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Photodynamic therapy of human lung cancer xenografts in mice

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ABSTRACT

Background: There is a need to develop novel therapies for non-small cell lung cancer (NSCLC). Photodynamic therapy has been used successfully for endobronchial palliation of NSCLC, and its role in early stages of disease is being explored. We hypothesized that a novel photosensitizer, PS1, would be more effective than the standard agent, porfimer sodium (Photofrin or PFII), in treating human lung cancer xenografts in mice.

Materials and methods: Patient-derived NSCLC xenografts were established subcutaneously in severe combined immune deficiency mice. Two groups of five mice were injected with PS1 (3-[1′-*m*-iodobenzyloxy]ethyl-3-devinylpyropheophorbide-a), a chlorophyll-a derivative, or PFII (a purified version of hematoporphyrin derivative) and then treated with nonthermal laser light. Four mice were treated with laser light without photosensitizer and six mice received no treatment at all. All mice were then observed for tumor growth. The tumor growth end point, time-to-1000 mm^3, was evaluated using standard Kaplan–Meier methods and the log-rank test. Tumor hematoxylin and eosin and caspase 3 staining was done to evaluate necrosis and apoptosis.

Results: The median time-to-1000 mm^3 was 12, 12, 26, and 52 d for the control, light only, PFII, and PS1 groups. There was a significant association between the tumor growth end point and treatment ($P < 0.05$). Hematoxylin and eosin staining revealed <1%, 0%, 67%, and 80% necrosis, and caspase 3 positivity was 2%, <1%, 17%, and 39%, respectively, in the same four groups.

Conclusions: The mice treated with PS1 exhibited a longer time for tumor regrowth and showed more tumor necrosis and apoptosis compared with the other treatment groups. Thus, the novel photosensitizer, PS1, was demonstrated to be more effective than porfimer sodium in this preclinical pilot study.

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1. Introduction

Lung cancer is the most frequent cause of cancer death in both men and women in the United States and accounted for about 27% of all estimated cancer deaths in 2014 [1]. It kills more people than breast, prostate, and colon cancer combined. Given the high incidence and mortality from lung cancer, there is a need to develop novel therapies for this disease. In photodynamic therapy (PDT), systemic administration of a light-activatable drug (i.e., photosensitizer) is followed by illumination of the target tissue with visible light resulting in tumor cell death, microvasculature damage, and local inflammation [2]. PDT has shown great promise in treating a variety of cancers, including lung cancer [2–4]. This treatment modality has remained a promising modality in thoracic oncology for quite some time. It is an established therapy for obstructive or premalignant lesions in the tracheobronchial tree [5–7]. The relative simplicity of this intervention and its highly reliable tumor ablative ability make it attractive as a therapeutic modality for broader application in lung cancer. However, the common adverse effect of skin photosensitivity and the relatively superficial nature of tumor necrosis produced by this modality have contributed to the limited application of PDT in lung cancer. Newer photosensitizers with long wavelength absorptions in the near infrared region with enhanced tumor-specificity and limited skin phototoxicity are in development to address the limitations associated with Photofrin (Pinnacle Biologics Inc. Bannockburn, IL) [8]. This report describes a pilot preclinical study of one such agent, photosensitizer PS1. On the basis of our previous study with PS1 in other tumor models [9], we hypothesized that this novel photosensitizer would be more effective than the current clinical standard, porfimer sodium (Photofrin or PFII), in treating human lung cancer xenografts in mice. This is because of the longer wavelength of absorption, which should result in deeper tumor necrosis when the activating light is delivered on the surface of the tumor. PS1 is still in preclinical trials and has not been used in humans yet.

2. Materials and methods

Patient-derived non-small cell lung cancer (NSCLC) xenografts were established subcutaneously in 20 severe combined immune deficiency (SCID) mice. These mice were part of a larger cohort established from 85 patients who underwent surgical resection of NSCLC from June 2000–June 2010. Samples of lung tumors of various histologies and stages were immediately implanted subcutaneously in non-obese diabetic (NOD) SCID mice (Charles River Laboratories International, Inc, Kingston, NY) under an Institutional Animal Care and Use Committee (IACUC)-approved protocol. Patients with lung tumors proven to be metastases from other primary malignancies were excluded. Of note, the histologies of the primary tumors were adenocarcinoma 46%, squamous cell carcinoma 37%, and others 17%. Also, 87% of the patients had stage I or II disease, whereas 13% had stage III or IV disease. Surgical specimens of patients' lung tumors were received shortly after resection through the Tissue Procurement Facility and cut into 2 mm × 2 mm pieces in tissue culture medium (Roswell Park Memorial Institute 1640 medium) under sterile conditions. SCID mice were then anesthetized with isoflurane and individual tumor pieces were implanted subcutaneously in the abdominal wall of three mice (first passage) and monitored for growth. The mice used in all experiments were 7- to 8-wk-old CB17 SCID mice with an average weight of 18–20 g. Tumor specimens that grew were surgically retrieved and subsequently passaged into recipient mice (second passage) and were considered to have successfully engrafted when these tumors grew. Pathologic diagnosis of patient specimens and evaluation of engrafted tumors were performed in collaboration with a staff pathologist.

There were four groups of mice—two treatment and two control groups. The mice had tumors, which were all 4–6 mm in size at the beginning of the experiment and had been established from an adenocarcinoma patient. Two groups of five mice were injected intravenously with PS1 (3-[1′-*m*-iodobenzyloxy]ethyl-3-devinylpyropheophorbide-a), a chlorophyll-a derivative, or PFII (a purified version of hematoporphyrin derivative). The dose of PS1 was 1.5 μmol/kg based on previous dose optimization studies [9], whereas the dose of PFII was 6 mg/kg based on the dose used clinically in humans. Twenty-four hours later, the subcutaneous tumors in these mice were treated with nonthermal laser light at a wavelength of 630 nm for PFII and 665 nm for PS1. These wavelengths of light were chosen based on the specific characteristics of those photosensitizers. The light source consists of tunable dye lasers (375; Spectra-Physics, Mt. View, CA) pumped by an argon-ion laser (either 171 or 2080; Spectra-Physics). The tumor-bearing mice were restrained without anesthesia in Plexiglas holders, designed to expose to light only the tumor and a 4–5 mm margin of skin. Four mice were treated with laser light with no photosensitizer (sham treatment—two at a wavelength of 630 nm and two at a wavelength of 665 nm) and six mice received no treatment at all. The mice were then observed for 60 d for tumor growth before euthanasia. The tumor volume was calculated by the following formula:

$$\text{Tumor volume } (\text{mm}^3) = \frac{1}{2}(\text{long diameter})\,(\text{short diameter})^2$$

One representative tumor in each group was cut and stained with hematoxylin and eosin and caspase 3 to evaluate necrosis and apoptosis, respectively. All slides were analyzed by one dedicated pathologist. Caspase 3 positive cells were identified using a modified Aperio image analysis cytoplasmic algorithm. Results were reported as percent of tumor necrosis and percent of cells positive for caspase 3.

The tumor volume was modeled as a function of treatment and time (in days) using standard time-series modeling. This type of model will account for the autocorrelated nature of the data, as observations were taken serially from the same mice. Bonferroni adjusted contrast was used to compare the tumor growth rates between treatment groups. The tumor growth end point, time-to-1000 mm^3, was evaluated using standard Kaplan–Meier methods and compared between groups using the log-rank test. All analyses were conducted in Statistical Analysis System (SAS) v9.3 (SAS, Cary, NC) at a significance level of 0.05.