Anticancer Effects of Novel Photodynamic Therapy with Glycoconjugated Chlorin for Gastric and Colon Cancer

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Abstract. Background/Aim: Photodynamic therapy (PDT) is an attractive, minimally invasive modality for cancer therapy that utilizes the interaction of light and photosensitizer. To improve the efficacy of PDT, development of cancer specificity of the photosensitizer is needed. Cancer cells consume more glucose than normal cells. In this study, the efficacy of PDT using a newly developed photosensitizer, glycoconjugated chlorin (H2TFPC-SGlc), was compared with Talaporfin, which is clinically used in Japan. Materials and Methods: Photosensitizers were administered to gastric and colon cancer cell lines, followed by irradiation of light, and the cell death-inducing effects were compared. Xenograft tumor mouse models were established and photosensitizer accumulation was assessed and antitumor effects analyzed. Results: In vitro, H2TFPC-SGlc was 30 times more cytotoxic to cancer cells than was Talaporfin. In vivo, H2TFPC-SGlc accumulation was higher in xenograft tumors and significantly suppressed tumor growth when compared with Talaporfin. Conclusion: This novel glycoconjugated chlorin is potentially useful in PDT.

Photodynamic therapy (PDT) is a promising non-invasive treatment for cancer (1-3). PDT involves the administration of a photosensitizer and visible light irradiation at a specific wavelength to produce reaction by the photosensitizer (1). Activation of the photosensitizer leads to a conversion of molecular oxygen into various highly reactive oxygen species (ROS), which directly kill tumor cells or damage tumor-associated vasculature (1, 2). PDT has several advantages over other conventional cancer treatments (4). It is relatively non-invasive because irradiation is limited to the tumor site (5), and it shows lower systemic toxicity and relatively selective destruction of tumors, partly due to preferential localization of photosensitizer within the tumor (2). Thus, PDT has been widely employed against various tumors to which irradiation can be applied directly, such as lung, esophageal, gastric, breast, head and neck, bladder and prostate carcinomas (1). When compared with other therapies, PDT often produces higher cure and lower recurrence rates (6).

However, as every technique has its limitations, PDT has been limited by the insufficient efficacy of photosensitizers. Despite being the most widely used in photosensitizer clinical settings, Photofrin, a first-generation photosensitizer, has several disadvantages, including long-term skin photosensitivity and a short absorption wavelength that limits tissue penetration (2, 5).

Some second-generation photosensitizers have been shown to improve efficacy and reduce side-effects when compared with first-generation photosensitizers (5, 6). Talaporfin, a second-generation photosensitizer, has several advantages over first-generation photosensitizers, such as reducing the photosensitization period and requiring a shorter interval between drug administration and laser light exposure as compared to photofrin (7). Talaporfin-mediated PDT has been examined in the treatment of several different type of solid tumor (4, 8). However, issues related to insufficient efficacy and skin photosensitivity remain unsolved; thus,
more effective photosensitizers are expected to be developed (3). Tumors consume higher levels of glucose than normal cells, a phenomenon known as the Warburg effect (9). Several glycoconjugated porphyrins have been synthesized and evaluated for photocytotoxicity (10), but there have been few reports on glycoconjugated chlorin. Yano and colleagues have developed several glycoconjugated chlorins and have reported excellent photocytotoxicity (11, 12).

In this study, we evaluated the efficacy of PDT with a novel photosensitizer, glycoconjugated chlorin (H2TFPC-SGlc), as compared with Talaporfin in vitro and in vivo.

### Materials and Methods

**Photosensitizers.** H2TFPC-SGlc [glycoconjugated chlorin: 5,10,15,20-tetrakis (4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl)-2,3-(methano(N-methyl) iminomethano) chlorin] (Figure 1A) was prepared by the method described elsewhere (12). Talaporfin sodium (mono-l-aspartyl chlorin6, Laserphyrin®) was purchased from Meiji Seika (Tokyo, Japan).

**Cell culture.** The human gastric cancer cell lines MKN28 (No. 0253; Japanese Cancer Research Resources Bank, Tokyo, Japan) and MKN45 (No. 0254; Japanese Cancer Research Bank, Tokyo, Japan) were cultured in RPMI1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) and 1% ampicillin and streptomycin. The human colon cancer cell lines HT29 (No. HTB-38; American Type Culture Collection, VA, USA) and HCT116 (No. CCL-247; American Type Culture Collection) were cultured in McCoy’s 5A Medium (Sigma-Aldrich) supplemented with 10% FBS and 1% ampicillin and streptomycin. Cells were cultured under an atmosphere of 5% CO2 at 37°C.

**In vitro PDT.** Gastric and colon cancer cells (MKN28, MKN45, HT29 and HCT116) were incubated with photosensitizer in culture medium for 24 h. Cells were washed once with, immersed in PBS, and irradiated with 10 J/cm2 (intensity: 37 mW/cm2) using a light emitting diode (LED) for 24 h. Cells were washed once with, immersed in PBS, and irradiated and HCT116) were incubated with photosensitizer in culture medium for 24 h. As shown in Figure 1B, PDT with H2TFPC-SGlc induced cell death in gastric and colon cancer cell lines.

**Flow cytometric analysis.** In order to label apoptotic cells, cells were incubated with Active Caspase-3-FITC (BD Pharmingen™, Tokyo, Japan) at 4°C for 30 min in the dark and were neutralized with binding buffer. Cells were then analyzed using a FACScan™ (BD Biosciences, Tokyo, Japan). Analyses were performed at 0, 1, 2, 4, 8, 12, 16, 20 and 24 h after PDT. At least 10,000 events were collected for each sample.
PDT with H$_2$TFPC-SGlc induced apoptosis via ROS production. We investigated the mode of cell death induced by H$_2$TFPC-SGlc-mediated PDT. Cells were incubated with 1 μM photosensitizer (H$_2$TFPC-SGlc or Talaporfin) for 24 h, followed by irradiation with 633-nm LED (16 J/cm$^2$). We measured the fluorescence intensity of active caspase-3 as a marker for apoptosis by FACS. An increase in mean fluorescence intensity was observed after 4 h, and this increase continued, until peaking at 16 h (Figure 1C). We then examined ROS induction by PDT with H$_2$TFPC-SGlc. Cells were incubated with 1 μM photosensitizer (H$_2$TFPC-SGlc or Talaporfin) for 24 h, followed by irradiation with 633-nm LED (16 J/cm$^2$). Figure 1C shows the data for MKN45 cells. The same trend was seen for the other cell lines (MKN28, HT29 and HCT116) (data not shown).

Table 1. IC$_{50}$ of the efficiency of photodynamic therapy.

<table>
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<th>Gastric cancer</th>
<th>Colon cancer</th>
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<tr>
<td>IC$_{50}$ (μM)</td>
<td>MKN28</td>
<td>MKN45</td>
</tr>
<tr>
<td>Talaporfin</td>
<td>12.7</td>
<td>18.6</td>
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<tr>
<td>H$_2$TFPC-SGlc</td>
<td>0.56</td>
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Accumulation of H$_2$TFPC-SGlc in xenograft tumor of colon cancer. We examined whether photosensitizer (H$_2$TFPC-SGlc or Talaporfin) accumulated in the xenograft tumors established by subcutaneously implanting colon cancer cells (HT29 and HCT116). Four hours after intravenous injection...
of photosensitizer (H$_2$TFPC-SGlc or Talaporfin) at a dose of 6.25 μmol/kg, the spectrum waveform from the xenograft tumors was analyzed by a spectrometer (VLD-M1). The spectrum waveform showed two peaks of fluorescence emission: one at 505 nm, corresponding to autofluorescence, and the other corresponding to photosensitizers (H$_2$TFPC-SGlc at 655 nm, and Talaporfin at 670 nm) (Figure 2A). Next, we measured the relative fluorescence intensity ratio of photosensitizers in tumors and normal tissue surrounding the tumor using a spectrometer. The relative fluorescence intensity ratios of both photosensitizers were highest at 4 h after drug administration (Figure 2B). H$_2$TFPC-SGlc accumulated in the tumor tissue to a significantly higher extent than in the normal tissue surrounding the tumor.

**Antitumor effects of H$_2$TFPC-SGlc in vivo.** In order to examine the effects of H$_2$TFPC-SGlc-mediated PDT on tumors in vivo, PDT was performed on xenograft tumor models established by subcutaneously implanting colon cancer cells (HT29, HCT116). When tumors grew to 75-150 mm$^3$, mice were given an intravenous injection (via tail vein) of photosensitizer (H$_2$TFPC-SGlc or Talaporfin) at a dose of 6.25 μmol/kg, followed by irradiation with a 633-nm LED.
light (37.5 $\text{J/cm}^2$). The xenograft tumors showed no changes in size immediately after PDT. However, at 48 h after PDT, the tumors were reduced in size. Little damage was observed in normal tissues surrounding the tumor (Figure 3A). Figure 3A shows mice injected with HCT116 cells. Similar results were obtained with the other cell line, HT29 (data not shown).

As shown in Figure 3B, the tumor volume increased by about 3-fold within 5 days in control mice (i.e. kept in darkness without photosensitizer administration, n=8), mice exposed to light alone (i.e. illuminated without photosensitizer administration, n=4) and mice exposed to H$_2$TFPC-SGlc alone (i.e. administered H$_2$TFPC-SGlc and kept in darkness, n=4). In contrast, tumor growth was significantly suppressed after H$_2$TFPC-SGlc-mediated PDT (n=8, $p<0.01$) and Talaporfin-mediated PDT (n=8, $p<0.01$). H$_2$TFPC-SGlc-mediated PDT was more potent than that of Talaporfin-mediated PDT in xenograft tumor models (HT29 tumors, $p<0.01$; HCT116 tumors, $p<0.01$) (Figure 3B). H$_2$TFPC-SGlc-mediated PDT resulted in continued decreases in tumor volume for 5 days post-treatment.

**Discussion**

H$_2$TFPC-SGlc was developed as a chlorine-based photosensitizer, and was expected to have a number of advantages, including significant reductions in dark cytotoxicity, improved water-solubility, greater cellular uptake, and sugar-dependent photocytotoxicity (11-13).
investigated whether H$_2$TFPC-SGlc could act as a potential photosensitizer of PDT in gastric and colon cancer in vitro as well as in vivo. In vitro, H$_2$TFPC-SGlc-mediated PDT is able to induce apoptosis and is about 30 times more cytotoxic than Talaporfin-mediated PDT. In xenograft tumor models, H$_2$TFPC-SGlc-mediated PDT suppressed tumor growth and had no adverse effects on surrounding tissues, as compared to light alone, H$_2$TFPC-SGlc alone and Talaporfin-mediated PDT. Our results indicate that PDT with H$_2$TFPC-SGlc offers a minimally invasive therapeutic modality for clinical treatment of gastric and colon cancer.

Many experiments have been performed in order to develop new photosensitizers that show preferential accumulation within the target tumor tissue for various active targeting approaches, such as peptide conjugates and antibodies (14-18), incorporation within liposomes (19, 20), and encapsulation within polymeric nanoparticles (21-26). For accumulation in the target tumor, H$_2$TFPC-SGlc was developed by linking glucose to the photosensitizer chlorin. The uptake of H$_2$TFPC-SGlc is greater than that of Talaporfin both in vitro (Sakuma et al., in submission) and in vivo (Figure 2A). These results indicate that the glucose-linked photosensitizer is a very useful drug delivery system for cancer cells.

Talaporfin has frequently been reported to shorten the in vivo retention that increases the risk of phototoxicity and the interval required between drug administration and laser treatment (27). The optimal interval length of H$_2$TFPC-SGlc was 4 h, which is the same as that of Talaporfin (Figure 2B). These findings suggest that H$_2$TFPC-SGlc-mediated PDT has the advantage of prolonged photosensitization, similarly to Talaporfin.

In cancer therapy, intense research has been performed to identify the molecules that regulate cross-talk between apoptosis and other major cell death subroutes (e.g. necrosis and autophagic cell death). The necrotic pathway is initiated by photodamage to the endoplasmic reticula/Golgi body, the apoptotic pathway by photodamage to the mitochondria, and the autophagic pathway also by photodamage to the endoplasmic reticula (28). We observed that PDT induced apoptotic cell death with H$_2$TFPC-SGlc in human gastric and colon cancer cells. Apoptosis was also observed in by H$_2$TFPC-SGlc-mediated PDT (Figure 1C). In human melanoma cells (COLO679), the subcellular localization of H$_2$TFPC-SGlc was investigated using fluorescence probes for intracellular organelles, and H$_2$TFPC-SGlc was found to accumulate in mitochondria, lysosomes, Golgi bodies and endoplasmic reticula (Sakuma et al., in submission). The same trend was seen in with human gastric cancer cell lines (data not shown), and it remains possible that PDT with H$_2$TFPC-SGlc induces cell death via necrosis and/or autophagy.

PDT with H$_2$TFPC-SGlc showed significant antitumor effects in vitro (Table I) and in vivo (Figure 3B). Talaporfin, a second-generation photosensitizer, shows improved efficacy when compared with first-generation photosensitizers (7). In this study, H$_2$TFPC-SGlc-mediated PDT was more effective than Talaporfin-mediated PDT. PDT with H$_2$TFPC-SGlc showed significant antitumor effects using relatively low light doses (37.5 J/cm$^2$). Several photosensitizers, including Photofrin and Talaporfin, have typically been used with over 100 J/cm$^2$ for carcinoma xenografts (3, 29-32), and low-dose light irradiation may reduce side-effects related to damage of adjacent normal tissue.

In conclusion, we demonstrated that H$_2$TFPC-SGlc-mediated PDT effectively suppress the growth of xenograft tumors, inducing apoptosis. In addition, H$_2$TFPC-SGlc had superior cancer cell selectivity and was able to reduce side-effects, such as prolonged skin phototoxicity. Based on the potential and characteristics of H$_2$TFPC-SGlc presented in this study, we confirm that H$_2$TFPC-SGlc is a potential photosensitizer for PDT in gastric and colon cancer.

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References

Tanaka et al: Novel PDT with Glycoconjugated Chlorin


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