

Highly Selective Photodynamic Therapy with a Short Drug-Light Interval Using a Cytotoxic Photosensitizer Porphyrus Envelope for Drug-Resistant Prostate Cancer Cells

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Abstract

Background: Photodynamic therapy (PDT) is a less invasive cancer treatment using photochemical reactions induced by light irradiation to a photosensitizer (PS). Highly selective PDT with fast accumulation of the PS in target site might be a promising treatment option for drug-resistant prostate cancer facing high incidence rate of elderly men who have no effective treatment options and require a minimally invasive treatment. Hemagglutinating virus of Japan envelope (HVJ-E) allows selective and fast drug delivery to the drug-resistant prostate cancer cells via rapid cell membrane fusion. PS named porphyrus envelope (PE) has been developed by insertion of lipidated protoporphyrin IX (PpIX lipid) into HVJ-E. In this study, we investigated the optimal conditions for PE preparation and laser irradiation for highly selective PDT using PE with a short drug-light interval. **Materials and Methods:** Human hormone refractory prostate cancer cell line PC-3 and human normal prostate epithelial cell line PNT2 were cultured. PpIX lipid uptake and cytotoxicity of PDT in the cells incubated with PE for 10 min were evaluated by measuring fluorescence intensity and by using a cell counting reagent 24 h after PDT, respectively. **Results:** PpIX lipid uptake and cytotoxicity of PDT were increased with PpIX lipid concentration. Cytotoxicity of PDT using PE was more than 9 times as strong as that with PpIX lipid and PpIX induced by 5-aminolevulinic acid. Much stronger cytotoxicity was induced in PC-3 cells

than PNT2 cells with the ratio of cell death rate for cancer to normal cells up to 4.64 ± 0.09 . **Conclusions:** Fast PS delivery with HVJ-E allows highly selective PDT with a short drug-light interval. Therefore, PDT using PE has a potential to shorten treatment period and reduce side effects of PDT.

Keywords

Photodynamic Therapy, Hemagglutinating Virus of Japan Envelope, Drug Delivery System, Short Drug-Light Interval, Drug-Resistant Prostate Cancer

1. Introduction

Prostate cancer is becoming the most common cancer due to the aging population [1]. Hormone therapy has been considered as an effective treatment since prostate cancer grows with androgen. However, cancer cells often become resistant to the therapy [2], and disease progress requires that patients undergo chemotherapy whose response rate is not high enough to radically cure such drug-resistant prostate cancer [3].

Photodynamic therapy (PDT) is a cancer treatment using photochemical reactions induced by light irradiation to a photosensitizer (PS). The PS is excited by a specific wavelength of light and reacts with surrounding substrates or oxygen to produce reactive oxygen species (ROS) that damages tissues where the PS is localized [4]. Unlike an anticancer drug, the PS alone exhibits no severe cell toxicity [5], and low power light irradiation allows patients to obtain therapeutic efficacy after repeated treatment [6]. These characteristics of drugs and laser irradiation suggest that PDT is a potential treatment for drug-resistant prostate cancer [7] [8] [9]. Selectivity in therapeutic efficacy of conventional PDT is mainly related to the selective accumulation of the PS in tumor tissue or neovasculature. In reality, however, the PS itself has poor selectivity, leading to the long interval lasting from administration of the PS to light irradiation for maximization of the gap of PS concentration in between tumor and normal tissue. This interval is known as “drug-light interval” and patients need to stay out sun and room light for 3 - 48 h [10]. Poor selectivity of PSs is also associated with side effects such as normal tissue damage and skin photosensitivity after treatment. Especially, photosensitivity results in prolong light shielding period for 1 - 4 weeks that increases treatment cost and makes patients feel uncomfortable about the therapy [11] [12].

To overcome these problems in clinical, a number of site-specific delivery of the PS has been received much attention. Replication-defective hemagglutinating virus of Japan (HVJ) envelope (HVJ-E) particle is considered to be a safe and effective carrier of DNA, proteins and drugs [13] [14] to a target cell. HVJ-E introduces those contents in cells thorough membrane fusion process via two types of viral proteins on its surface called hemagglutinin-neuraminidase (HN) and fusion (F) protein [15]. HN protein binds to its receptor gangliosides on

host cell membrane, and then F protein-mediated membrane fusion occurs [16]. HVJ-E has two characteristics suitable as a carrier of the PS to drug-resistant prostate cancer cells. First, receptors of HN protein are more expressed in human drug-resistant prostate cancer cell lines PC-3 and DU145 compared with the normal prostate epithelial cell line PNT2 [17]. This cancer-specific expression of receptors enables HVJ-E to selectively deliver the PS to such prostate cancer cells. Second, membrane fusion takes place immediately after the adsorption of HVJ-E to the cells within a few minutes [18], and localization of the PS in the cell membrane significantly enhances therapeutic efficacy of PDT by inducing substantial membrane disruption upon laser irradiation even at a low PS concentration [19]. Taken together, rapid membrane fusion has a possibility to induce strong cytotoxicity in cancer cells at a short drug-light interval.

These characteristics of HVJ-E suggest that delivery of the PS by HVJ-E induces selective cytotoxicity of PDT in drug-resistant prostate cancer at a short drug-light interval. We have previously developed a PS named porphyrus envelope (PE) by insertion of lipidated protoporphyrin IX (PpIX lipid) into HVJ-E [20] [21]. Previous study showed that PE undergoes membrane fusion with PC-3 cells by 10 min incubation [22] and PpIX lipid localizes in the cell membrane [23]. However, the optimal conditions for PE preparation and light irradiation for PDT with a short drug-light interval have not been clarified and selectivity in PDT using PE remains unclear. In this study, we examined uptake kinetics of PE-mediated PpIX lipid and cytotoxicity of PDT in both PC-3 and PNT2 cells with the 10 min drug-light interval in order to determine the optimal concentration of PpIX lipid loaded in PE and light irradiation energy density for highly selective PDT against drug-resistant prostate cancer cells.

2. Materials and Methods

2.1. Cell Culture

Hormone refractory human prostate cancer cell line PC-3 and human normal prostate epithelial cell line PNT2 were purchased from the American Type Culture Collection (USA), respectively. The cell lines were cultured in Dulbecco's modified Eagle's medium (D6046, Sigma-Aldrich, USA) with fetal bovine serum (S1820, Biowest, France) and 100 units/mL penicillin-streptomycin (P4458, Sigma-Aldrich). The cells were incubated at 37°C humidified atmosphere containing 5% CO₂. PC-3 and PNT2 cells were reseeded after reaching confluence of 80% and 70%, respectively.

2.2. Photosensitizers

5-aminolevulinic acid hydrochloride (5-ALA, A7793, Sigma-Aldrich) was diluted in the culture medium to a concentration of 1 mM. An aqueous solution of 5 mMPpIX lipid was prepared by synthesizing two lithium groups and two lipid chains to PpIX in order to enhance its water-solubility and be inserted into HVJ-E as previously reported [20] [21]. PpIX lipid solution was diluted in the

culture medium to a concentration of a 28.5 μM . HVJ-E was prepared by irradiation of ultraviolet light to HVJ at an energy density of 99 mJ/cm^2 in order to make its RNA genome fragmented and inactivate its replication activity. HVJ-E was diluted in 1 mL of Dulbecco's phosphate buffered saline (D-PBS, D8537, Sigma-Aldrich) to a titer of 2500 hemagglutination units (HAU) and purified by centrifugation ($20,000 \times g$, 4°C , 10 min). The pellet of HVJ-E was suspended in 1 mL of PpIX lipid solution which was diluted in D-PBS to the concentration range of 0 - 24.1 μM . The suspension was heated to 37°C for 5 min, followed by insertion of PpIX lipid into HVJ-E by centrifugation ($20,000 \times g$, 4°C , 10 min) with ratios of the amount of PpIX lipid to titer of HVJ-E for 0 - 9.65 pmol/HAU. After centrifugation, PE was prepared by suspending the pellet in 833 μL of the culture medium. In all the experiments, the culture medium and PSs were administered to the cells at a dose of 50 μL .

2.3. Light Source and Optical Setup

A laser diode (VLM 500, Sumitomo Electric Industries, Japan) emitting continuous wave light with a wavelength of 405 nm was used as a light source. The wavelength corresponds to the highest absorption peak of PE [21] and the effects of absorption and scattering of the light at wavelengths of around 400 nm are hardly observed *in vitro* experiment which does not use biological tissue but only cultured cells. The optical setup for PDT experiment is shown in **Figure 1**. Laser light guided by an optical fiber (FT600EMT, Thorlabs, USA) was collimated to a diameter of 6.6 mm using an aperture and was irradiated to a clear bottom of a 96-well cell culture plate with a black wall (Falcon® 353219, BD, USA) where the cells were maintained at the temperature of 37°C using a plate warmer (KM-1, Kitazato Science, Japan). The distance between the tip of the fiber and a bottom of the cell culture plate was vertically adjusted to 90 mm. Each well of the plate was irradiated with a laser light at an average power density of $100 \text{ mW}/\text{cm}^2$. The position of the cell culture plate was automatically controlled by a two-axis motorized linear stage (SGSP26-150(XY), Sigmakoki, Japan) and changed when each irradiation time had finished.

2.4. Dependence of PS Uptake and PDT Efficacy in PC-3 Cells on PpIX Lipid Concentration

2.4.1. Evaluation of Cellular PS Uptake

PC-3 cells were seeded into a 96-well cell culture plate (Falcon® 353219, BD) at a concentration of 5.0×10^3 cells/100 μL in each well and incubated at 37°C containing 5% CO_2 for 24 h. Subsequently, the cells were incubated with the culture medium, or PE suspension in the PpIX lipid concentration range of 0.6 - 9.65 pmol/HAU for 10 min, and were washed with 100 μL of D-PBS. After washing, 100 μL of 0.1% sodium dodecyl sulfate (SDS, 31606-75, NacalaiTesque, Japan) solution in D-PBS was added to the cells in order to dissolve cellular lipids and proteins, followed by incubation of cells for 10 min. Fluorescence intensity of

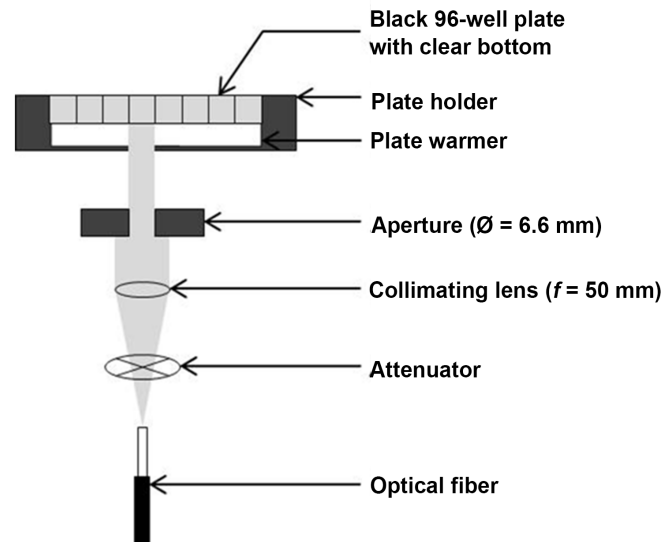


Figure 1. Schematic diagram of the optical setup for *in vitro* PDT. Laser light with a wavelength of 405 nm was guided by an optical fiber, was collimated to a diameter of 6.6 mm, and was irradiated to a clear bottom of a 96-well cell culture plate with a black wall. The distance between the tip of the fiber and a bottom of the cell culture plate was vertically adjusted to 90 mm. The position of the plate was controlled by a two-axis motorized linear stage and the temperature of the plate was maintained at 37°C using a plate warmer.

cell lysate was measured by a fluorescence microplate reader (SpectraMAX Gemini, Molecular Devices, USA) at excitation and fluorescence wavelengths of 401 nm and 625 nm, respectively.

2.4.2. PDT Procedure

PC-3 cells were incubated as described above. PpIX lipid loaded in PE was prepared to the concentration range of 1.75 - 9.5 pmol/HAU. Subsequently, the cells were washed with 100 μ L of D-PBS and a 100 μ L of the fresh culture medium was added to the cells. Immediately after addition of the medium, cells were irradiated using a laser light for 60 s at an energy density of 6 J/cm².

2.4.3. Comparison of PDT Efficacy of PE with That of PpIX Lipid Alone and 5-ALA-Induced PpIX

PC-3 cells were incubated as described above, and incubated with the culture medium, PpIX lipid, 5-ALA solution, or PE suspension with 9.5 pmol/HAU PpIX lipid for 10 min, 30 min, 1 h, or 2 h. Subsequently, the cells were washed with D-PBS, and the fresh culture medium was added. Then laser light was irradiated as described above.

2.5. Evaluation of Selectivity

2.5.1. Evaluation of Cellular PS Uptake

PC-3 and PNT2 cells were seeded into a 96-well cell culture plate (Falcon®

353219, BD) at a concentration of 5.0×10^3 cells/100 μL in each well and incubated at 37°C containing 5% CO_2 for 24 h. Subsequently, cells were incubated with the culture medium or PE in the PpIX lipid concentration range of 1.19 - 9.5 pmol/HAU for 10 min, 30 min, or 1 h. After incubation, the cells underwent washing with D-PBS, adding of 0.1% SDS solution in D-PBS, and measurement of fluorescence intensity of its lysate, as described in 2.4.1.

2.5.2. PDT Procedure

PC-3 and PNT2 cells were incubated as described above but with culture medium or PE for only 10 min, respectively. Subsequently, the cells underwent washing with D-PBS and adding of the fresh culture medium as described in 2.4.2. Immediately after addition of the medium, cells were irradiated using a laser light for 0 - 180 s in energy density range of 0 - 18 J/cm^2 .

2.6. Cell Survival Assay

Cells irradiated with a laser light were incubated at 37°C containing 5% CO_2 for 24 h, and the culture medium in each well was replaced with the mixture of 100 μL of the fresh culture medium and 10 μL of a cell counting reagent (07553-44, NacalaiTesque, Japan) containing water-soluble tetrazolium salt (WST-8). Cells were further incubated for 1 h, and the optical density (OD) of the culture medium containing formazan dye produced upon reduction of WST-8 by dehydrogenases in a living cell was measured at a wavelength of 450 nm using an absorbance microplate reader (VersaMAX, Molecular Devices). Cell survival rate was defined as the ratio of OD of the cells exposed to the PS and laser light, to that of the cells which were exposed to laser light only.

2.7. Statistical Analysis

Statistical analysis was conducted using a two-tailed unpaired Student's t-test for detecting the significant difference between two samples and probability values of $P < 0.05$ were considered to indicate statistical significance.

3. Results

3.1. PpIX Lipid Uptake and Cytotoxicity of PDT Increased with PpIX Lipid Concentration in PE

To determine the PpIX lipid concentration required for PDT using PE with the 10 min drug-light interval against PC-3 cells, the amount of PpIX lipid and cytotoxicity of PDT were assessed using PE with different PpIX lipid concentrations. Concentration-dependent PpIX lipid uptake was observed at PpIX lipid concentration higher than 1.21 pmol/HAU (**Figure 2(a)**). The cytotoxicity of PDT increased with the concentration of PpIX lipid loaded in PE, and survival rate of PC-3 cells decreased to 10% at a concentration of 9.5 pmol/HAU (**Figure 2(b)**). These results suggested that increase of PpIX lipid concentration in PE

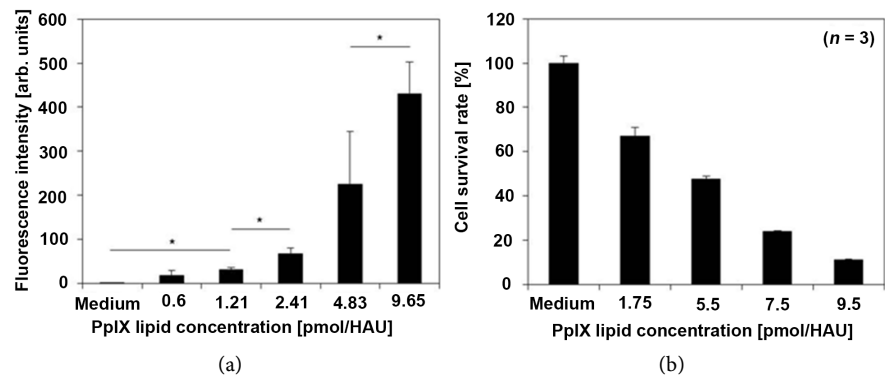


Figure 2. (a) Fluorescence intensity of PpIX lipid in PC-3 cells incubated with PE in the PpIX lipid concentration range of 0.6 - 9.65 pmol/HAU for 10 min. Concentration-dependent increase in PpIX lipid uptake was observed at the concentration higher than 1.21 pmol/HAU; (b) cytotoxicity of PDT in PC-3 cells incubated with PE in the PpIX lipid concentration range of 1.75 - 9.5 pmol/HAU for 10 min. Concentration-dependent enhancement in the cytotoxicity of PDT was observed, and the cell survival rate decreased to 10% at a PpIX lipid concentration of 9.5 pmol/HAU. The data represent the mean \pm standard deviation. *, significance at $P < 0.05$.

can induce strong photo-cytotoxic effect of PDT in PC-3 cells at the 10 min drug-light interval.

3.2. PDT Using PE Induced Much Stronger Photo-Cytotoxicity than That of Other PpIX-Mediated PDT

To confirm whether PDT using PE could induce stronger photo-cytotoxicity at the 10 min drug-light interval than that of other PpIX-mediated PDT, PC-3 cells were incubated with the culture medium, PE, PpIX lipid alone, or 5-ALA which is metabolized into PpIX in a cellular mitochondria. At the 10 min drug-light interval, survival rates of PC-3 cells incubated with PpIX lipid alone and 5-ALA were 100% and 90%, respectively, and the rates decreased with the increase of drug-light interval, while survival rate of the cells incubated with PE was lower than 10%, and time-dependent decrease in cell survival rate was hardly observed in the drug-light interval of between 10 min and 2 h (Figure 3).

3.3. PE Allowed Selective Delivery of PpIX Lipid Even at a Short Incubation Period

To examine the selectivity for uptake kinetics of PpIX lipid, the amounts of PpIX lipid in PC-3 and PNT2 cells were assessed under different PpIX lipid concentrations in PE and incubation periods. Significant increases in fluorescence intensities were observed in PC-3 cells in all PpIX lipid concentrations and incubation periods (Figure 4). The tumor to normal cell ratio (T/N ratio) of fluorescence intensity at an incubation period of 10 min was $1.19 \pm 0.07 - 2.10 \pm 0.20$, and time-dependent increase in T/N ratio was hardly, or not observed in each PpIX lipid concentration. These results suggested that cytotoxicity of PDT using PE can be selectively induced in cancer cells with a short drug-light interval.

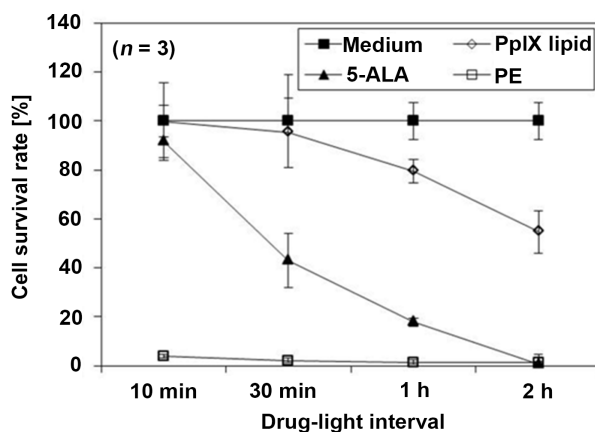


Figure 3. Cytotoxicity of PDT using PE in PC-3 cells was compared with that of other PpIX-mediated PDT at the 10 min drug-light interval. The cells were incubated with the culture medium, PpIX lipid alone, 5-ALA, or PE, respectively. Distinct time-dependent decreases in cell survival rates were observed in PDT using PpIX lipid alone and 5-ALA, respectively, while the cells incubated with PE hardly showed decrease in the survival rate in drug-light interval of between 10 min and 2 h. Moreover, survival rate of the cells incubated with PE for 10 min was 10-folds and 9-folds lower than that with PpIX lipid alone and 5-ALA, respectively. The data represent the mean \pm standard deviation.

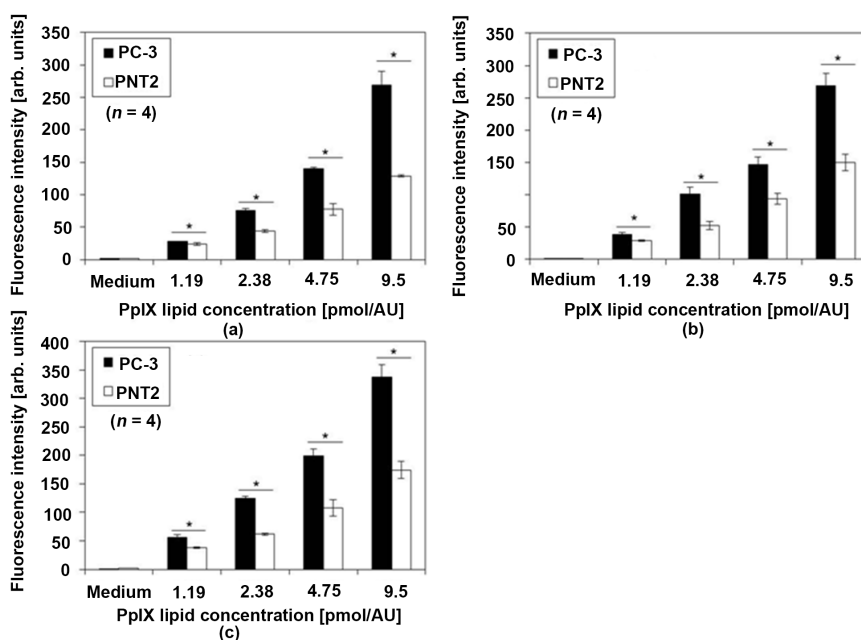


Figure 4. Fluorescence intensity of PpIX lipid in PC-3 and PNT2 cells incubated with PE in the PpIX lipid concentration range of 1.19 - 9.5 pmol/HAU for (a) 10 min; (b) 30 min, and (c) 1 h. Fluorescence intensities of PpIX lipid in PC-3 cells were significantly higher than that in PNT2 cells in all PpIX lipid concentrations and incubation periods. The tumor to normal cell ratio (T/N ratio) of fluorescence intensity was $1.19 \pm 0.07 - 2.10 \pm 0.20$. There was little or no time-dependent increase in selectivity for PpIX lipid uptake. The data represent the mean \pm standard deviation. *, significance at $P < 0.05$.

3.4. PE-Mediated PDT Induces Highly Selective Cytotoxicity with a Short Drug-Light Interval

To determine doses of PpIX lipid and laser light which are required for highly selective PDT using PE with a short drug-light interval, the cytotoxicity of PDT in PC-3 and PNT2 cells were assessed using PE with different PpIX lipid concentrations and light irradiation energy densities at the 10 min drug-light interval. When the cells were not exposed to laser light, or incubated with PE at a PpIX lipid concentration of 1.19 pmol/HAU, survival rates of PC-3 and PNT2 cells were 40% - 50% and 70% - 80%, respectively (**Figure 5**). Survival rates of PC-3 cells decreased with the increase of irradiation energy density at the PpIX lipid concentration higher than 2.38 pmol/HAU, while no energy density dependent decrease in survival rate of PNT2 cells was observed at a PpIX lipid concentration of 2.38 pmol/HAU. Laser irradiation at energy densities of 12 J/cm² and 6 J/cm² induced photo-cytotoxic effects in PNT2 cells at PpIX lipid concentrations of 4.75 pmol/HAU and 9.5 pmol/HAU, respectively. The decreases in survival rates of PNT2 cells incubated with PE at PpIX lipid concentrations of 4.75 pmol/HAU and 9.5 pmol/HAU were also observed by laser irradiation to the cells in the energy density ranges of 8 - 12 J/cm² and 1 - 4 J/cm², respectively (data not shown). Ultimately, laser irradiation at energy densities of 18 J/cm² and 6 J/cm² with PpIX lipid concentrations of 2.38 pmol/HAU and 4.75 pmol/HAU, respectively, obtained the highest T/N ratios of cell death in PDT using PE at the 10 min drug-light interval. The ratios in each PDT conditions were estimated to be 4.64 ± 0.09 and 4.54 ± 0.01 , respectively (**Table 1**).

4. Discussion

As shown in **Figure 4**, PE showed the selective uptake in all PpIX lipid concentrations and incubation periods, and distinct time-dependent increases in selectivity were hardly observed. We consider that rapid and cancer-selective PS delivery by HVJ-E allowed selective accumulation of the PS even at a short incubation period [24] [25]. Based on this, we performed PDT using PE with high concentration of PpIX lipid (9.5 pmol/HAU) at the 10 min drug-light interval and revealed that PE induced much stronger cytotoxicity in PC-3 cells than PpIX lipid alone and 5-ALA (**Figure 3**) probably due to the rapid delivery of PpIX lipid into the cell membrane and effective induction of cell death [19] [21] [22] [23].

In this study, we also evaluated the selectivity of cell death induced by PDT using PE with the 10 min drug-light interval. As shown in **Table 1**, T/N ratio of cell death in PDT was higher than that of PE uptake. It is known that selective apoptosis in PC-3 cells but not in PNT2 cells is induced via recognition of fragmented HVJ-E's RNA genome by intracellular retinoic acid-inducible gene-I whose signaling pathway selectively activates apoptosis-inducing ligands in PC-3 cells [26]. In this study, survival rates of PC-3 cells were lower than those of PNT2 cells by 30% - 40% in all PpIX lipid concentrations without laser irradiation. Previous study reported that PE also selectively induced cytotoxicity in

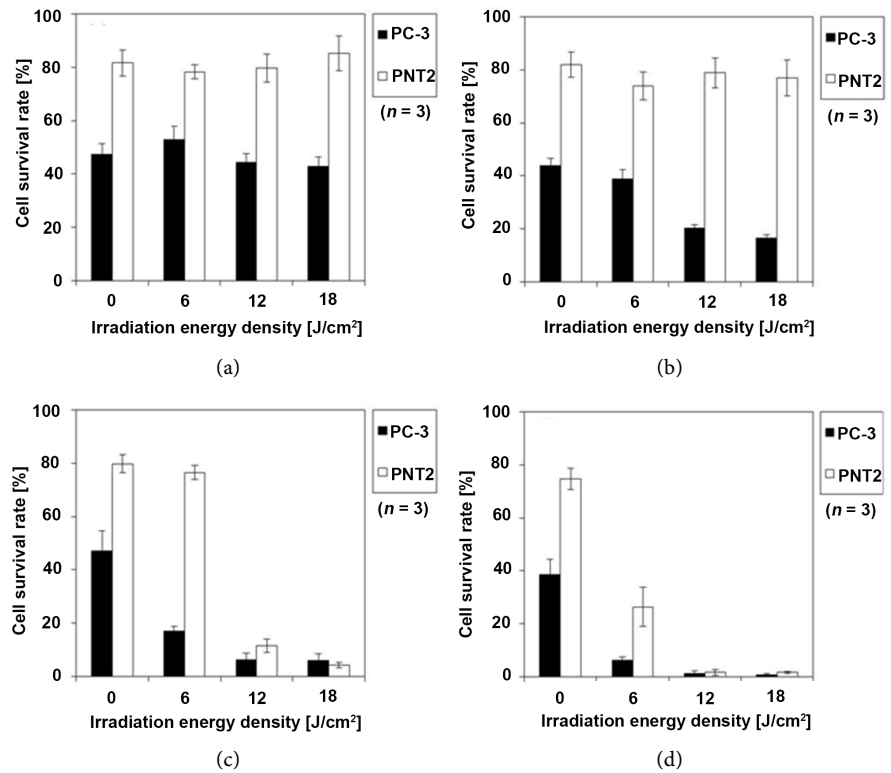


Figure 5. Cytotoxicity of PDT in the laser irradiation energy density range of 0 - 18 J/cm² at the 10 min drug-light interval in PC-3 and PNT2 cells incubated with PE at the PpIX lipid concentrations of (a) 1.19 pmol/HAU; (b) 2.38 pmol/HAU; (c) 4.75 pmol/HAU, and (d) 9.5 pmol/HAU. Survival rates of PC-3 and PNT2 cells were 40% - 50% and 70% - 80%, respectively when the cells were not exposed to laser light, or incubated with PE at a PpIX lipid concentration of 1.19 pmol/HAU. Survival rates of PC-3 cells were decreased with the increase of energy density at the PpIX lipid concentrations higher than 2.38 pmol/HAU, while phototoxicity-mediated cell deaths in PNT2 cells incubated with PE at PpIX lipid concentrations of 2.38 pmol/HAU and 4.75 pmol/HAU were not observed after laser irradiation at energy density up to 18 J/cm² and 6 J/cm², respectively. The data represent the mean ± standard deviation.

Table 1. T/N ratio of cell death in PDT using PE with the 10 min drug-light interval under different PpIX lipid concentrations and irradiation energy densities. The ratio was calculated by division of the cell death rate for PC-3 cells to PNT2 cells based using the cell survival rates shown in Figure 5. Laser irradiation at energy densities of 18 J/cm² and 6 J/cm² with PpIX lipid concentrations of 2.38 pmol/HAU and 4.75 pmol/HAU, respectively, showed the highest T/N ratios of cell death of 4.64 ± 0.09 and 4.54 ± 0.01, respectively.

Energy density [J/cm ²]	PpIX lipid concentration [pmol/HAU]			
	1.19	2.38	4.75	9.5
0	1.72 ± 0.04	1.87 ± 0.03	1.69 ± 0.06	1.94 ± 0.05
6	1.48 ± 0.04	1.91 ± 0.03	4.54 ± 0.01	4.31 ± 0.01
12	1.80 ± 0.03	3.91 ± 0.02	1.82 ± 0.00	1.50 ± 0.00
18	2.00 ± 0.04	4.64 ± 0.09	0.69 ± 0.03	2.28 ± 0.03

PC-3 cells but not in PNT2 cells, and the cytotoxicity was maintained even after PE had been subjected to laser light at an energy density of 6 J/cm² with the same light source and power density, as described in this study [23]. Other study reported that there was threshold of cellular PpIX amount for cell killing and T/N ratio of cellular PpIX uptake did not strictly correspond to that of cell death [27]. In this study, when PNT2 cells were incubated with PE at PpIX lipid concentrations of 2.38 pmol/HAU and 4.75 pmol/HAU and were irradiated with laser light at energy densities of 0 - 18 J/cm² and 0 - 6 J/cm², respectively, cancer-selective decreases in cell survival rates were observed with the increase of laser irradiation energy density. This result suggested that the amount of cellular PpIX lipid loaded in PE at those concentrations did not show the threshold amount in PNT2 cells for inducing cell death. Taken together, higher selectivity of cell death than that of PpIX lipid uptake was possibly owed to HVJ-E's derived cancer-selective cytotoxicity and low PpIX lipid amount in PNT2 cells which did not show threshold amount for cell killing.

Finally, the highest T/N ratios of cell death were observed in following conditions:

- 1) PpIX lipid concentration of 2.38 pmol/HAU and laser energy density of 18 J/cm²,
- 2) PpIX lipid concentration of 4.75 pmol/HAU and laser energy density of 6 J/cm².

In clinical, PSs are not always distributed homogeneously in tissue. This indicates that long laser irradiation period possibly gives damages to normal cells where high concentration of PSs accumulates. In this study, PE with a PpIX lipid concentration of 4.75 pmol/HAU induced substantial decrease in survival rate of PNT2 cells at laser irradiation energy densities of 12 J/cm² and 18 J/cm², while no energy density-dependent decrease in the cell survival rate was observed in PE at a PpIX lipid concentration of 2.38 pmol/HAU. These results suggest that PDT using PE should be conducted with low PpIX lipid concentration of PE and long irradiation period, as shown in condition (1), in order not to generate acute cytotoxicity to normal cells since high incidence rate of drug-resistant prostate cancer is observed in elderly people over 65 years [1] and patients often need minimally invasive cancer treatments.

Much stronger cytotoxicity was induced in PC-3 cells than PNT2 cells with the T/N ratio of 4.64 ± 0.09 under condition (1). There have been some reports that have shown the photo-cytotoxicity in between PC-3 cells and normal prostate cell lines, however, the lower T/N ratios of cell death were observed by as much as 1.5 - 2 in other PSs [28] [29]. This suggested that PE has the possibility to allow highly selective treatment for drug-resistant prostate cancer with a much shorter drug-light interval.

Previous studies have shown the safety and efficacy of intratumoral administration of HVJ-E to drug-resistant prostate cancer tissue in the phase of pre-clinical and clinical trials [30] [31]. It has been reported that intratumoral

administration of the PS induces high level of PS concentration in tumor tissue at a shorter drug-light interval with an advantage of the much higher tumor to normal skin ratio for accumulation of the PS [32] [33]. Furthermore, tumor-specific PS delivery is known to be a promising strategy to further improve the accumulation efficacy and retention of the PS which is directly administrated into tumor tissue [34] [35]. Therefore, the possibility of intratumoral administration of PE *in vivo* trials probably provides higher selectivity in PDT with a short drug-light interval than *in vitro* experiments, as reported in this study.

5. Conclusion

In this study, we focused on the fast delivery of the PS by HVJ-E and have demonstrated highly selective cytotoxicity of *in vitro* PDT using PE against drug-resistant prostate cancer cells with a short drug-light interval. This result suggests that PE-mediated PDT can shorten drug-light interval and reduce PDT-mediated side effects, and gives the possibility to apply PDT to a novel treatment modality for drug-resistant prostate cancer in clinical.

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