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In-vivo singlet oxygen dosimetry of clinical 5-aminolevulinic acid photodynamic therapy

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Abstract

Photodynamic therapy (PDT) is a viable treatment option for a wide range of applications, including oncology, dermatology, and ophthalmology. Singlet oxygen is believed to play a key role in the efficacy of PDT, and on-line monitoring of singlet oxygen during PDT could provide a methodology to establish and customize the treatment dose clinically. This work is the first report of monitoring singlet oxygen luminescence *in vivo* in human subjects during PDT, demonstrating the correlation of singlet oxygen levels during PDT with the post-PDT photobiological response.

Keywords

photodynamic therapy; singlet oxygen; luminescence

Photodynamic therapy (PDT) is a viable treatment option for a variety of applications, including oncology, dermatology, and ophthalmology.[1] In particular, 5-aminolevulinic acid (ALA)-PDT is widely used to treat a range of dermatologic conditions.[2] PDT is based on the interaction of a photosensitizer (PS), light, and oxygen, in which photoactivation of PS generates cytotoxic molecular species. Customized dosimetry could, in principle, impact the efficacy of treatment outcome and of the effective use of resources. Dosimetry in PDT is complex, as the treatment effect is generated by an interaction of multiple components.

A number of dose metrics have been evaluated to monitor the outcome of ALA-PDT in dermatological treatment.[3,4] Since singlet oxygen ($^1O_2$), which phosphoresces to the ground triplet state, is believed to be a key cytotoxic species in PDT,[1] its direct monitoring is of great interest. Until now, *in-vivo* optical detection of $^1O_2$ luminescence at 1270 nm remained elusive because of its low signal yield. In recent pioneering works, Niedre et al.[5] and Yamamoto et al.[6] measured PDT-generated $^1O_2$ luminescence *in vivo* in mice, and demonstrated its correlation with treatment outcome. Recently, we have developed a fiber-

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based $^1O_2$ monitoring device[7] that is compatible with human studies. We present the first clinical trial measuring PDT-generated $^1O_2$ levels in skin of healthy volunteers before and after ALA application, and correlating the $^1O_2$ luminescence signal with the photobiological effects of ALA-PDT, possibly indicating treatment outcome.

A total of 18 healthy subjects (14 males and 4 females) were enrolled with written, informed consent in the clinical study approved by our internal Institutional Review Board to ensure adherence to the Declaration of Helsinki protocols. Power analysis indicated that the sample size of 18 subjects provided 80% power ($\alpha = 0.05$, $\beta = 0.20$) to detect a significant linear relationship between the change in $^1O_2$ luminescence signal and photobiological skin response following PDT using repeated-measures mixed model regression analysis (version 6.0, nQuery Advisor, Statistical Solutions, Saugus, Massachusetts). A newly developed, entirely fiber-based $^1O_2$ dosimeter was used to detect $^1O_2$ luminescence *in vivo*.[7] The device uses a low power, time-resolved diode laser of 635 nm (10-kHz repetition rate, 5-μs pulse duration, less than 1 μJ/pulse) for PS activation. $^1O_2$ signal was measured at the end of each laser pulse to reduce background fluorescence signal. Simultaneously, three optical signal strengths at 1.22, 1.27, and 1.315 μm are recorded using narrow bandpass filters and a photon multiplier tube (PMT) (Hamamatsu H9170-45) with a fast photon counter (model MSA-300, Becker and Hickl, Berlin). The average signal from 1.22 and 1.315 is used to estimate the background signal and then subtracted from the $^1O_2$ signal at 1.27 μm to eliminate back-ground noise. Measurements typically take only a few seconds.

Each subject had two treatment sites on the right upper arm outlined and randomly assigned for ALA-PDT (Levulan Kerastick, DUSA Pharmaceuticals, Wilmington, Massachusetts) with either one-hour (I_{short}) or three-hour (I_{long}) incubation periods. ALA incubation time was varied to control PS accumulation, keeping in mind that longer incubation times result in increased PpIX levels,[8] leading to different $^1O_2$ production and treatment outcomes. $^1O_2$ measurements were done before ALA application (pre-ALA) when no exogenous PS was administered and immediately before the therapeutic light dose (pre-PDT) following PpIX production from ALA application.

Following $^1O_2$ measurements, each treatment site was irradiated with a therapeutic light dose of 20 J/cm^2 (irradiance 100 mW/cm^2) with a separate fiber-based continuous diode laser at 635 nm (HPD 7401, High Power Devices, North Brunswick, New Jersey).

Standardized digital photographs of the treatment sites were taken within 15 min and at 24 h after this treatment and used for the evaluation of phototoxic reactions by four blinded investigators. Each investigator rated the degree of erythema and edema between a scale of 0 (no response) to 10 (most pronounced). Median of the four readings is reported to minimize any potential interobserver variability. The photobiological response to the I_{long} treatment sites were more pronounced and more clinically significant compared to I_{short} treatment sites (Fig. 1). Consistent with Ref. [9], edema and erythema response peaked at 15 min and 24 h following PDT, respectively. Subsequent analysis was performed using the maximal photobiological skin response: acute edema at 15-min post-PDT and erythema at follow-up 24-h post-PDT. PS-induced $^1O_2$ signal was defined by change in $^1O_2$ luminescence between pre-ALA (time of minimum PpIX concentration) and pre-PDT (time of maximum PpIX concentration) time points.

Several researchers have shown that the degree of erythema and edema following ALA-PDT is predictive of clinical outcome in various dermatologic conditions.[4,10] To evaluate the relationship between $^1O_2$ signal and photobiological skin responses (edema and erythema), we applied repeated-measures linear mixed model analysis.[11] Using this model, the $^1O_2$

luminescence signal significantly correlated with acute edema in I_{long} sites (P=0.01) and 24-h follow-up erythema for I_{long} and I_{short} combined (P=0.028) (Table 1).

Equality of slopes for I_{short} and I_{long} sites was assessed with an F-test to decide whether a common slope parameter could be modeled to describe this correlation.[12] The relationship between erythema and $^1O_2$ signal showed no significant difference in slopes between I_{short} and I_{long} sites (P=0.527). As a result, correlation between follow-up erythema and $^1O_2$ signal was investigated with a common slope fitted to the combined dataset for the I_{short} and I_{long} sites (Fig. 2). The interpretation is that the percent change in $^1O_2$ signal from baseline is positively correlated for both sites in a similar way, and thus can be described using a common slope.

On the other hand, the correlation between acute edema and $^1O_2$ signal was modeled separately for I_{short} and I_{long} sites because they had unequal slopes (P=0.009) (Fig. 2). In fact, greater edema was significantly correlated with greater percent change from baseline in $^1O_2$ signal but only for the I_{long} sites (P=0.01), not I_{short} sites (P=0.747) (Table 1). Interestingly, only I_{long} sites showed a significant correlation between $^1O_2$ signal and acute edema (Fig. 2). It is possible that the minor acute edema at the I_{short} sites (median score of 0.25 out of 10) may not have been clearly discernable by the blinded investigators in a 2-D photograph, and thus may not have correlated significantly with the quantitative $^1O_2$ signal.

Unexpectedly, a decrease in $^1O_2$ signal after the incubation period was observed in some treatment sites. This might be related to modification of skin optical properties by ALA and its carrier.[13] A carrier-only control treatment site could potentially provide additional information when incorporated in future studies.

This is the first study showing the feasibility of monitoring PS-generated $^1O_2$ signal *in vivo* in human subjects. $^1O_2$ signal measured immediately prior to PDT light irradiation correlated significantly with the photobiological response to ALA-PDT in normal skin. As a result, monitoring $^1O_2$ production in the skin is predictive of the clinical ALA-PDT outcome and is a helpful tool for customizing the clinical ALA-PDT treatment.

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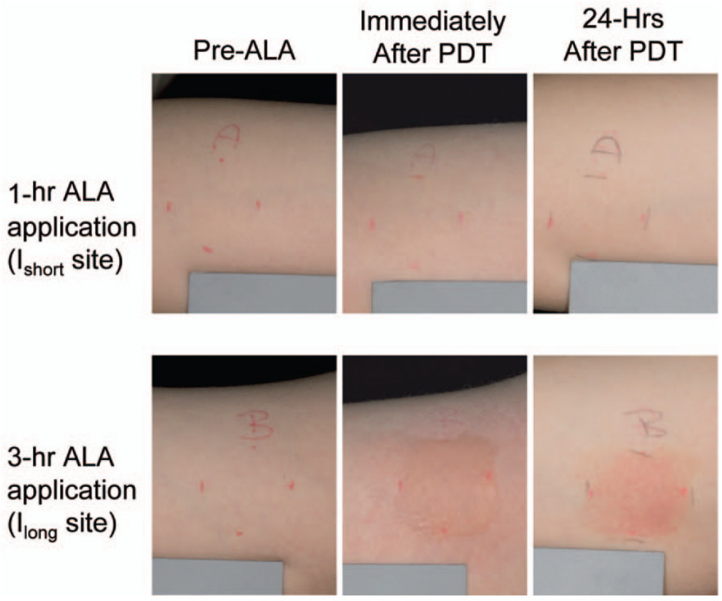

Fig. 1.

Representative images of treatment sites with 1 h (I_{short}) or 3 h (I_{long}) of ALA application time. The phototoxic reaction in I_{long} sites (edema immediately after PDT and erythema 24 h afterward) is more intense than in I_{short} sites.

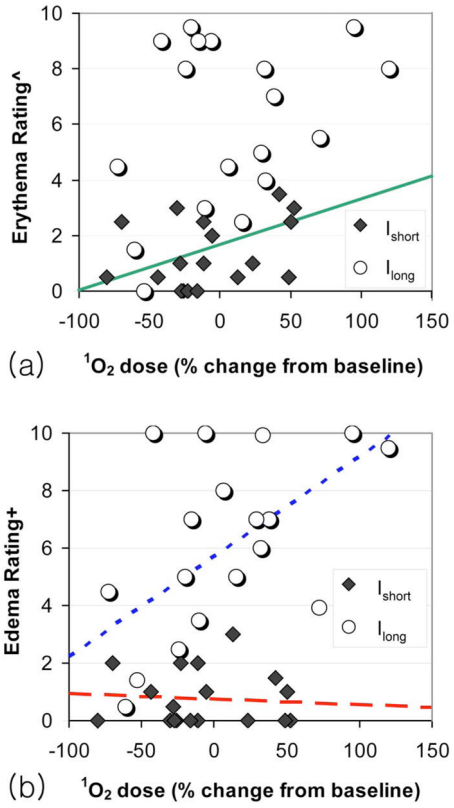

Fig. 2.

Scatter plots illustrating the relationship between $^1O_2$ luminescence signal versus (a) acute edema rating and (b) erythema rating 24 h after PDT. Regression-based fits to the I_{short}, I_{long}, and combined data are shown by the red dashed line, the blue dotted line, and the solid green line, respectively. (Color online only.)

Table 1

Effect of $^1O_2$ signal on photobiological responses based on a linear mixed model regression analysis of the data. Slope of the linear correlation between $^1O_2$ signal and photobiological response in either I_{short} sites, I_{long} sites, or the combined data are reported. Edema is measured at post-PDT; erythema is measured at follow-up. The slope test shows the comparison of slopes estimated from I_{short} sites and I_{long} sites using an F-test. ST is statistically significant. CI=confidence interval.

	Edema[+]		Erythema[×]	
$^1O_2$ signal	**Slope (95% CI)**	***P* value**	**Slope (95% CI)**	***P* value**
Slope test[†]		0.009 (ST)		0.527
I_{short} sites only	−0.002 (−0.015, 0.011)	0.747		
I_{long} sites only	0.035 (0.011, 0.059)	0.010 (ST)		
Combined sites			0.017 (0.002, 0.031)	0.028 (ST)