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Dosimetry study of PHOTOFRIN-mediated photodynamic therapy in a mouse tumor model

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Abstract

It is well known in photodynamic therapy (PDT) that there is a large variability between PDT light dose and therapeutic outcomes. An explicit dosimetry model using apparent reacted 1O_2 concentration ($[^1O_2]_{rx}$) has been developed as a PDT dosimetric quantity to improve the accuracy of the predicted ability of therapeutic efficacy. In this study, this explicit macroscopic singlet oxygen model was adopted to establish the correlation between calculated reacted $[^1O_2]_{rx}$ and the tumor growth using Photofrin-mediated PDT in a mouse tumor model. Mice with radiation-induced fibrosarcoma (RIF) tumors were injected with Photofrin at a dose of 5 mg/kg. PDT was performed 24h later with different fluence rates (50, 75 and 150 mW/cm²) and different fluences (50 and 135 J/cm²) using a collimated light applicator coupled to a 630nm laser. The tumor volume was monitored daily after PDT and correlated with the total light fluence and $[^1O_2]_{rx}$. Photophysical parameters as well as the singlet oxygen threshold dose for this sensitizer and the RIF tumor model were determined previously. The result showed that tumor growth rate varied greatly with light fluence for different fluence rates while $[^1O_2]_{rx}$ had a good correlation with the PDT-induced tumor growth rate. This preliminary study indicated that $[^1O_2]_{rx}$ could serve as a better dosimetric predictor for predicting PDT outcome than PDT light dose.

Keywords

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

1. Introduction

Photodynamic therapy (PDT) is a relatively new therapeutic modality for certain cancers, precancerous diseases and benign diseases [1-3]. The mechanism of PDT is unique in that during PDT, the three elements, the photosensitizer, the oxygen, and the light, are dynamic changing and interacting. In a typical type II process, upon light activation, the excited photosensitizer will transfer energy to oxygen to produce singlet oxygen (1O_2), resulting in cell death or survival, which is highly dependent on the amount of 1O_2 [4].

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Establishment a dosimetry metric based on cumulative singlet oxygen dose to predictive efficacy of PDT is of great importance for the safety and effectiveness of the clinical application of PDT, which is a very challenging and active area of study for PDT [5-7]. Direct $^1\text{O}_2$ measurements based on the 1270 nm phosphorescence of $^1\text{O}_2$, called singlet oxygen luminescence dosimetry (SOLD) has shown promising result in *in vitro* studies, but *in vivo* it is very difficult due to the weak signal of singlet oxygen luminescence[8]. A microscopic model involves the dynamic interaction of the three components of PDT (the light, drug and oxygen) as well as the effect of the dynamic change of blood flow to these three elements. Although the mathematical model has shown improvement in predicting the PDT outcome in *in vivo* studies, it is still impossible in the clinical setting due to the complicated of describing the complete 3D tumor microvascular environment and the vast computational need. In order to facilitate the clinical practice, an explicit macroscopic singlet oxygen model was developed [9]. In this model, the light dose, *in vivo* photosensitizer concentration, the initial oxygen concentration and the tumor optical properties were all taken into account. Instead of $^1\text{O}_2$, an apparent reacted singlet oxygen, $[\text{O}_2]_{\text{rx}}$, was proposed as the dosimetry quantity. In this study, the ability of the $[\text{O}_2]_{\text{rx}}$ as a dosimetric predictor for Photofrin-mediated PDT was evaluated in a mouse RIF tumor growth study.

2. Materials and Methods

2.1 Macroscopic Singlet Oxygen Model

The aim of this section is to introduce the empirical macroscopic singlet oxygen model that was adopted in this study which can be simplified and expressed as following four PDT kinetic equations (1-4) [7]. This model was derived from reaction rate equations for a type II PDT mechanism. The derivation for these equations has been described in detail elsewhere [10,11].

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

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

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

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

Here, ϕ is the light fluence rate, S is the source term, μ_a and μ_s' are the absorption and reduced scattering coefficients of the tumor tissue. ξ , σ , and β are three photochemical parameters. ξ , represents the specific oxygen consumption rate, σ is the specific

photobleaching ratio, and β is the oxygen quenching threshold concentration. δ is defined as the low concentration correction of photosensitizer, and g is the maximum oxygen perfusion rate.

2.2 Determine the model parameters

The photochemical parameters β and δ were obtained from the literature [12,13], the other model parameters (σ , g , ξ and $[^1\text{O}_2]_{\text{rx,sh}}$) were obtained from a former study by using two interstitial catheters to induce partial treatment [9].

For tumor optical properties (μ_a and μ_s'), a central catheter in the tumor was used to insert a 2mm point source coupled to a 630 nm diode laser (Biolitec, Inc., East Longmeadow, MA, USA), the peripheral catheter placed 3 mm away contained an isotropic detector to collect the light fluence rate profile along the length of the tumor. Using light fluence data prior to PDT treatment, Eq. (1) for a point source is used to obtain μ_a and μ_s' using a nonlinear optimization algorithm based on a diffusion theory model [14,15]. Both the diffuse approximation method and the monte carlo method were used to simulate the spatial distribution of light fluence rate with measured optical property variation in mice (Figure 1A), as demonstrated in Figure 1B, the maximum variation between these two methods was $\sim 25\%$.

The *in vivo* Photofrin concentration was obtained by measured fluorescence spectra and compared with phantoms studies with known Photofrin concentrations [16].

Then PDT was performed with a serial of conditions to induce a partial treatment response. The tumor was harvested 24h after PDT and the necrotic radius of tumor was obtained from H & E stained tissue sections. A range of values for the concentration of $[^1\text{O}_2]_{\text{rx, sd}}$ was used as an initial guess. A differential evolution algorithm adjusted g and ξ to vary the $[^1\text{O}_2]_{\text{rx}}$ at the necrotic radius to match the assigned $[^1\text{O}_2]_{\text{rx, sd}}$. The objective function of the fitting algorithm was the maximum relative difference between measurement-based $[^1\text{O}_2]_{\text{rx}}$ and calculated threshold singlet oxygen concentration, and the fitting was terminated when the maximum deviation had less than 10% variation of $[^1\text{O}_2]_{\text{rx, sd}}$ based on the uncertainty of the threshold dose measurement. All these calculations were done using Matlab (Natick, Massachusetts, United States). The values of the parameters used in the macroscopic model are listed in Table 1.

2.3 In vivo mouse tumor model for PDT study

All studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Radioactively induced fibrosarcoma (RIF) cells were injected subcutaneously into the right shoulders of 6-8 week old female C3H mice (NCI-Frederick, Frederick, MD) 5-10 days before PDT at a concentration of 3×10^5 cells/ml. When the tumors reached about 2-9 mm in diameter and 2-5 mm in height, PDT was performed. Before PDT, the fur in the irradiation area was depilated with Nair.

PDT was carried out 24h after the injection of Photofrin at a dosage of 5mg/kg by tail vein with a series of PDT fluence rates (50, 75 and 150 mW/cm²) and total fluences (50 and 135J/cm²). As shown in figure 2, two mice were irradiated simultaneously with a 630 nm

diode laser with a spot size of 1 cm. A microlens was fitted at the end of the laser fiber to produce uniform collimated light.

The tumor volume was measured by a caliper daily until 12-14 days post PDT. The volume of the tumor was calculated by the following formula $V = \pi/6 \times a^2 \times b$. Here a and b refer to the width and length of the tumor respectively. The tumor growth rate (k) was obtained by fitting the tumor volume to an exponential growth equation, $V = A \cdot \exp(k \cdot d)$. A is the amplitude and d represents the number of the days after PDT treatment. Tumor-bearing mice with neither light irradiation nor Photofrin were used as controls.

2.4 Statistical Analysis

Tumor growth factor of each condition is expressed as the mean $\pm$ standard deviation of the measurements. Before PDT, the tumor volumes in each of the two groups were compared using Kruskal-Wallis tests to find if there was any difference between each group. After PDT, Wilcoxon tests were used to evaluate whether the tumor growth rate in control and each PDT group had significantly different. Analyses were carried out using SPSS 22.0 software. Statistical significance was defined at $p < 0.05$ level (95% confidence level).

3. Results and Discussion

Table 2 summarized the results for all PDT treatment conditions with different light fluence of 50 and 135 J/cm² at fluence rates of 50, 75 and 150 mW/cm², measured drug concentration, tumor growth rate and $[^1\text{O}_2]_{\text{rx}}$. Before PDT, the tumor volumes in each of the two groups had no significant difference from each other ($p=0.448$). Comparing with the control group, each PDT group had the ability to inhibit the tumor regrowth after PDT ($p<0.05$ for all). Among them, PDT at 135 J/cm² with a fluence rate of 75 mW/cm² reached complete cure, while partial tumor growth inhibition was found in all other PDT treatment conditions groups.

Based on this series of PDT treatment conditions, the average $[^1\text{O}_2]_{\text{rx}}$ concentration at a 3mm depth inside the RIF tumor was calculated. The result indicated that amount of $[^1\text{O}_2]_{\text{rx}}$ was affected by PDT light dose (fluence and fluence rate). As demonstrated by figure 3B, at the same fluence of 50J/cm², the calculated $[^1\text{O}_2]_{\text{rx}}$ was different with the variation of fluence rate, the highest $[^1\text{O}_2]_{\text{rx}}$ was found at the fluence rate of 75mW/cm², the lowest $[^1\text{O}_2]_{\text{rx}}$ was found at the fluence rate of 150mW/cm². But as shown in figure 4A, there was a very good correlation between the calculated $[^1\text{O}_2]_{\text{rx}}$ and the tumor growth with the $R^2=0.98$. In all the experiment PDT conditions in this study, with the increasing of $[^1\text{O}_2]_{\text{rx}}$, the tumor growth factor was decreased accordingly.

As for PDT light dose, PDT efficiency on tumor growth was varied with fluence and fluence rate (Figure 4B). For example, at the same fluence of 50 J/cm² with the fluence rate of 50, 75, and 150 mW/cm², the tumor growth rate after PDT was at 0.31 ± 0.02 , 0.30 ± 0.07 and 0.32 ± 0.01 , respectively. At the higher fluence of 135 J/cm², among these three fluence rates, the highest tumor growth rate of was found at 150 mW/cm², while the lowest tumor growth rate was with 75mW/cm². While at the same fluence rate of 75mW/cm², the tumor growth rate after PDT with a fluence of 50 and 135J/cm² was at 0.30 ± 0.07 and 0, respectively.

Furthermore, the linear fitting of fluence to the tumor growth rate showed the goodness of $R^2=0.71$, which indicated that the poor relation between fluence and PDT efficiency.

Together, these preliminary results indicate that PDT light dose alone is not accurate to predict the PDT outcome, while $[^1O_2]_{rx}$ had a better relation with the PDT-induced tumor growth inhibition than that of PDT light dose.

4. Conclusion

Based on the established macroscopic singlet oxygen model and Photofrin PDT induced RIF tumor necrosis radius, the *in vivo* $[^1O_2]_{rx}$ was calculated for a range of PDT fluence. The correlation between $[^1O_2]_{rx}$ and the PDT outcome (the tumor growth rate) was also determined and compared with that of PDT light dose (fluence and fluence rate). Preliminary results showed that $[^1O_2]_{rx}$ could serve as a better dosimetric predictor than PDT light dose. More PDT conditions with a wide range of PDT fluence and fluence rate as well as a larger sample of mice will be needed to further verify the predictive ability of this macroscopic single oxygen model.

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Figure 1.

Light fluence rate (ϕ) distribution due to RIF tumor optical properties (A) The ratio of the fluence at a tissue depth and at air (ϕ_{air}) versus the tumor depth. DA is the diffuse approximation simulation, while MC is the monte carlo simulation. (B) Ratio of the DA mean fitting to the MC simulation, the maximum variation is $\sim 25\%$.

Figure 2. Setup for superficial Photofrin-PDT irradiation. Two mice were irradiated simultaneously with a 630 nm diode laser with a light spot size of 1cm.

Figure 3.

PDT effect on a treatment condition of 50 J/cm^2 with fluence rates of 50, 75, and 150 mW/cm^2 . (A) Exponential fitting of tumor volumes after Photofin-PDT within 14 days in this condition. (B) Calculated singlet oxygen at the same PDT condition.

Figure 4.

(A) Linear fitting of tumor growth rate versus [¹O₂]_{rx} in each PDT group. (B) Linear fitting of tumor growth rate versus fluence in each PDT group. The grey area shows the upper and lower bounds of the fit with 95% confidence level.

Table 1
The value of the model parameters for the Photofrin-PDT study([7,10,11])

Parameter	Definition	Value
ξ	Specific oxygen consumption rate $\xi = S_{\Delta} \left(\frac{k_5}{k_5 + k_3} \right) \frac{\varepsilon}{h\nu} \frac{k_7[A]/k_6}{k_7[A]/k_6 + 1}$	$3.7 \times 10^{-3} \text{ cm}^2\text{s}^{-1}\text{mW}^{-1}$
σ	Specific photobleaching ratio $k_1/k_7[A]$	$7.6 \times 10^{-5} \text{ }\mu\text{M}^{-1}$
β	Oxygen quenching threshold concentration k_4/k_2	$11.9 \text{ }\mu\text{M}$
δ	Low concentration correction	$33 \text{ }\mu\text{M}$
g	Macroscopic oxygen maximum perfusion rate	$0.76 \text{ }\mu\text{M/s}$
$[^1\text{O}_2]_{\text{rx,sh}}$	Singlet oxygen threshold dose	$0.74 \pm 0.25 \text{ mM}$

Table 2
The tumor growth rate, drug concentration, and calculated [$^1\text{O}_2$]_{rx} of each group

No. of mice	Fluence rate (mW/cm ²)	Exposure time(s)	Fluence (J/cm ²)	Drug concentration (μM)	n	Growth Rate, k(days ⁻¹)	Mean [$^1\text{O}_2$] _{rx} (mM)
4	50	1000	50	3.53		0.31±0.02 [*]	0.35
5	75	666	50	5.97		0.30±0.07 [*]	0.40
4	150	333	50	4.93		0.32±0.01 [*]	0.24
4	50	2700	135	2.97		0.19±0.13 [*]	0.66
5	75	1800	135	7.18		0 [*]	1.05
4	150	900	135	3.47		0.22±0.04 [*]	0.53
4	Control ^a	0	0	0		0.405±0.05	0

^a Mice without photosensitizer or PDT treatment.

^{*} ($p < 0.05$) indicates significant statistical difference between the PDT group and the control group.