Basic study on pulse-intensity-domain depth-controlled Photodynamic Therapy for transurethral prostate cancer

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ABSTRACT

Photodynamic therapy (PDT) is promising modality for cancer. Prostate cancer is the most common cancer in USA. We proposed transurethral prostate cancer treatment using the pulse-intensity-domain depth-controlled PDT to preserve urethra wall. We have found that photocytotoxicity has been suppressed under high-intensity pulsed excitation with the second generation photosensitizers. We aim to apply this effect to form intact portion on the surface of the irradiated field. Irradiation condition dependence of photocytotoxicity of rat prostate cancer cell line R3327-AT-1 was investigated with two clinical photosensitizers, Porfimer sodium and Talaporfin sodium. A pulsed laser was irradiated with the power energy density ranging from 1.25 to 10 mJ/cm^2. Near-infrared luminescence from singlet oxygen in the solution of those two photosensitizers was measured transiently. We performed PDT against a rat subcutaneous prostate tumor mode with Talaporfin sodium (2mg/kg) injected intravenously 1 h prior to the irradiation. The laser was irradiated with the power energy density 2.5 or 10 mW/cm^2, with the total fluence of 50 J/cm^2. Photocytotoxicity *in vitro* and the singlet oxygen generation were both suppressed with the 10mJ/cm^2 irradiation with Talaporfin sodium, while these with Porfimer sodium were kept relatively constant. The surface of the irradiated field of 1mm in thickness remained intact, while the tumor damaged layer of 1.3 mm in thickness was obtained in the case of 10mJ/cm^2 irradiation. We think Talaporfin sodium has high sensitivity to the pulse energy density, which might be useful to realize urethra preserved PDT for prostate cancer.

Keywords: pulsed excitation, high intensity, surface prevention, prostate cancer

1. INTRODUCTION

Photodynamic Therapy (PDT) is the photochemical treatment for various malignant tumors and lesions with neovascularization [1]-[3]. This therapy is based on the cell and/or vascular damage by singlet oxygen, which is generated when the photosensitizer accumulated in a lesion is excited by the light source with the proper wavelength. Porfimer sodium is the most popular photosensitizer approved worldwide. This photosensitizer has been applied various malignant tumors [4]-[6]. It takes 48 – 72 h to establish selective accumulation of photosensitizer in a lesion, besides, 1 month to excrete from a body. Long hospitalization term is required to avoid severe sunlight sensitivity, which has been main issue to prevent this therapy from being popular. The hydrophilic new photosensitizers have been developed to reduce the excretion term [7] [8]. Talaporfin sodium (mono-L-aspartyl chlorin e_6: NPe6) is one of these photosensitizers approved for early lung cancer with a continues diode laser in Japan in 2004 [9] and under clinical trial in U.S.A [10]. Talaporfin sodium is hydrophilic chlorin photosensitizer derived from chlorophyll and has high photosensitizing properties *in vitro* and *in vivo* [11]-[13]. It reported to localize in lysosome in cells [14]. Absorbance peak of Talaporfin sodium is at 664nm to obtain PDT effect at deeper portion, while that of Porfimer sodium was around 630 nm. The excretion term of Talaporfin is 2 weeks, which is a half of the excretion term of Porfimer sodium of 1 month. Regarding to a light source of PDT, the effectivenesses of continuous wave and pulsed lasers have been discussed [15]-[18]. One of the important advantages in pulsed laser irradiation is to treat deeper portion by high-intensity photonflux [19]. The internal energy kinetics of PDT with pulsed irradiation is sill unclear and implies to have complex mechanism. One of the authors reported that the surface layer of a rat subcutaneous renal tumor model remained intact with over MW/cm^2 of high pulsed peak power irradiation PDT with chlorin photosensitizer PAD-S31, while the deeper portion of the tumor was well treated [20]. This photocytotoxicity suppression induced by the high-intensity irradiation may be available to control treatment depth of PDT to preserve healthy internal wall of a hollow organ.

We studied photocytotoxicity suppression under the high-intensity pulsed irradiation with clinical photosensitizers. We performed *in vitro* cell experiment with Porfimer sodium and Talaporfin sodium. The pulse peak power density

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dependence of the photocytotoxicity of rat prostate cancer cells with Porfimer sodium and Talaporfin sodium was investigated. To study progress of Type-II reaction, singlet oxygen generation under various pulse energy densities was measured. We demonstrate the pulse excitation PDT on a rat subcutaneous prostate cancer model with Talaporfin sodium.

2. MATERIALS AND METHODS

2.1 Photocytotoxicity of two photosensitizer on R3327-AT-1 cells

Porfimer sodium was purchased from Takeda Phamaceutical Company Ltd. (Tokyo, Japan). Talaporfin sodium was provided from Meiji Seika Kaisha Ltd. (Tokyo, Japan). The photosensitizers were dissolved in medium of RPMI-1640 without phenol red supplemented with 10 % fetal bovine serum, 20 IU/ml penicillin, 20 μIU/ml streptomycin (all from Invitrogen Corp., Carlsbad, CA, USA) and dexamethasone (ICN Biomedicals Inc. OH, USA). The measured absorbance spectra were shown in Figure 1. The absorption peak wavelength in the red region of each photosensitizer in the medium was, 625 and 664 nm.

The rat prostate cancer cells R3327-AT-1 (ATCC, VA, USA) were placed in 96well plates at $1x10^4$ cells/ 0.2 ml/well for 24 h at 37 °C under 5 % CO_2. Then the cells were incubated with the photosensitizers (the concentration: 5, 10,15 μg/ml) for 1 h. As a pulsed light source for the two photosensitizers, the XeCl excimer laser pumped dye laser (EDL-1, Hamamatsu Photonics K. K., Japan) was employed. The wavelength was tuned to the proper wavelength for each photosensitizer, 626±3nm for Porfimer sodium and 669±3nm for Talaporfin sodium by changing the dye mixture. The pulse duration was 7ns in FWHM. The experimental set-up for the laser irradiation was shown in Figure 2. The laser beam was irradiated perpendicularly from the bottom side of the 96 well plate. The pulse energy density was varied from 1.25 to 10 mJ/cm^2. The repetition rate and the total fluence were fixed at 40 Hz and $20J/cm^2$. After the irradiation, the medium with the photosensitizer was replaced by the medium without the photosensitizer. The cell lethality was measured by the WST assay 24 h after the laser irradiation. The cell lethality was calculated as the percentage to the number of the cells in the well without the laser irradiation.

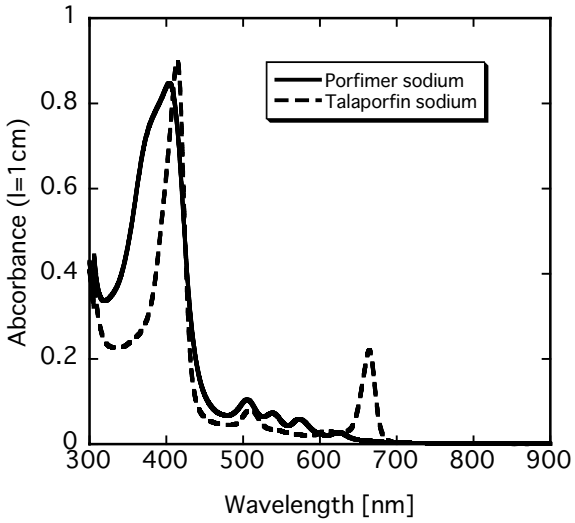


Figure 1. The absorbance spectra of the photosensitizers at the concentration of 6μg/ml in the medium (RPMI-1640 with 10% fetal bovine serum) (Optical length: 1cm)

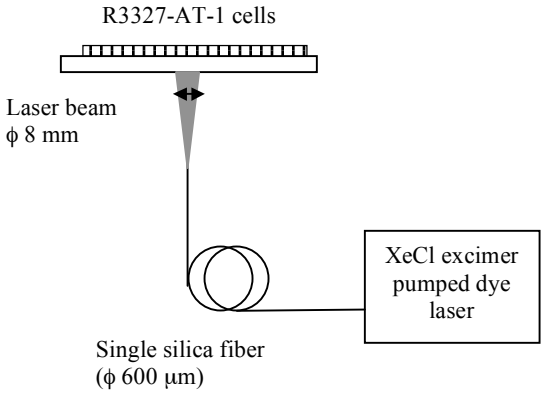


Figure 2. The set-up for the laser irradiation to the cells

2.2 Detection of near-infrared luminescence of singlet oxygen in the photosensitizer solution

To investigate the singlet oxygen generation during the laser irradiation, the singlet oxygen luminescence was measured. The concentration of the photosensitizers in the medium was 45 μM. The photosensitizer solution of 1.1 mm in depth was contained in the plastic dish of 35 mm in diameter. The laser was irradiated with the pulse energy density up to 10 mJ/cm^2 at the pulse repetition rate of 80 Hz. The transient luminescence of the singlet oxygen was measured by photon counting measurement system with a liquid nitrogen-cooled photomultiplier tube (R5509-43, Hamamatsu Corp., Japan). The luminescence from $^1O_2$ ($^1\Delta_g$) to $^3O_2$ ($^3\Sigma_g$) transition appeared around the wavelength of 1270nm. The luminescence was measured from 1220 nm to 1320 nm in wavelength by a monochromater (CT-25C, Jasco Corp., Japan) with the Numerical Aperture of 0.22 and the wavelength resolution of 4 nm. The sampling volume was 2.3 mm^2 (the area of the surface of the solution) x 1.1 mm (the depth). During the measurement, the solution was stirred by a stirrer located outside of the measurement field to prevent hypoxia.

2.3 Photocytotoxicity of Talaporfin sodium *in vivo*

The rat subcutaneous tumor model was prepared. The R3327-AT-1 cells at the concentration of 1x10^6 cells/0.2 ml were placed at femoral region of a Copenhagen rat (male, 8 weeks). Ten days after the injection, tumor reached about 10 mm in height. The Talaporfin sodium was injected intravenously at the dose of 2mg/kg. The dye laser was irradiated at the pulse energy density of 2.5 or 10 mJ/cm^2, 1 h after the photosensitizer injection. The tumor was removed 48 h after the laser irradiation and was made to the H-E stained thin section. The damaged area was determined by the microscopic observation of the section.

3. RESULTS AND DISCCUSIONS

3.1 Photocytotoxicity dependence on pulse energy density

Table 1 shows the cell lethality at the fluence of 20 J/cm^2, the pulse energy density ranged from 1.25 to 10 mJ/cm^2. In the case of Talaporfin mediated PDT, the cell lethality decreased significantly from 64 to 16% with the pulse energy density increasing at the concentration of 15μg/ml. In case of Porfimer sodium mediated PDT, the photocytotoxicity was kept constant about 60 % to the pulse energy density of 5 mJ/cm^2 at the concentration of 5μg/ml.

We found dependences of the photocytotoxicity on pulse energy density were different between the Porfimer sodium mediated PDT and the Talaporfin sodium mediated PDT. Porfimer sodium indicated to have high photocytotoxicity on the prostate cancer cell under our procedure of PDT. The Porfimer sodium was more lipophilic photosensitizer that might be easier to bond closer to the cell membrane. In the Porfimer sodium of mediated, the photocytotoxicity was kept constant until the pulse energy density of 5 mJ/cm^2. In the case of the Talaporfin sodium mediated PDT, the photocytotoxicity was indicated to easier to be suppressed.

Table 1. The cell lethality at various pulse energy density
(Total fluence: 20 J/cm^2, n=8)

Pulse energy density [mJ/cm^2]		1.25	5	10
Cell lethality [%]	Talaporfin sodium (15 μg/ml)	64±8	56±8	16±9
	Porfimer sodium (5μg/ml)	60±7	60±4	30±20

3.2 Detection of near-infrared luminescence of singlet oxygen in the photosensitizer solution

The transitional intensity of singlet oxygen luminescence is shown in Figure 3. The intensity of the singlet oxygen luminescence was calculated as the integral of the measured time-resolved spectrum. The intensity was divided by the irradiated pulse energy density and then was normalized by the intensity at the condition of the pulse energy density of 0.63 mJ/cm^2 with Talaporfin sodium. The relative intensity of the singlet oxygen luminescence was listed up in Table 2. The luminescence efficiency with Talaporfin sodium was higher than that with Porfimer sodium at the same pulse energy density. The decrease of the efficiency with Talaporfin sodium with the pulse energy density increasing was more significant than that of Porfimer sodium. Photochemical reaction with Talaporfin sodium was indicated to be easier to be suppressed under the high intensity pulsed irradiation.

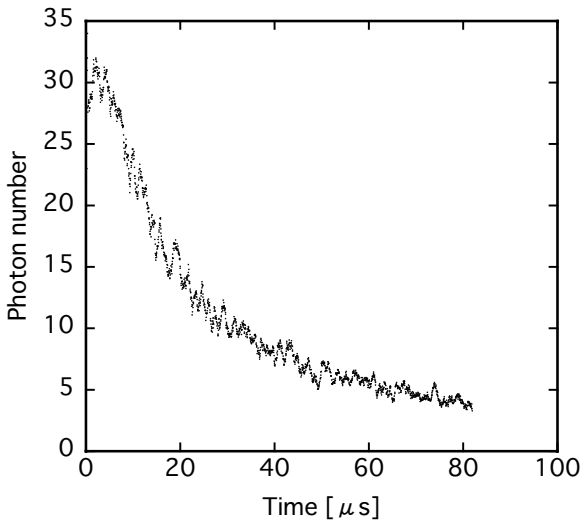


Figure 3. The transitional measurement of singlet oxygen luminescence (Talaporfin, 45ug/ml, 10 mJ/cm^2)

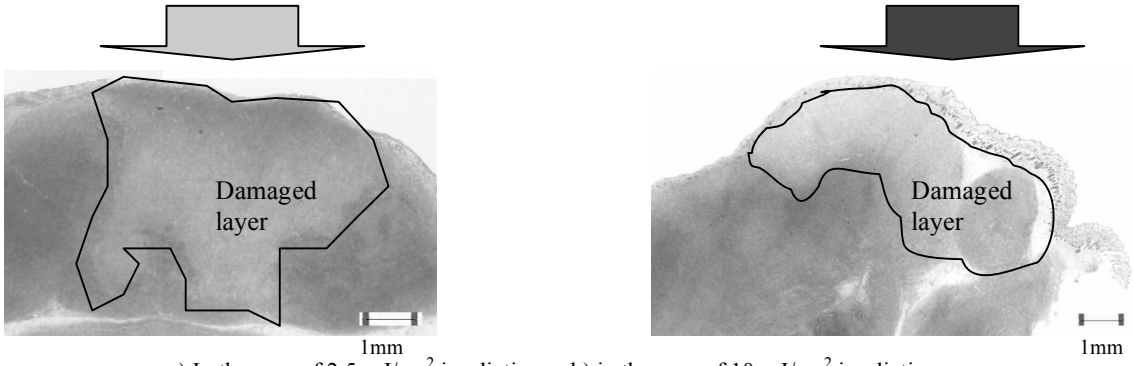
Table 2. Normalized singlet oxygen luminescence efficiency at various pulse energy density

Pulse energy density [mW/cm^2]		1.25	5	10
Normalized Singlet oxygen luminescence efficiency	Talaporfin sodium	1	0.19	0.09
	Porfimer sodium	0.13	0.06	0.04

3.3 PDT effect under high pulse energy density *in vivo*

We performed the pulsed excitation PDT against a rat subcutaneous prostate cancer model with Talaporfin sodium, which indicated to have significant dependency on the irradiated pulse energy. The pictures of the H-E stained section of

the subcutaneous rat prostate tumor are shown in Figure 4. The depth of damage layer was 2.0±1.0 mm was obtained in the case of 2.5mJ/cm^2, 50J/cm^2 irradiation (n=7). On the other hand, in the case of the 10mJ/cm^2 irradiation, the surface of the irradiated field within the 1 mm thickness was intact, while the deeper layer up to the depth of 2.3±1.0 mm was damaged (n = 11). We could obtain the surface intact area in the PDT with the high energy pulsed irradiation. The pulse energy density at the depth of 1 mm from the surface under the 10mJ/cm^2 irradiation was calculated to 2.2 mJ/cm^2 based on the tissue absorbance obtained in literature [21]. This fact indicated that the pulsed excitation PDT was efficient under the pulse energy density around 2.5 mJ/cm^2.



a) In the case of 2.5 mJ/cm^2 irradiation. b) in the case of 10 mJ/cm^2 irradiation.
Figure 4. The images of H-E stained section of the tumor with PDT [22]

4. CONCLUSION

We studied the dependence of PDT effect against pulse energy density to realize the pulse-intensity-domain depth-controlled PDT. Irradiation condition dependence of photocytotoxicity of the rat prostate cancer cell R3327-AT-1 was investigated with Porfimer sodium and Talaporfin sodium. We had significant photocytotoxicity suppression from 64 to 16% with the pulse energy density increasing in the case of Talaporfin sodium mediated PDT. The luminescence from the singlet oxygen in the solution of those two photosensitizers was measured. The decrease of the luminescence efficiency with Talaporfin sodium was more significant than that of Porfimer sodium. Photochemical reaction with Talaporfin sodium was indicated to be easier to be suppressed under the high intensity pulsed irradiation.

We performed PDT against prostate cancer model with Talaporfin sodium, which indicated has significant dependency on the irradiated pulse energy. In the case of 10mJ/cm^2 irradiation, the surface of the irradiated field of 1mm in thickness remained intact, while the deeper area in thickness of 1.3±1 mm was damaged. We think urethra preserved PDT for prostate cancer might be realized by our pulse excitation PDT effect suppression induced by high intensity irradiation.

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