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Reactive Oxygen Species Explicit Dosimetry (ROSED) of a Type 1 Photosensitizer

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Abstract

Type I 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 oxygen species (ROS). The goal of this study is to develop a model to calculate reactive oxygen species concentration ($[ROS]_{rx}$) after Tookad®-mediated vascular PDT. Mice with radiation-induced fibrosarcoma (RIF) tumors were treated with different light fluence and fluence rate conditions. Explicit measurements of photosensitizer drug concentration were made via diffuse reflective absorption spectrum using a contact probe before and after PDT. Blood flow and tissue oxygen concentration over time were measured during PDT as a mean to validate the photochemical parameters for the ROSED calculation. Cure index was computed from the rate of tumor regrowth after treatment and was compared against three calculated dose metrics: total light fluence, PDT dose, reacted $[ROS]_{rx}$. The tumor growth study demonstrates that $[ROS]_{rx}$ serves as a better dosimetric quantity for predicting treatment outcome, as a clinically relevant tumor growth endpoint.

Keywords

photodynamic therapy; reactive oxygen species explicit dosimetry; PDT dose; light fluence

INTRODUCTION

Photodynamic therapy (PDT) is a treatment modality that uses a light sensitive drug which is known as a photosensitizer along with near infrared (NIR) visible light of the appropriate wavelength to damage malignant or abnormal tissues^{1,2}. PDT has been approved by the US Food and Drug Administration for the treatment of microinvasive lung cancer, obstructing lung cancer, and obstructing esophageal cancer, for premalignant diseases such as actinic keratosis, as well as for non-oncologic conditions such as age-related macular degeneration³. PDT is not only “dynamic” but also multifaceted. There are three principal components of photosensitizer, light, and oxygen ($^3O_2$), all of which interact on time scales relevant to a single treatment. Ground state photosensitizer molecule is excited to its triplet state by

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absorbing a photon with appropriate wavelength. The excited triplet state photosensitizer can undergo two types of photochemical reactions, characterized as type II and type I reactions, which create cytotoxic singlet oxygen ($^1\text{O}_2$) and other reactive oxygen species (ROS) that ultimately result in photodynamic damage². Both type I and type II reactions can occur simultaneously and the ratio between these processes depends on the type of photosensitizer, the concentration of substrate and the availability of oxygen⁴. *In vivo* detection of singlet oxygen luminescence (SOL) has been demonstrated and validated as a dose metric for PDT treatment^{5–7}. However, SOL methods are not applicable for type I photosensitizers since singlet oxygen is not present. An empirical reactive singlet oxygen explicit dosimetry (ROSED) model has been developed to calculate ROS (including $^1\text{O}_2$) based on the temporal and spatial distribution of light fluence rate (ϕ), photosensitizer concentration ($[\text{S}_0]$), and tissue oxygenation uptakes ($[\text{O}_2]$)^{2,8}. Several *in vivo* mouse studies have been performed using type II photosensitizers, (Photofrin^{9,10}, HPPH^{11,12} and BPD^{13,14}) and ROSED calculated $[\text{O}_2]_{\text{rx}}$ values were found to be correlated well with PDT outcomes. The validity of the ROSED in predicting treatment outcome of a type I PDT has not been established.

In this study, ROSED was implemented using measured values of light fluence rate, photosensitizer concentration, $[\text{O}_2]$, and blood flow changes to evaluate the treatment outcomes of Tookad®-mediated PDT in mice bearing radiation-induced fibrosarcoma (RIF) tumors. Tookad® is a vascular targeting photosensitizer which is known to have low quantum yield of singlet oxygen *in vivo* but with high quantum yield of superoxide anion which is a precursor for most other ROS produced in type I reactions. Cure index was computed from the rate of tumor regrowth after treatment and was considered as a measure for PDT treatment effectiveness. ROSED for the prediction of treatment outcome is also compared with those obtained using dosimetry quantities of light fluence and PDT dose. PDT dose was calculated by the integral of ϕ and Tookad® concentration over time.

2. MATERIALS AND METHODS

2.1 Tumor models administered with Tookad®

30 μl of Radiation-induced fibrosarcomas (RIF) cells, at a concentration of 1×10^7 cells/ml, were injected in the right shoulders of 6 weeks old female C3H mice (NCI-Frederick, Frederick). Tumors were treated when they were ~3 mm in diameter. The treatment area was depilated with Nair (Church & Dwight Co., Inc., Ewing, New Jersey, US) and 4 mg/kg Tookad® was injected via tail vein for most mice. For a subset of mice, 1 – 6 mg/kg Tookad® were injected. PDT treatments were performed with different light fluence rates and light doses after a drug-light interval (DLI) of 15 minutes for most mice. Photosensitizer concentration was measured before and after PDT treatment. Tissue oxygenation and blood flow changes were monitored during the course of PDT treatment. 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.

2.2 Measurement of the Tookad® concentration in tumors

Absolute Tookad® concentrations were obtained from diffuse reflectance spectra measured using DRS contact probe, as described elsewhere¹⁵, from the tumor before and after PDT treatment. A multi-wavelength algorithm based on diffusion approximation equation was employed to fit all reflectance spectra between 600 nm to 900 nm simultaneously using multiple source-detector separations to extract $\mu_a(\lambda)$ and $\mu_s'(\lambda)$. Then, the concentration of Tookad® (and other chromophores) were obtained from $\mu_a(\lambda)$ using $\mu_a(\lambda) = \sum_i c_i \epsilon_i(\lambda)$, where $\epsilon_i(\lambda)$ is the extinction coefficient of i th chromophore and $c_i(\lambda)$ is the molar concentration of the i th chromophore. The major chromophores in the spectral region of interest are oxy-hemoglobin (HbO_2), deoxy-hemoglobin (Hb), water, and Tookad®. The extinction coefficient of Tookad® are measured with spectrophotometer and the extinction coefficients of the other three chromophores are obtained from the literature¹⁶. The concentration of Tookad® is reconstructed directly using a nonlinearly constrained optimization method, *fminsearch*, implemented in Matlab and is used for ROSED calculations.

2.3 Measurement of the tumor tissue oxygenation

A third generation, multi-channel dissolved oxygen (pO₂) and temperature monitor (OxyLite Pro, Oxford Optronix, Oxford, UK) with an oxygen-only bare-fiber sensor (fluorescence-based) (NX-BF/O/E, Oxford Optronix, UK) was used to measure the tissue oxygenation pO₂ for each tumor before, during and after the PDT. Then, ³O₂ concentration was calculated by multiplying the measured pO₂ with ³O₂ solubility in tissue, which is 1.295 $\mu\text{M}/\text{mmHg}$ ¹⁷.

2.4 Measurement of the blood flow changes during PDT

A noninvasive optical technology, known as Diffuse Correlation Spectroscopy (DSC), was used to monitor the blood flow changes during PDT^{18,19}. DCS derives flow information by quantifying the temporal intensity fluctuation of multiple scattered light. DCS employs coherent near-infrared light that travels diffusively through tissue and scattered multiple times before emerging from tissue surface. During each scattering event, the phase of the scattered light is altered. Multiple light fields with different phases interfere constructively and destructively at the tissue surface and create a speckle pattern. The dynamic scattering due to the movements of red blood cells in tissues causes the speckle pattern to change in time. As these temporal intensity fluctuations of the detected light are sensitive to the motions of red blood cells in the tissue microvasculature, the DCS signals provides a direct measure of blood flow. Tumor blood flow index (*BFI*) is obtained by fitting the measured intensity autocorrelation function to the solution of the correlation diffuse equation in the semi-infinite geometry.

A noncontact DSC system was used in this study which includes a 785 nm laser source (CrystaLaser Inc., Reno, NV) with long coherence length, operating in continuous mode, one source and four detector fibers mounted at the detector plane of a camera to deliver excitation light and collect reflected diffuse light from the tumor surface. Bandpass optical filters are used to attenuate the treatment light and prevent the detectors from saturation.

2.5 PDT treatment

An optical fiber with a microlens attachment was coupled with a 763 nm diode laser with a maximum output power of 4 W (Biolitec AG, Jena, Germany) to produce a collimated beam with a diameter of 1 cm on the surface of the tumor. Immediately before starting the PDT, Tookad® concentration and initial tissue oxygenation level ($[^3\text{O}_2]_0$) was measured for each mouse. Mice were treated with in-air light fluence rate of 50 to 667 mW/cm² with different treatment time between 333 to 1400 seconds to deliver a total fluence in the range of 30–800 J/cm². Tumor-bearing mice with no Tookad® and no light excitation were used as controls.

2.6 Tumor re-growth after PDT

Tumor volumes were tracked daily after PDT. Width (a) and length (b) were measured with slide calipers, and tumor volumes (V) were calculated using $V = \pi \times a^2 \times b / 6$.²⁰ Since initial tumor volumes at the time of treatment were not identical among mice, daily tracked volumes were scaled relative to an initial normalized tumor volume of $\sim 28.6 \text{ mm}^3$ (the average of all initial tumor volume). This process provided for consistent comparisons among treatment groups. PDT was performed at small tumor volume to ensure complete treatment of the entire tumor (light can penetrate 5–7 mm depth), providing the potential for a curative response. Tumor volume was fitted to an exponential growth equation, $V = Ae^{kd}$ to obtain the tumor regrowth rate (k). A is the initial tumor volume and d represents the number of days after PDT. Tumor cure index (CI) was calculated using $\text{CI} = 1 - (k/k_{\text{control}})$ where k_{control} is the tumor regrowth rate for the control mice without PDT. Cure index is plotted against three different dosimetry quantities, light fluence, PDT dose, and $[\text{ROS}]_{\text{rx}}$ for comparison.

2.7 Simulation of the spatial distribution of ϕ in RIF tumors

The average μ_a and μ_s' for RIF tumor tissue administered with 4 mg/kg Tookad® was measured to be $0.14 \pm 0.03 \text{ cm}^{-1}$ and $9.65 \pm 0.59 \text{ cm}^{-1}$. These values were obtained based on the monitoring of the ϕ profile along a catheter inserted into the tumors from a subset of 5 mice treated in this study. The detailed description of optical measurement technique can be found elsewhere²¹. An empirical six-parameter analytic expression²² was used to calculate the *in vivo* light fluence rate distribution within tumor. Light fluence rate at 3mm was used to calculate light fluence and for ROSED calculation in this study to represent the doses of different metrics that are delivered to the bottom of the tumor.

2.8 Reactive oxygen species explicit dosimetry

PDT process can be described by a set of kinetic equations which can be simplified to describe the creation of $[\text{ROS}]_{\text{rx}}$.² These equations are dependent on the temporal and spatial distribution of ϕ , photosensitizer concentration ($[S_0]$), ground state oxygen concentration ($[^3\text{O}_2]$), oxygen supply rate (g), and the photosensitizer-specific reaction-rate parameters (δ , β , σ , and ξ). The relevant equations are:

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

$$\frac{d[{}^3\text{O}_2]}{dt} = -\frac{[{}^3\text{O}_2]}{[{}^3\text{O}_2] + \beta} \phi[S_0]\xi + g\left(1 - \frac{[{}^3\text{O}_2]}{[{}^3\text{O}_2]_0}\right) \quad (2)$$

$$\frac{d[\text{ROS}]_{rx}}{dt} = \xi \frac{[{}^3\text{O}_2]}{[{}^3\text{O}_2] + \beta} \phi[S_0] \quad (3)$$

For the calculation of $[\text{ROS}]_{rx}$, the spatial distribution of ϕ in tumors obtained using 6-parameter analytic expression, as well as $[S_0]$, $[{}^3\text{O}_2]$, and $[{}^3\text{O}_2]_0$ measured before, during and after the PDT were passed to the Eqs. (1) – (3). The value of parameters σ and g are obtained by fitting the Tookad® concentration and tissue oxygen measured from a separate set of 5 mice using Eqs. (1) and (2), as shown in Figure 1. The value of Tookad® photochemical parameters ξ , β and δ are obtained from the literature (see Table 1) as they are not sensitive in the fitting. Higher dose of Tookad®, 10mg/kg, was administered to these mice for 3 hour drug-light interval, followed by PDT treatment with light fluence rate of 150mWcm^{-2} for 1334 seconds to deliver a total light fluence of 200Jcm^{-2} . Rapid blood clearance has been reported for Tookad®²³. However, the measured Tookad® concentration before and during PDT in these mice shows there is little uptake of Tookad® by tumor.

3. RESULTS

Mice bearing RIF tumor were treated with a total fluence in the range of 30–800 J/cm². The *in vivo* Tookad® concentration, and the changes of the blood flow and tissue oxygenation were measured before, and during PDT. The tissue oxygenation profile (blue solid line) and the spectra of the blood flow changes (red and green lines recorded by using two detectors) during PDT have been shown for two mice in Figs. 2(a) and (b).

The magnitude of blood flow changes, scaled by the conversion factors of 1.5×10^9 , is found to correlate well with the measured tissue oxygen for all mice. High variation in the tissue oxygenation level is observed. The mean initial tissue oxygen level $[{}^3\text{O}_2]_0$ is 26.1 μM with a standard deviation of 20.2 μM .

Figure 3 shows the ratio of light fluence rate on tissue and in tumor to the in-air light fluence rate (ϕ/ϕ_{air}) for 5 mice with the measured tumor optical properties. ϕ/ϕ_{air} was calculated using the six parameters analytic expression for 1-cm beam²². The mean optical properties of the mice are $\mu_a = 0.14 \pm 0.03 \text{ cm}^{-1}$ and $\mu_s' = 9.65 \pm 0.59 \text{ cm}^{-1}$ and the spatial distribution of the average light fluence rate is represented as dashed line in Figure 3. The *in vivo* light fluence rate at 3 mm depth is found to be ~2.13 times higher than the in-air light fluence rate at the mean tumor optical properties.

To understand the pharmacokinetics of Tookad® in mice tumor, we injected 4 RIF-bearing mice with 10mg/kg Tookad® via tail vein and measured the *in vivo* PS concentration in tumor over 3 hours. The mice received no PDT light illumination and the reduction in

measured Tookad® is solely due to blood clearance and not photobleaching. Figure 4 demonstrates the rapid blood clearance of Tookad® concentration in control mice and the average Tookad® concentration at 3 hours after injection is $0.36 \pm 0.13 \mu\text{M}$.

Figure 5 shows the temporal changes of Tookad® concentration and $[\text{ROS}]_{\text{rx}}$ during PDT calculated using ROSED for RIF tumor of mouse #5-5. The measured initial Tookad® concentration and $[\text{}^3\text{O}_2]$ were used in the calculation, and the values of the photochemical parameters used are presented in table 1.

Figs. 6(a), (b) and (c) show the comparison of fluence, PDT dose and $[\text{ROS}]_{\text{rx}}$ as dose metric against cure index for 23 mice that received 4mg/kg of Tookad® 15 minutes before PDT. The dashed lines show the best fit to the data with functional form $\text{CI} = 1.018/(1+15.69e^{-0.0039x})$, $\text{CI} = 1.056/(1+16.95e^{-0.00845x})$, and $\text{CI} = 1.014/(1+57.78e^{-0.1947x})$ with $R^2 = 0.7534, 0.7751$ and 0.9729 for (a), (b) and (c), respectively.

Figs. 7(a), (b) and (c) show the comparison of fluence, PDT dose and $[\text{ROS}]_{\text{rx}}$ as dose metric against cure index for 23 mice injected with 4mg/kg of Tookad® and an additional 5 mice that received different Tookad® dose at 1, 2 and 6 mg/kg, as tabulated in table 2. The dashed lines show the best fit to the data with functional form $\text{CI} = 1.1/(1+11.71e^{-0.0026x})$, $\text{CI} = 1.058/(1+16.63e^{-0.0084x})$, and $\text{CI} = 1.017/(1+50.98e^{-0.1873x})$ with $R^2 = 0.6101, 0.7872$ and 0.9712 for (a), (b) and (c), respectively.

Table 2 summarized the treatment parameters for 28 mice. In-air light fluence rate was measured using isotropic detector and light dosimeter and the light fluence rate at 3 mm depth was calculated by multiplying the in-air fluence rate with a conversion factor of 2.13. Light fluence delivered at 3mm depth was calculated and used as a metric dose. PDT dose at 3 mm was calculated by the integral of the Tookad® concentration and $\phi_{3\text{mm}}$ over time. $[\text{ROS}]_{\text{rx}}$ at 3 mm were calculated using measured Tookad® concentration and tissue oxygenation $[\text{}^3\text{O}_2]$.

4. DISCUSSION AND CONCLUSION

Fluence, PDT dose, and $[\text{ROS}]_{\text{rx}}$ at 3 mm were compared as dosimetric quantities to estimate the outcome of Tookad®-mediated PDT for RIF tumors on a mouse model. The outcome was evaluated by the calculation of CI. No tumor regrowth up to 14 days after treatment resulted in a CI of 1. Figs 6(a), (b) and (c) show that cure index for mice that received 4mg/kg of Tookad® (N=23) can be fitted to a sigmoidal function when plotted against fluence, PDT dose and $[\text{ROS}]_{\text{rx}}$ with $R^2 = 0.7534, 0.7751$ and 0.9729 , respectively. Both fluence and PDT dose are reasonably good dosimeter quantities for predicting PDT outcome as shown in Figs. 6(a) and (b) with comparable R^2 values. The measured *in vivo* PS concentrations before PDT are found to be highly consistent across all 23 mice injected with the same concentration of Tookad® (4mg/kg). The mean *in vivo* Tookad® concentration for mice 15 minutes after injection of 4 mg/kg of Tookad® is $1.36 \pm 0.22 \mu\text{M}$. Due to the small variation in *in vivo* PS concentration, PDT dose does not show profound improvement in the treatment outcome prediction as compared to fluence alone. Among the three dose metrics,

$[\text{ROS}]_{\text{rx}}$ is the best dosimetry quantity in predicting treatment outcome, with a goodness of fit R^2 of 0.9729 as shown in Fig. 6(c), as variations in tissue oxygen are taken into account.

To test the our model calculation in a more clinically relevant situation, we performed PDT treatment on 5 mice which received different amount of Tookad® at 1, 2, and 6mg/kg to introduce heterogeneity in the *in vivo* PS concentrations. These mice are treated after 15 minutes DLI and with different treatment parameters as listed in table 2. Figs. 7(a), (b) and (c) show the comparison of fluence, PDT dose and $[\text{ROS}]_{\text{rx}}$ at 3 mm as dosimetric quantities to estimate treatment outcome of Tookad®-mediated PDT for all mice (N=28). The treatment outcome prediction using fluence alone is worsen due to the heterogeneity in Tookad® concentration as the goodness of fit reduces from $R^2 = 0.7534$ (Fig. 6(a)) to $R^2 = 0.6101$ (Fig. 7(a)). On the other hand, the variation in the Tookad® concentration has not affected PDT dose as dosimetric quantity to predict PDT treatment outcome. The goodness of fit of Fig. 6(b) and Fig. 7(b) are comparably good with $R^2 = 0.7751$ and 0.7872, respectively. This result suggests that PDT dose is a better dosimetric quantity than light fluence alone when there is heterogeneity in *in vivo* PS concentration. Similar to Fig. 6(c), Fig. 7(c) show that $[\text{ROS}]_{\text{rx}}$ is the best dosimetric quantity, with little to no change in R^2 values (0.9729 and 0.9712). Our results demonstrate that light fluence could be use as a dose metric for vascular targeting PS with short DLI, in which the variation in the *in vivo* PS concentration is rather small and highly correlated to the amount of PS administered. PDT dose is a better dosimetry quantity than light fluence alone when the information of tissue oxygenation are not available as it takes into account the variation in *in vivo* PS concentration due to the heterogeneity in pharmacokinetics and tumor uptakes. $[\text{ROS}]_{\text{rx}}$ is the best dosimetric quantity. $[\text{ROS}]_{\text{rx}}$ is the best dosimetric quantity as the three principal components of PDT processes, the light, PS and oxygen, are all included in the model calculation of the reacted reactive oxygen species.

The results of Figs. 6 and 7 indicate that $[\text{ROS}]_{\text{rx}}$ is the most effective dosimetric quantity to predict PDT treatment outcome. Threshold dose can be estimated from Figs. 6(c) and 7(c) when the CI = 0.5. The mean $[\text{ROS}]_{\text{rx}}$ threshold concentration for this study is 20.81 ± 1.75 mM. This value is about 20 times higher than those published threshold $[\text{}^1\text{O}_2]_{\text{rx}}$ values for Photofrin (1.1 mM) [10], HPPH (1.0 mM) [12], and BPD (1.0 mM) [14]. This study is the first time that a threshold $[\text{ROS}]_{\text{rx}}$ value is computed and directly compared with published threshold $[\text{}^1\text{O}_2]_{\text{rx}}$ values in the literature. Given that vascular targeting photosensitizers are known to be more potent than tissue targeting photosensitizers in term of PDT treatment outcome, higher threshold dose requires by Tookad®-mediated PDT, which is a type I photosensitizer, suggest that ROS is >20 times less effective than $\text{}^1\text{O}_2$ in inflicting tissue damage to the targeted tumors. However, it should be pointed out that the values of the photochemical parameters used in the calculation of $[\text{ROS}]_{\text{rx}}$ in this study are adopted from the literature as tabulated in table. 1. These numbers are based on theoretical calculations and speculations and there is so far very limited literature that reported these values in the *in vivo* and clinical relevant environment. Future works should be focused on identifying these parameters for all photosensitizers and clinical conditions to establish ROS explicit dosimetry as a reliable method in PDT dosimetry.

5. CONCLUSION

Although PDT is an established modality for the cancer treatment, the medical application of this technique has been limited due to inaccurate PDT dosimetric methods. In this study, we suggest a calculated $[\text{ROS}]_{\text{rx}}$ based on the explicit dosimetry of the photosensitizer concentration, tissue optical properties, tissue oxygenation level, and blood flow changes during PDT to predict treatment outcome of a Tookad®-mediated PDT, in which the primary cytotoxic agent is superoxide anions produced by Type I photochemical reactions. Assessing tumor cure index across three dosimetric quantities of light fluence, PDT dose, and $[\text{ROS}]_{\text{rx}}$ in RIF tumor model demonstrates that $[\text{ROS}]_{\text{rx}}$ is the most reliable dosimetric quantity. The threshold dose of PDT dose $[\text{ROS}]_{\text{rx}}$ to achieve CI = 0.5 is found to be 20.81 ± 1.75 mM which is approximately 20 times larger than other photosensitizers which produce $^1\text{O}_2$ as the primary cytotoxic agent through Type II photochemical reactions. PDT dose can be a good dose metric if tissue oxygenation or blood flow information is not available.

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Figure 1.

(a) Measured and model calculated (a) Tookad® concentration and (b) tissue oxygen from 5 mice injected with 10mg/kg Tookad®, 3 hours DLI and treated with 150 mWcm^{-2} , for 1334 seconds to a total light fluence of 200 Jcm^{-2} . The values of photochemical parameters ξ , σ , and δ obtained from [2] are used to fit for σ and g as shown in Table 1.

Figure 2.

Correlation of the blood flow spectrum (blue line) and concentration of the tissue oxygenation (red line) during Tookad®-mediated PDT for (a) mouse 1-2 and (b) mouse 4-2. Blood flow spectra in (a) and (b) have been scaled by the conversion factors of 1.5×10^9 .

Figure 3.

Mathematical calculation of light fluence rate, ϕ/ϕ_{air} on tissue surface and in tumor for 5 mice with different measured optical properties. The dashed line represents the spatial distribution of ϕ/ϕ_{air} at the mean optical properties. 'X' represents the ratio of measured light fluence rate on tissue surface to in-air light fluence rate, ϕ_{air} .

Figure 4. Temporal changes of PS concentration measured from 4 RIF-bearing mice injected with 10mg/kg Tookad® and received no PDT treatment.

Figure 5.

Mathematical simulation of the (a) temporal changes of Tookad® concentration and (c) corresponding temporal changes of the reactive singlet oxygen concentration during PDT in a RIF tumor. Tookad® concentration and $[\text{ROS}]_{\text{rx}}$ was simulated based on the measured tissue oxygenation of mouse 5-5.

Figure 6.

CI plotted against (a) fluence at 3 mm tumor depth, (b) calculated PDT dose at 3 mm tumor depth, and (c) reacted reactive oxygen species at 3 mm depth ($[\text{ROS}]_{\text{rx}}$). All mice (N=23) received initial Tookad® injection of 4mg/kg 15 minutes before PDT.

Figure 7.

CI plotted against (a) fluence at 3 mm tumor depth, (b) calculated PDT dose at 3 mm tumor depth, and (c) reacted reactive oxygen species at 3 mm depth ($[\text{ROS}]_{\text{rx}}$). 23 mice received initial Tookad® injection of 4 mg/kg and 5 mice received initial Tookad® injection of 1, 2 and 6 mg/kg, 15 minutes before PDT.

Table 1

Photochemical parameters of Tookad®-mediated PDT used in the reactive oxygen species explicit dosimetry.

Parameters	Definition	Value	References
ξ (cm ² s ⁻¹ mW ⁻¹)	Specific oxygen consumption rate	122×10^{-3}	[2]
β (μM)	Oxygen quenching threshold concentration	11.9	[2]
δ (μM)	Low concentration correction	33	[2]
σ (μM ⁻¹)	Specific photobleaching ratio	2.6×10^{-5}	This study
g (μM/s)	Macroscopic oxygen maximum perfusion rate	3	This study

Table 2

In-air light fluence rate, light fluence rate and fluence at 3mm, treatment time, administered Tookad® dose, *in vivo* Tookad® concentration, initial tissue oxygenation, PDT dose, calculated reactive oxygen species concentration and cure index.

Mouse #	Φ_{ir} (mW/cm ²)	Φ_{3mm} (mW/cm ²)	Fluence at 3mm (J/cm ²)	Time (s)	[Tookad®] injected (mg/kg)	[Tookad®] ₀ (μM)	[³ O ₂] ₀ (μM)	PDT dose (μMJ/cm ²)	[ROS] (mM)	Cure index
4-2	162	345.1	114.9	333	4	1.57	5.4	112.2	4.3	0.12
4-3	164	349.3	116.3	333	4	1.34	1.0	97.9	0.9	0.12
4-4	152	323.8	107.8	333	4	1.12	3.8	77.2	2.3	0.04
4-1	163	347.2	115.6	333	4	1.62	40.8	127.0	12.0	0.13
1-5	160	340.8	113.5	333	4	1.68	43.0	125.4	12.0	0.12
2-4	52	110.8	66.5	600	4	1.43	10.4	45.5	2.6	0.11
3-3	53.3	113.5	68.1	600	4	1.58	1.7	46.8	0.7	0.11
2-5	55.4	118.0	70.8	600	4	1.46	9.8	48.2	2.7	0.04
3-2	52.4	111.6	67.0	600	4	1.27	43.8	41.0	3.9	0.04
4-5	53.3	113.5	68.1	600	4	1.31	42.8	42.2	4.0	0.06
5-1	453	964.9	964.9	1000	4	1.22	10.4	402.3	22.9	0.47
5-2	406	864.8	864.8	1000	4	1.43	55.9	446.3	44.9	1
5-4	397	845.6	845.6	1000	4	1.50	59.8	432.3	44.0	1
5-5	386	822.2	822.2	1000	4	1.31	3.1	373.1	9.5	0.15
6-2	565.3	1204.1	1444.9	1200	4	1.61	29.9	647.6	56.5	1
6-3	672.4	1432.2	1718.7	1200	4	1.35	9.5	731.4	39.6	1
6-4	678.2	1444.6	1733.5	1200	4	1.46	43.6	735.3	70.5	1
6-5	683.2	1455.2	1746.3	1200	4	1.43	53.7	719.8	71.9	1
7-1	297	632.6	632.6	1000	4	1.25	31.2	238.4	21.1	0.57
7-2	305	649.7	649.7	1000	4	1.34	47.6	261.4	25.5	0.90
7-3	310	660.3	660.3	1000	4	1.43	11.4	302.7	18.1	0.30
7-4	289	615.6	615.6	1000	4	1.27	27.3	231.0	19.6	0.43
7-5	303	645.4	645.4	1000	4	1.22	6.0	252.5	10.3	0.11
8-2	337	717.8	717.8	1000	2	0.41	39.7	99.8	9.4	0.15
8-3	404	860.5	1075.7	1250	2	0.38	56.8	116.8	11.8	0.15

Mouse #	ϕ_{ir} (mW/cm ²)	ϕ_{3mm} (mW/cm ²)	Fluence at 3mm (J/cm ²)	Time (s)	[Tookad®] injected (mg/kg)	[Tookad®] ₀ (μM)	³ O ₂ ₀ (μM)	PDT dose (μMJ/cm ²)	[ROS] (mM)	Cure index
8-5	135	287.6	383.6	1334	6	1.77	28.6	185.3	16.0	0.21
9-4	253.2	539.3	755.0	1400	2	0.76	31.5	139.1	12.3	0.2
9-5	251.8	536.3	750.9	1400	1	0.29	16.9	56.7	4.1	0.10