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Reactive oxygen species explicit dosimetry to predict local tumor control for Photofrin-mediated photodynamic therapy

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Abstract

Although photodynamic therapy (PDT) is an established modality for cancer treatment, current dosimetric quantities, such as light fluence and PDT dose, do not account for the differences in PDT oxygen consumption for different fluence rates (ϕ). A macroscopic model was adopted to calculate reactive oxygen species concentration ($[\text{ROS}]_{\text{rx}}$) to predict Photofrin-PDT outcome in mice bearing radiation-induced fibrosarcoma (RIF) tumors. Singlet oxygen is the primary cytotoxic species for ROS, which is responsible for cell death in type II PDT, although other type I ROS is included in the parameters used in our model. Using a combination of fluences (50–250 J/cm²) and ϕ (50 – 150 mW/cm²), tumor regrowth rate, k , was determined for each condition by fitting the tumor volume vs. time to $V_0 \cdot \exp(k \cdot t)$. Treatment was delivered with a collimated laser beam of 1 cm diameter at 630 nm. Explicit dosimetry of initial tissue oxygen concentration, tissue optical properties, and Photofrin concentration was used to calculate $[\text{ROS}]_{\text{rx,cal}}$. ϕ was determined for the treatment volume based on Monte-Carlo simulations and measured tissue optical properties. Tissue oxygenation is measured using an oxylite oxygen probe to throughout the treatment to calculate the measured $[\text{ROS}]_{\text{rx,mea}}$. Cure index, $\text{CI} = 1 - k/k_{\text{ctr}}$, for tumor growth up to 14 days were determined as an endpoint using five dose metrics: light fluence, PDT dose, and $[\text{ROS}]_{\text{rx,cal}}$, and $[\text{ROS}]_{\text{rx,mea}}$. PDT dose was defined as the product of the time-integral of photosensitizer concentration and ϕ at a 3 mm tumor depth. Preliminary studies show that $[\text{ROS}]_{\text{rx,mea}}$ best correlates with CI and is an effective dosimetric quantity that can predict treatment outcome.

Keywords

photodynamic therapy; macroscopic singlet oxygen model; explicit PDT dosimetry; Photofrin-mediated PDT

1. INTRODUCTION

Photodynamic therapy (PDT) is currently undergoing intensive clinical investigations as an adjuvant treatment for proliferative disorders including cancer.^{1–3} PDT involves the administration of a photosensitizer, which preferentially accumulates in diseased cells, followed by light excitation at a specific wavelength.^{3,4} PDT is dynamic and multifaceted

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with the interactions between a treatment light at a particular wavelength, a photosensitizer, and tissue oxygenation ($[^3\text{O}_2]$).² At Photofrin-PDT, the photosensitizer undergoes mostly type II processes upon photoexcitation in which the triplet state transfers energy to $^3\text{O}_2$ to produce singlet oxygen (ROS).^{5,6} Generation of reactive $^1\text{O}_2$ ($[\text{ROS}]_{\text{rx}}$) causes cytotoxicity and eventually cell death and/or therapeutic effects⁶.

The results of our study using additional real-time measurements of Photofrin concentration and $[^3\text{O}_2]$ result in reduced uncertainties for correlations between cure index at 14 days and the measured reactive oxygen species, $[\text{ROS}]_{\text{rx,mea}}$.

2. MATERIALS AND METHODS

2.1 Tumor Model and PDT Treatment Conditions

Radiation-induced fibrosarcomas (RIF) cells were cultured and injected in the right shoulder regions of 6–8 weeks old female C_3H mice (NCI-Frederick, Frederick, MD). 30 μl were injected at a concentration of 1×10^7 cells/ml, as described previously.^{8–11} 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 treatment region was clipped prior to cell inoculation, and the treatment area was depilated with Nair (Church & Dwight Co., Inc., Ewing, NJ) at least 24 hours prior to measurements and treatment. Tumors were treated when they were 3–5 mm in diameter. Mice were given a chlorophyll-free (alfalfa-free) rodent diet (Harlan laboratories Inc., Indianapolis, IN) at least 10 days prior to treatment to eliminate the fluorescence signal from chlorophyll-breakdown products, which have a similar range to the photosensitizer spectra obtained in this study. The photosensitizer fluorescence was used to determine the in vivo concentrations in this study, using methods described previously.^{7–9}

Treatment delivery was done using an optical fiber with a microlens attachment coupled to a diode laser with the appropriate wavelength for each photosensitizer. A 630 nm laser (Biolitec, Inc., East Longmeadow, MA, USA) was used for Photofrin after a 18–24 hours drug-light interval. The in-air fluence rate (ϕ_{air}) is defined as the calculated irradiance determined by the laser power divided by the treatment area (1 cm diameter spot size). The in-air fluence was calculated by multiplying the in-air fluence rate by the treatment time. RIF tumor-bearing mice with no photosensitizer and no light excitation were used as controls ($n = 5$). Treatment conditions are summarized in Table 1.

2.2 Photodynamic Therapy Protocol

Photofrin (Pinnacle Biologics, Chicago, Illinois) at a dosage of 5 mg/kg was injected through the mouse tail vein as described previously.^{12,13} At a 24-h drug–light interval, superficial irradiation of the tumor was performed with a 630-nm laser (Biolitec AG., A-1030, Vienna). A microlens fiber was coupled to the laser to irradiate the tumor uniformly. Animals were assigned to four light dose groups, and each group was comprised of 2 to 3 subgroups with different ϕ . There were a total of 5 treatment groups: 50 J/cm² at 150 mW/cm², 250 J/cm² at 75 mW/cm², 135 J/cm² at 75 mW/cm², 75 J/cm² at 75 mW/cm²,

and 100 J/cm^2 at 150 mW/cm^2 . Tumor-bearing mice that received neither light irradiation nor Photofrin were used as controls.

2.3 Photofrin Concentration

Following the drug-light interval of 18~24 hours, measurements of light fluence rate, photosensitizer concentration and $[\text{O}_2]$ were performed. Photofrin fluorescence spectra was obtained using a custommade multi-fiber contact probe before and after PDT.¹⁴ The probe is connected to a 405 nm laser (Power Technology Inc., Little Rock, AR, USA) for the fluorescence excitation of Photofrin and a multichannel CCD spectrograph (InSpectrum, Princeton Instruments, Trenton, NJ, USA) for the collection of the spectra. The in vivo photosensitizer concentration was obtained by comparing the in vivo Photofrin spectra with those of phantoms with known photosensitizer concentrations. The attenuation of the fluorescence signal due to the light absorption and scattering by tissues was corrected by applying an empirical correction factor described elsewhere. The accuracy of in vivo measurements was validated by ex vivo measurements in separate mice.^{15,16}

2.4 Oxygen Measurements

The in vivo tissue oxygen partial pressure $p\text{O}_2$ was measured during PDT treatment using a phosphorescence-based $^3\text{O}_2$ probe (OxyLite Pro, Oxford Optronix, Oxford, United Kingdom). A barefiber-type probe (NX-BF/O/E, Oxford Optronix, Oxford, United Kingdom) was placed inside the tumor at a 3 mm depth from the treatment surface. The oxygen concentration ($[\text{O}_2]$) was calculated by multiplying the measured $p\text{O}_2$ with the O_2 solubility in tissue, which is $1.295 \mu\text{M/mmHg}$.⁵ Measured $[\text{O}_2]_0$ and $[\text{O}_2](t)$ was used to calculate reacted oxygen species using the macroscopic singlet oxygen model.^{4,5}

2.5 Tumor Regrowth Rate Analysis

Tumor volumes were measured daily after PDT. Width (a) and length (b) were measured with slide calipers, and tumor volumes (V) was calculated using $V = \pi \times a^2 \times b/6$.¹⁷ Tumor volumes were tracked for 14 days, and the tumor regrowth factor (k) was calculated by the best exponential fit [with a form $f(d) = Ae^{kd}$] to the measured volumes over the days (d). CI was calculated for each treatment group as

$$CI = 1 - \frac{k}{k_{ctr}} \quad (1)$$

where k is the tumor regrowth factor for each group and k_{ctr} is the regrowth factor for the control group, which consisted of tumors to neither Photofrin nor light illumination.

2.6 Reactive Oxygen Species Explicit Dosimetry

Type II PDT process can be described by a set of kinetic equations which can be simplified to describe the creation of $[\text{ROS}]_{rx}$.^{18,19} These equations are dependent on the temporal and spatial distribution of ϕ , photosensitizer concentration ($[\text{S}_0]$), ground state oxygen concentration ($[\text{O}_2]$), oxygen supply rate (g), and the photosensitizer-specific reaction-rate parameters (δ , β , σ , and ξ). The relevant equations are:

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

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

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

$$[ROS]_{rx} = \int_{t=0}^T \xi \frac{[{}^3O_2]}{[{}^3O_2] + \beta} \phi[S_0] dt \quad (5)$$

where ϕ is the light fluence rate, S is the source term, μ_a and μ_s' are the absorption and reduced scattering coefficients, respectively. The five parameters involved in the kinetic equations are photosensitizer-specific and details of each can be found elsewhere.¹⁸ ξ is the photochemical oxygen consumption rate per light fluence rate and photosensitizer concentration under ample 3O_2 supply. σ is the probability ratio of a 1O_2 molecule to react with ground state photosensitizer compared to the 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 . δ is the low concentration correction factor, and g is the maximum macroscopic oxygen perfusion rate. The reacted oxygen species concentration ($[ROS]_{rx}$) used as a dosimetric measure in other studies was calculated and compared using an initial ground state oxygen concentration of ($[{}^3O_2]_0$) of 40 μM , the measured $[{}^3O_2]_{(t)}$ throughout treatment.

For each spatial location, $[S_0]_{(t)}$ and $[{}^3O_2]_{(t)}$ can be calculated by solving the coupled differential equations [Eqs. (2) and (4)] using the initial conditions for $[S_0]_0$ based on fluorescence measurement before PDT and its assumed spatial homogeneous, the initial $[{}^3O_2]_0$ value (from Figure 2), and $\phi(d)$. The light fluence rate does not change with time. Finally, $[{}^1O_2]_{rx}$ is calculated using Eq. (4)^[15,22]. We also measure the temporal value $[{}^3O_2]_{(t)}$. And then we use the calculated $[{}^3O_2]_{(t)}$ and measure $[{}^3O_2]_{(t)}$ in the Eq.(5), and get the $[ROS]_{rx \text{ cal}}$ and $[ROS]_{rx \text{ mea}}$.

3. RESULTS AND DISCUSSION

Photofrin-mediated PDT with various in-air fluences, ϕ_{air} , and exposure times were performed in mouse models bearing RIF tumors. Tissue optical properties, photosensitizer concentration, and tissue oxygenation were measured to PDT dose, and $[ROS]_{rx}$. Table 1 summarizes all of the treatment conditions, as well as the measured and calculated quantities using the photochemical parameters summarized in Table 2.

Photofrin concentration ($[Photofrin]$) was measured both before and after PDT treatment. And some of them were measured in process PDT treatment. Measured $[Photofrin]$ was compared to model calculated values for all of the treatment conditions and is shown in Figure. 1. The symbols represent the measured values, and the solid lines are model-calculated photosensitizer concentration during treatment.

Measured $[^3O_2]$ was used to refine the photochemical parameters previously determined²⁰ for the reactive singlet explicit dosimetry model used to calculate $[ROS]_{rx}$. Individually measured $[^3O_2]_{(t)}$ for each mouse were fit with the model-calculated values. Measured data are shown with symbols and calculated $[^3O_2]$ are shown as dashed lines in Figure 2.

Fluence, PDT dose, calculated $[ROS]_{rx,cal}$ at 3 mm and measured $[ROS]_{rx,mea}$ at 3 mm were compared as dosimetric quantities to estimate the outcome of photofrin-mediated PDT for RIF tumors. Outcome was quantified by the calculation of CI. No tumor regrowth up to 14 days after treatment resulted in a CI of 1. PDT dose is calculated using the product of PS uptake and measured light fluence rate at 3 mm. The fluence rate at 3 mm is determined. We used the Eq.(6)²² and the preliminary photochemical parameters shown in Table 2⁷ to calculate $[ROS]_{rx,cal}$ and measured $[ROS]_{rx,mea}$ using model calculated $[^3O_2]$ and measured $[^3O_2]_{(t)}$, respectively. The goodness of the fit and the corresponding upper and lower bounds of the fits (gray area) to the fluence, PDT dose, calculated $[ROS]_{rx,cal}$ and the measured $[ROS]_{rx,mea}$ are presented in Figure 3. Figure 3(a) shows that, while fluence correlates sigmoidal with the PDT outcome, it exhibits large uncertainties as defined by the large bounds of the gray area, as well as by the low value of $R^2=0.777$. As shown in Figure 3(b), PDT dose allows for reduced subject variation and improved predictive efficacy as compared to fluence, calculated $[ROS]_{rx,cal}$ and measured $[ROS]_{rx,mea}$. PDT dose showed a better correlation with CI with a higher value of $R^2=0.8744$ and a narrower band of gray area as it accounts for both light dose and tissue $[photofrin]$ levels. However, PDT dose overestimates $[ROS]_{rx}$ in the presence of hypoxia as it does not account for the oxygen dependence of ROS quantum yield. The goodness of fit $R^2=0.93$, $R^2=0.972$ and the narrowest gray area in Figure 3(c), (d) shows that the calculated $[ROS]_{rx,cal}$ and the measured $[ROS]_{rx,mea}$ correlates the best with CI. $[ROS]_{rx}$ accounts for the key quantities of light fluence, photosensitizer concentration, and tissue oxygen level, respectively. The result that R^2 value of the measured $[ROS]_{rx,mea}$ is better than the calculated $[ROS]_{rx,cal}$.

The PDT dose threshold (439 mJ/cm^2) due obtain in this study is comparable to results published studies in literature 306 mJ/cm^2 in in-vitro condition^{20,21}. But it is substantially smaller than that obtain from our previous study (1200 mJ/cm^2)²². The reason for this different is because the tumor size in the current study is substantially smaller

and less variable than the previous study (100 cc vs. 400 cc). This also explains why the relationship between CI vs. ϕ or CI vs. PDT dose has a sigmoid shape in the current study while no threshold dose behavior was observed in the previous.

4. CONCLUSION

The response of mouse RIF tumors to PDT depends on tissue oxygenation, photosensitizer uptake, total energy delivered, and the ϕ at which treatment is delivered. An accurate dosimetry quantity for the evaluation of treatment outcome

should account for all of these parameters. This study evaluated the efficacy and outcomes of different PDT treatments and how fluence, PDT dose, and $[\text{ROS}]_{\text{rx}}$ compare as dosimetric quantities. The correlation between CI and $[\text{ROS}]_{\text{rx}}$ suggests that $[\text{ROS}]_{\text{rx}}$ at 3 mm is the best quantity to predict the treatment outcome for a clinically relevant tumor regrowth endpoint. PDT dose is a better dosimetric quantity than fluence, but it is worse than $[\text{ROS}]_{\text{rx}}$ as it does not account for the consumption of $[\text{}^3\text{O}_2]$ for different ϕ . For Photofrin in RIF tumors, the temporal dependence of in-vivo oxygen concentration during PDT can't be well modeled by our macroscopic model (for measurement $[\text{}^3\text{O}_2]$, $R^2 = 0.972$). This implies that it is not necessary to make $[\text{}^3\text{O}_2]$ measurements during PDT to obtain $[\text{ROS}]_{\text{rx}}$, and use these values in conjunction with our model. This study validated the model and photochemical parameters for Photofrin-mediated PDT for an endpoint that is clinically relevant. This is being reported for the first time.

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Figure 1.
Photofrin concentration versus fluence at 3 mm tumor depth for various treatment conditions. The lines indicate the calculated change in photosensitizer concentration during light delivery for PDT.

Figure 2. Temporal changes in $^3\text{O}_2$ concentration during light for PDT with various treatment conditions. The lines represent the calculated changed in $^3\text{O}_2$ concentration during treatment.

Figure 3.

CI plotted as a function of (a) fluence at a 3 mm tumor depth, (b) calculated PDT dose at 3 mm tumor depth, (c) calculation ROS at 3 mm tumor depth (d) mean reacted singlet oxygen at 3 mm depth ($[\text{ROS}]_{\text{rx}}$) calculated using Eqs. (3)~(6) and the parameters summarized in Table 2. The solid lines show the best-fit to the data with functional forms

$$CI = \frac{1}{1 + 223.4e^{-0.05269x}}, CI = \frac{1}{1 + 9971e^{-0.02098x}}, CI = \frac{1}{1 + 144.6e^{-5.221x}} \text{ and } \frac{1}{1 + 6480e^{-9.151x}}$$

with $R^2 = 0.777, 0.8744, 0.93$ and 0.972 for (a), (b), (c) and (d), respectively. The gray region indicates the upper and lower bounds of the fit with 95% confidence level.

In-air light fluence, in-air light fluence rate, photofrin concentration in tumors, initial tissue oxygenation, PDT dose, as well as calculated reactive oxygen species concentration. Methods to determine the calculated and measured $[ROS]_{rx, mea}$ and $[ROS]_{rx, calc}$ will be discussed in Section 2.6.

Table 1.

Index	Mice #	Φ (mW/c m ²)	Time(s)	Photofrin pre(μ M)	Photofrin post (μ M)	Fluence(Mw/cm ²)	PDT Dose(μ MJ/c m ²)	$[ROS]_{rx, calc}$ (mM)	$[ROS]_{rx, mea}$ (mM),	CI
1	#10-1	150.00	333.00	6.80	3.07	28.99	136.00	0.29	0.27	0.03
2	#10-2	150.00	333.00	3.07	1.19	43.36	87.00	0.235	0.27	0.00
3	#11-5	150.00	333.00	5.84	2.80	42.42	175.00	0.39	0.21	0.01
4	#12-5	150.00	333.00	5.10	2.30	38.18	134.00	0.32	0.38	0.06
5	#11-1	75.00	3333.00	4.50	2.20	212.52	527.00	1.87	1.20	1.00
6	#11-2	75.00	3333.00	6.47	3.02	195.78	673.00	2.33	1.40	1.00
7	#11-3	75.00	3333.00	4.20	2.44	197.43	524.00	1.73	1.41	1.00
8	#11-4	75.00	3333.00	4.54	2.48	205.21	560.00	1.89	1.39	1.00
9	#12-1	75.00	1800.00	6.90	4.90	103.44	551.00	1.39	1.13	1.00
10	#12-2	75.00	1800.00	6.80	3.35	116.56	476.00	1.24	0.96	0.56
11	#12-3	75.00	1800.00	5.20	3.00	114.90	399.00	1.07	1.00	0.64
12	#12-4	75.00	1800.00	5.67	3.50	104.00	414.00	1.09	1.10	0.82
13	#13-1	75.00	1500.00	6.50	2.80	103.25	413.00	0.89	0.82	0.20
14	#13-2	75.00	1500.00	6.70	3.70	88.36	387.00	0.93	0.73	0.22
15	#13-3	75.00	1500.00	6.00	3.01	97.94	354.00	0.87	0.65	0.13
16	#13-4	150.00	666.00	6.40	2.50	82.95	327.00	0.69	0.77	0.15
17	#13-5	150.00	666.00	7.20	3.54	83.57	409.00	0.81	0.79	0.25
18	#14-1	150.00	666.00	7.84	4.88	72.14	431.00	0.8	0.88	0.42
19	#14-2	150.00	666.00	6.79	3.49	85.31	402.00	0.81	0.78	0.27

Table 2:

Preliminary photochemical parameters obtained from literature.¹⁰

Parameter	Definition	Value
ξ (cm ² s ⁻¹ mW ⁻¹)	Specific oxygen consumption rate	3.7×10^{-3}
σ (μM ⁻¹)	Specific photobleaching ratio	7.6×10^{-5}
β (μM)	Oxygen quenching threshold concentration	11.9
δ (μM)	Low concentration correction	33
g (μM/s)	Macroscopic oxygen maximum perfusion rate	0.76