Triplet-State Lifetime Measured by Time-resolved Luminescence from $^1\text{O}_2$ Generated by PDT under High-Intensity Pulsed Excitation

Kensuke MASUDA, 1 Sayaka OHMORI, 1 and Tsunenori ARAI1

1 Keio University, School of Fundamental Science and Technology, Graduate School of Science and Technology, 3-14-1 Hiyoshi, Kouhoku-ku, Yokohama, Kanagawa 223-8522

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To estimate the triplet state lifetime of Photodynamic therapy (PDT) under pulsed excitations, we measured the photosensitizer triplet state lifetimes by time resolved measurement of the singlet oxygen generated by PDT using the clinical photosensitizers, Talaporfin and Photofrin II(R). By fitting the measured curve to nonlinear least-squares statistics, the photosensitizer triplet state lifetime was obtained. For Talaporfin, the triplet state lifetime measured at a high intensity of 1.67 MW/cm$^2$ was 4.3 times longer than that measured at a low intensity of 0.20 MW/cm$^2$. In the case of Photofrin II, the measured lifetime was increased to 1.1 times. These results indicated that there might be oxygen depletion at high pulsed intensities in the case of Talaporfin, which was not observed with Photofrin II.

Key Words: Photodynamic therapy, Triplet-state lifetime, Singlet oxygen luminescence, Pulsed excitation, Clinical photosensitizers

1. Introduction

Photodynamic therapy (PDT) is minimally invasive treatment for cancer and other diseases.1) The main cytotoxic agent in PDT is singlet oxygen ($^1\text{O}_2$).2,3) Therefore, it is important to measure density of dissolved oxygen around the photosensitizer.4) In PDT, $^1\text{O}_2$ is produced by the following Type-II photochemical pathway5):

\[ S_0 + h\nu \rightarrow S_1 \]  
\[ S_1 \rightarrow T_1 \]  
\[ T_1 + ^3\text{O}_2 \rightarrow S_0 + ^1\text{O}_2 \]

where, $S_0$, $S_1$, $T_1$ are the photosensitizer ground state, the first excited singlet state and the first excited triplet state, respectively. $^3\text{O}_2$, $^1\text{O}_2$ are the ground state and the excited singlet state of oxygen, respectively. If the lifetime of the first excited triplet state density ($T_1$) was obtained, we could estimate the density of the dissolved oxygen under PDT. To investigate the behavior of dissolved oxygen under pulsed excitation, we measured luminescence from the singlet oxygen and obtained the lifetime of $T_1$: $\tau_T$. Rate equations of PDT could be expressed as the following formula from reaction of (1)-(3)5):

\[ \frac{d[S_0]}{dt} = \phi S_0 - \frac{1}{\tau_T}[S_0] [S_0] \]  
\[ \frac{d[T_1]}{dt} = \frac{[S_1]}{\tau_T} - \frac{1}{\tau_T}[T_1] \]  
\[ \frac{d[^1\text{O}_2]}{dt} = \frac{[T_1]}{\tau_T} - \frac{1}{\tau_T}[^1\text{O}_2] \]

where, $\phi$ is the local fluence rate (photons per second per square centimeter), $\sigma$ is the photosensitizer ground state absorption cross-section (cm$^2$), $\Phi_T$ is the photosensitizer triplet state quantum yield, $\Phi_{^1\text{O}_2}$ is the singlet oxygen quantum yield. $\tau_S$ is the photosensitizer singlet-state lifetime, $\tau_T$ is the photosensitizer triplet-state lifetime and $\tau_D$ is the singlet oxygen lifetime. For an excitation pulse, $\phi = N\delta(t)$, where $N$ is number of photons per square centimeter at time $t = 0$, the singlet oxygen density at time $t$ is showed as follows.6)

\[ \frac{d[S_0]}{dt} = \phi [S_0] - \frac{1}{\tau_T}[S_0] \]

This equation approximates the singlet oxygen luminescence time resolved curve under $\tau_{\text{pulse}} \ll \tau_T$ and $\tau_D < \tau_T$, where $\tau_{\text{pulse}}$ is pulsedwidth of the incident light. By fitting the rising curve with nonlinear least-squares method, we obtained photosensitizer triplet state lifetime.

2. Material and method

Figure 1 shows the experimental set-up for the singlet oxygen detection system. Talaporfin (Meiji Seika Kaisha LDT.) and Photofrin II(R) (Takeda Pharmaceutical Co. Ltd.) were used for photosensitizers. The photosensitizer solutions were prepared by dissolving at the concentration of 45 $\mu$M in culture medium of RPMI-1640 without phenol red (Invitrogen Corp., Carlsbad CA, USA) supplemented with 10 % fetal bovine serum. A XeCl excimer laser pumped dye laser (EDL-1, Hamamatsu Corp., Japan) of which wavelength was adjusted to 666 nm for Talaporfin and 630 nm for Photofrin II, was employed as a light source. The photosensitizer solutions were kept in a plastic dish and were irradiated by the pulse peakpower density ranging from 0.2 to 1.7 MW/cm$^2$ with the pulsed repetition rate fixed at 80 Hz. The
luminescence from $^1\text{O}_2$ to $^3\text{O}_2$ transition appeared around the wavelength of 1270 nm.

The IR luminescence of the singlet oxygen was measured by a liquid nitrogen-cooled photomultiplier tube (R5509-43, Hamamatsu Corp., Japan). The IR-PMT output was amplified by pre-amplifier (20 dB, -50 MHz, C6438, Hamamatsu Corp., Japan) and connected to multi channel scaler (SR430, Stanford Research Systems,) for time-resolved single photon counting with 2000 times integration. The luminescence was measured from 1220 nm to 1320 nm in wavelength with the resolution of ± 1 nm. The sampling field was 2.3 mm² on the surface of the solution with 1.1 mm in depth. We observed quenching of measured luminescence at 1270 nm in wavelength putting sodium azide solution in the photosensitizer solution. By fitting the rising curve with nonlinear least-squares method, we obtained photosensitizer triplet state lifetime.

3. Results and discussion

Figure 2 shows the 1270 nm time-resolved luminescence of singlet oxygen for Talaporfin and Photofrin II. The peak time of the luminescence were 4.3-5.2 µs after excitation, and the decay-lifetime were 10-20 µs. Figure 3 shows the time integrated (0-80 µs) spectrum for each photosensitizer. The measured curve could be explained by the addition of singlet oxygen luminescence (90 %) and photosensitizer phosphorescence curve (10 %) in case of Talaporfin. Same dependence was appeared in case of Photofrin II, at the ratio of 3:1. Then, we fitted the measured curve with equation (8), which is the addition curve of singlet oxygen luminescence and the tail of photosensitizer phosphorescence, by nonlinear least-squares method.

$$f(t) = A \left[ \exp\left(\frac{-t}{\tau_d}\right) - \exp\left(\frac{-t}{\tau_T}\right) \right] + B \exp\left(\frac{-t}{\tau_T}\right)$$  (8)

The best fits of equation (8) are showed in Fig. 2. The correlation coefficient of the measured curve and the fitted curve was 0.91, and standard error of the mean was ± 3.7 photon counts. Figure 4 shows the peakpower density dependence of photosensitizer triplet state lifetime. In case of Talaporfin, photosensitizer triplet state lifetime at high intensity (1.67 MW/cm²) was 4.3 times longer than those at low intensity (0.20 MW/cm²). In case of Photofrin II, lifetime was increased 1.1 times. Based on Stern-Volmer equation, this lifetime stretch may corresponds to 71 % and 29 % of the oxygen density decrease in case of Talaporfin and Photofrin II, respectively. There might be oxygen depletion at high pulsed intensity. The difference of reduction degree of oxygen density could be explained by the absorbance of photosensitizers. Absorbance of Talaporfin and Photofrin II for excitation wavelength are 0.68 cm⁻¹, 0.11 cm⁻¹ at 45 µM.

4. Conclusion

By measuring the time-resolved singlet oxygen luminescence, we obtained the photosensitizer triplet state lifetime under the pulsed excited PDT. There might be oxygen depletion at high pulsed intensity in case of Talaporfin rather than Photofrin II.
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References