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Synergic effect of photodynamic therapy using talaporfin sodium with conventional anticancer chemotherapy for the treatment of bile duct carcinoma

Yoshikazu Nonaka, MD,[a] *Atsushi Nanashima, MD,*[a,*] *Takashi Nonaka, MD,*[a] *Masataka Uehara, MD,*[b] *Hajime Isomoto, MD,*[c] *Takafumi Abo, MD,*[a] *Takeshi Nagayasu, MD*[a]

[a] *Division of Surgical Oncology, Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[b] *Department of Regenerative Oral Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[c] *Department of Gastroenterology and Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*

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ABSTRACT

Background: Photodynamic therapy (PDT) is an effective laser treatment for locally treating advanced bile duct carcinoma (BDC). The study objective was to evaluate the synergic effect of PDT using a new photosensitizer, talaporfin sodium (Laserphyrin), in combination with conventional anticancer drug treatments.

Methods: The range of the necrotic area, the percentage of apoptosis-positive cells, the vascular endothelial growth factor expression quantification, and the proliferating cell nuclear antigen–labeling index, as treatment effects, were examined in the BDC cell line (NOZ) *in vitro* and *in vivo* (4-wk-old male BALB/c mice).

Results: Tumor viability was determined by an *in vitro* MTS assay. PDT with a single treatment of 5-fluorouracil, gemcitabine, oxaliplatin, and *cis*-diamminedichloroplatinum showed a significantly lower viability compared with the control or the PDT-alone group ($P < 0.05$). Furthermore, administering PDT combined with two anticancer drugs showed a further decline in the tumor viability. A treatment of PDT combined with oxaliplatin and gemcitabine showed the least viability ($P < 0.05$). Thus, this regimen was administered in the *in vivo* study. The tumor necrotic area, apoptosis positivity, and the vascular endothelial growth factor expression rate were higher in the PDT with anticancer drugs group compared with those of the other groups ($P < 0.05$). The proliferating cell nuclear antigen–labeling index results in the PDT with the anticancer drugs group were significantly lower than those of the other groups ($P < 0.05$).

Conclusions: A treatment of PDT combined with gemcitabine and oxaliplatin showed the best synergic effect for necrosis, apoptosis, and cytostatic alterations for the treatment of BDC.

1. Introduction

Photodynamic therapy (PDT), a cancer-specific treatment based on using light-activated photosensitizers and inducing cytotoxicity in targeted cancer cells, has been widely applied in various cancer treatments [1]. PDT is technically feasible and is a useful modality for treating non-resectable or resectable bile duct carcinomas (BDCs) [2–5].

* *Corresponding author*. Division of Surgical Oncology, Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8581, Japan. Tel.: +81 95 819 7304; fax: +81 95 819 7306.
E-mail address: a-nanasm@nagasaki-u.ac.jp (A. Nanashima).
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Remarkably, PDT treatment induces a powerful antitumor immunologic response [6]. In two randomized controlled trials, PDT provided a longer survival [4,5]. PDT treatment benefits have been reported for treating the targeted area in BDC patients who are receiving chemotherapy or adjuvant chemotherapy after surgery [7,8]. Thus, PDT should be a promising treatment modality to augment the conventional anticancer chemotherapy and brachytherapy as recommended in the 2009 Japanese BDC treatment guidelines [9].

The first clinically approved photosensitizer, porfimer sodium (Photofrin; Wyeth Pharmaceuticals, Collegeville, PA and Wyeth K.K, Tokyo, Japan), is a hematoporphyrin derivative and has a very powerful cytocidal effect on BDC [1–8]. However, the antitumor effect was limited to the shallow bile duct wall because the 630-nm laser used in the treatment had a low permeability [10]. Furthermore, the long period of skin photosensitivity required the patients to be kept away from strong sunlight for several weeks after the drug administration [10]. Therefore, we evaluated a new and effective photosensitizer, mono-L-aspartyl chlorin e6 (talaporfin sodium [TPS]; NPe6, Laserphyrin; Meiji Seika Pharma Co, Ltd, Tokyo, Japan), which has been used for treating malignant tumors, such as bronchial cancer [11–13]. The 664-nm semiconductor laser light activates TPS and penetrates into deep tissue to a depth of >10 mm [14]. Furthermore, Laserphyrin PDT (L-PDT) has a lower skin phototoxicity compared with Photofrin PDT because TPS degrades rapidly *in vivo* [15,16]. Based on the demonstrated clinical effectiveness and the photosensitivity principles, a study that compared L-PDT and Photofrin-PDT for the treatment of human biliary cancer cells was examined [17,18]. The study demonstrated that L-PDT was a more powerful and effective anticancer treatment and had a higher percentage of tumor necrosis and apoptosis, a lower cancer cell proliferation activity, and a higher antiangiogenic activity. Based on these results, a clinical trial evaluating the L-PDT treatment in BDC patients has begun (not published in English). In the BDC patients, various anticancer drugs, such as gemcitabine (Eli Lilly and Co, Indianapolis, IN), have been adopted worldwide, which has resulted in a longer survival period for patients with non-resectable BDC [19,20].

In the future, combining a systemic chemotherapy and an effective local treatment, such as PDT, would be feasible and necessary for BDC treatment. Therefore, we hypothesized that L-PDT combined with a systemic anticancer treatment would show a greater synergic effect to control cancer tissue compared with PDT-alone or systemic chemotherapy–alone treatments. To evaluate our hypothesis, the cytotoxic and angiogenic effects of L-PDT combined with various well-known anticancer drugs were examined in a BDC cell line (NOZ). The percentage of tumor necrosis, terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay to assess the extent of apoptosis, labeling index (LI) of proliferating cell nuclear antigen (PCNA) to determine the cancer proliferative activity, and vascular endothelial growth factor (VEGF) expression quantification as an index of oxygenation of tumor tissue *in vitro* and *in vivo* were the effects evaluated in the present study.

2. Materials and methods

2.1. In vitro *studies of photosensitizer properties*

2.1.1. *Cancer cell culture*

NOZ cells, a human biliary cancer cell line (JCRB1033; Japanese Collection of Research Bioresources, Tokyo, Japan), were cultured in the Dulbecco Modified Eagle Medium (DMEM; Nissui Centical Co, Tokyo, Japan) with 10% fetal bovine serum, glutamine (0.6 mg/mL), penicillin (100 U/mL), and streptomycin (100 mg/mL) at 37°C under a humidified atmosphere of 5% CO_2 in air.

2.1.2. *Cell viability assay*

The effect of PDT on NOZ cell viability was investigated using a novel tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) (Promega Co, Madison, WI). Cells were cultured in 96-well microplates and were irradiated for 24 h. Subsequently, 20 μL of MTS solution (317 μg of MTS/mL phosphate-buffered saline [PBS]) was added to each well, followed by a 4-h incubation. After a complete dye solubilization by vortexing the plate, the absorbance was read on an Immunoreader (model NJ-2000; Nihon Inter Med, Tokyo, Japan) at 490 nm. A cell suspension (1×10^5 cells/mL) was made, and 100 μL per well of this suspension was incubated in 16 wells per plates at 37°C overnight. The cells were decanted and washed twice in PBS. A 100-μL aliquot of each anticancer drug was diluted with DMEM and fetal bovine serum and placed in well plate followed by another overnight incubation at 37°C.

2.1.3. *Photodynamic therapy*

At day 3, PDT was performed for 24 h on the incubated NOZ cell mixture that had previously been treated with an anticancer drug. The NOZ cell mixture was exposed for 24 h to 20 μg/mL of TPS (NPe6, Laserphyrin) diluted with DMEM and irradiated using a semiconductor laser apparatus (ZH-L5011HJP; Panasonic Healthcare Co, Ltd, Tokyo, Japan) that was tuned to 664 ± 2 nm and a 10-Hz frequency (energy density range, total 60 J/cm^2). The estimated TPS dose was 20 μg/mL as determined by our preliminary study [18]. The administered solution was incubated at 37°C for 3 h, later decanted, and then washed thrice in PBS. To induce 50% lethal dose PDT conditions, a laser power of 12 J/cm^2 was chosen to irradiate the NOZ cells. The laser was irradiated for 1 min. Apoptotic induction of the NOZ cells in each group was investigated after the cell treatment under the 50% lethal dose conditions as determined in our preliminary study [18]. Each sample was again incubated overnight at 37°C, and 20 μL of MTS solution (CellTiter96 Aqueous One Solution Cell Proliferation Assay; Promega Co) was added. Incubation at 37°C for 4 h was performed, and the viability of each sample of 16 wells per plate was analyzed thrice using a 96-well plate reader at a 490-nm absorbance.

The anticancer chemotherapeutic drugs that were coadministered *in vitro* with PDT were 5-fluorouracil (5-FU; Kyowa Hakko Kogyo Co Ltd, Tokyo, Japan), gemcitabine (Gemzar; Ei Lilly & Co, Indianapolis, IN), oxaliplatin (Eiplat: Yakutt Housha Co, Ltd, Tokyo, Japan) (Tokyo Chemical Industry, Co, Ltd, Tokyo,