comparing the values of each two groups. In all cases $p < 0.001$ showing a statistically significant difference with 95% confidence level.