Choline Molecular Imaging with Small-animal PET for Monitoring Tumor Cellular Response to Photodynamic Therapy of Cancer

Baowei Fei [1,2,*], Hesheng Wang[2,1], Chunying Wu[3], Joseph Meyers[2], Liang-Yan Xue[4], Gregory MacLennan[5], Mark Schluchter[6]

[1] Department of Radiology, Emory University, Atlanta, GA
Departments of Biomedical Engineering[2], Radiology[3], Radiation Oncology[4], Pathology[5], Epidemiology and Biostatistics[6], Case Western Reserve University, Cleveland, Ohio
* Corresponding author: bfei@emory.edu

ABSTRACT

We are developing and evaluating choline molecular imaging with positron emission tomography (PET) for monitoring tumor response to photodynamic therapy (PDT) in animal models. Human prostate cancer (PC-3) was studied in athymic nude mice. A second-generation photosensitizer Pc 4 was used for PDT in tumor-bearing mice. MicroPET images with ^{11}C-choline were acquired before PDT and 48 h after PDT. Time-activity curves of ^{11}C-choline uptake were analyzed before and after PDT. For treated tumors, normalized choline uptake decreased significantly 48 h after PDT, compared to the same tumors pre-PDT ($p < 0.001$). However, for the control tumors, normalized choline uptake increased significantly ($p < 0.001$). PET imaging with ^{11}C-choline is sensitive to detect early tumor response to PDT in the animal model of human prostate cancer.

INTRODUCTION

Photodynamic therapy (PDT) is a relatively new therapy that has shown promising for treating various cancers [1]. PDT requires exposure of tumor tissues or cells to a photosensitizing drug followed by irradiation with low-power laser light of the appropriate wavelength. Upon absorption of a photon, the photosensitizer generates singlet oxygen and other reactive oxygen species that are toxic to cells [2]. Suitable animal tumor models and tumor response measurement techniques can be helpful to develop and evaluate new PDT drugs.

In our previous study, we used both MRI and positron emission tomography (PET) to image tumor-bearing mice before and after PDT [3-5]. We used a second-generation PDT drug phthalocyanine 4 (Pc 4) that has been evaluated for treating various human cancers in animal models and is currently under clinical trials [6]. In our MR study, for the treated tumors, the T2 values significantly increased ($p < 0.002$) 24 hours after PDT, compared to the pre-PDT values. For the control tumors, there was no significant difference between the pre-PDT and 24-hour post-PDT values. PET can be used to measure the tumor response to therapy at the cellular level. PET with ^{18}F-fluorodeoxyglucose (FDG) is routinely used to assess tumor response to therapy in oncologic patients. Small animal PET imaging with FDG has been used to monitor changes in glucose uptake after PDT in animals as reported by others [7,8] and we reported [4,5]. A decrease of FDG uptake was observed in treated tumors after PDT.

In this study, we explored the potential of using PET imaging with radiolabeled *choline* as an imaging marker to detect prostate tumor response to PDT. Although PET with radiolabled choline

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has been reported for detection of cancers including prostate cancer [9-12], it has not been applied to study photodynamic therapy. Our experiments included Pc 4-PDT in tumor-bearing mice, microPET imaging with radiolabeled choline before and after PDT, and quantitative analysis of PET data. In the following sections, we report our methods, results and potential clinical applications.

MATERIALS AND METHODS

Pc 4 Formulation

We use a second-generation photosensitizing drug, the silicon phthalocyanine Pc 4, [$HOSiPcOSi(CH_3)_2(CH_2)_3N(CH_3)_2$], that was developed and evaluated for treating a variety of cancers. The chemical synthesis of Pc 4 was described earlier [13]. A stock solution (1 mg/mL) was made by dissolving Pc 4 in 50% Cremophor EL, 50% absolute ethanol, then adding 9 volumes of normal saline with mixing. For injection, the Pc 4 stock solution was mixed with an equal volume of 5% Cremophor EL, 5% ethanol, and 90% saline to give a final concentration of 0.05 mg/mL (0.07 mM).

Tumor Model

The PC-3 cell line is derived from a primary malignant human prostate tumor [14]. PC-3 cells were grown as monolayers in E-MEM supplemented with 15% fetal bovine serum at 37°C. Cells were harvested by trypsinization in ethylenediaminetetraacetic acid/trypsin, washed in Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}, and centrifuged at 150 x g for 5 min. Cells were counted in a hemacytometer using 0.4% trypan blue, and the cell suspension was brought to a final concentration of 1×10^6 cells/mL and kept on ice for immediate injection.

Male athymic nude mice of 4-8 weeks old were housed under pathogen-free conditions. They were maintained under controlled conditions (12-h dark-light cycles; temperature 20-24˚C) with free access to sterilized mouse chow. Two tumors were initiated in each mouse by injection of 50 μL containing 5×10^4 PC-3 cells intradermally on each flank at least 20 mm apart and as far from the lung and heart as possible to minimize motion effects in imaging.

Experimental Protocol

Tumors were treated and imaged when they reached 8-10 mm in diameter, which typically required 2-4 weeks after implantation. A volume of Pc 4 solution was injected intravenously into the tail vein to give 0.6 mg/kg (e.g., 240 μL to a 20 g mouse), a dose that we found to be optimal in another xenograft model (OVCAR-3 ovarian epithelial carcinoma) [15]. Appropriate controls of photosensitizer without light or light without photosensitizer produced no response. Forty-eight hours after photosensitizer injection, the animals were taken to the small-animal imaging facility for imaging and PDT. For PDT, a diode laser (Applied Optronics Corp., Newport, CT) delivered 672-nm light, the longest wavelength absorption maximum of Pc 4. The laser was coupled to a fiber optic cable terminating in a microlens. The treatment light covered the entire tumor and was distributed uniformly throughout the treatment field. One of the two tumors on each animal was irradiated with a fluence of 150 J/cm^2 and an irradiance of 100 mW/cm^2, that has been shown to produce a complete response and some cures in other tumor models [16,17]. The low power of the laser light precludes thermal effects. The other tumor in each animal served

as a control (receiving photosensitizer but no light). Mice were euthanized 48 hours after PDT to measure early histologic responses to Pc 4-PDT.

Radiosynthesis of ^{11}C-Choline

^{11}C-choline instead of ^{18}F-labeled choline analogues was used for the experiments because this study focused on choline metabolic response to PDT. The synthesis method for ^{11}C-Choline was previously reported [18]. ^{11}C-Carbon dioxide was produced by a Scanditronix MC17 cyclotron and bubbled into a reaction vial previously filled with $LiAlH_4$ in tetrahydrofurane (THF) solution (0.1 mol/L, 1 mL) at room temperature. After THF was completely evaporated, hydriodic acid (HI, 57%, 1 mL) was added, and the reaction vial was heated to 120°C. $^{11}C\text{-}CH_3I$ obtained by this "wet" chemistry was then distilled, dried and trapped onto an Accell Plus CM Sep-Pak cartridge which was previously loaded with precursor N,N-dimethylaminoethanol (60 µL) at room temperature. The methylation reaction took placed immediately. The final product was eluted from the cartridge by saline after being washed with ethanol and water and then passed through a 0.2-µm sterile filter. The radiolabeling yield was about 80% (corrected to $^{11}C\text{-}CH_3I$). The radiochemical purity was greater than 99% determined by high-performance liquid chromatography (HPLC) (Partisil SCX cation exchange column, 250 mM NaH_2PO_4/ CH_3CN (9:1, v/v), flow rate: 1.8 mL/min).

PET Studies

MicroPET images were acquired from each mouse forty-eight hours after the injection of the photosensitizer Pc 4 but before PDT (Day 0). A group of four mice was scanned 48 h after PDT. The mice were imaged mo more than two days after PDT because our study focuses on detecting the early response to PDT. A dedicated microPET imaging system (R4, Siemens Preclinical Solutions, Knoxville, TN) was used in this study. Approximately 18.5 MBq of ^{11}C-choline in 0.1 mL of physiological saline were injected into each animal via the tail vein. Mice were immediately scanned for 60 min with a list-mode acquisition that allowed retrospective determination of time-binning of dynamic data. During each imaging session, the animals were taped onto a plastic holder and were provided with a continuous supply of 2% isoflurane (EZAnesthesia, Palmer, PA) in air. Animal respiration rates were monitored throughout the entire experiment; typically, the respiration rate was maintained at 40/min.

Quantitative Image Analysis

The percentage of the injected dose per gram of tissue (%ID/g) was obtained using ASIPro (Acquisition Sinogram and Image Processing, software package installed with the microPET system) and our in-house software. Localization of ^{11}C-choline accumulation in the PET images in relation to anatomical structures was aided by visually comparing PET images with transmission images. From the PET image, each tumor was manually segmented on each image slice. A three-dimensional (3D) region of interest (ROI) was drawn around the tumor regions. A separate 3D ROI was used for each time point because the tumor size tended to change.

Histopathology

Mice were euthanized 48 h after PDT to measure histologic responses to Pc 4-PDT. Eight tumors (4 PDT-treated, 4 controls) were dissected one day after PDT. Dissected tumors were sliced into

2-3 slices and excised tissues were fixed in a large volume of 10% formalin overnight. All tumors were stained with hematoxylin and eosin (H&E) for histopathologic assessment of tumor features. Tissue sections of the entire specimen were then examined with an Olympus BX40 microscope at magnifications ranging from 40X to 400X.

RESULTS

Forty-eight hours after PDT, visible changes were observed on the treated tumor. Fig.1. showed pictures of a mouse before and 48 h after the treatment. The treated tumor showed visible necrosis, and the untreated tumor did not show obvious change. On microPET images, both treated and control tumors were visible before and after PDT in Fig.2. The microPET images showed that the ^{11}C-choline activity within the PDT-treated tumor decreased 48 h after PDT. Within the tumor region, the image demonstrated heterogeneity but the overall ^{11}C-choline activity within the entire tumor decreased after therapy. The control tumor did not show visible change on the PET images before and after therapy.

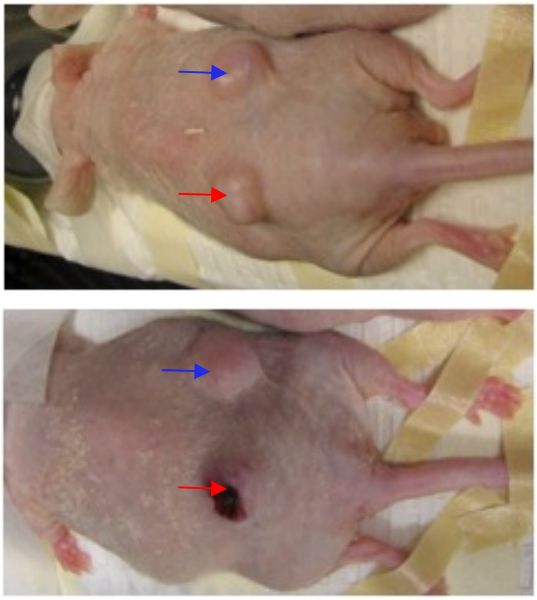

Fig. 1. Pictures of a tumor-bearing mouse before and 48 h after PDT. Top is the mouse before the treatment and the bottom is the picture 48 h after the treatment. The red arrows indicate the treated tumor before and after PDT, which clearly shows the treatment effect. The blue arrows indicate the control tumor that was intact without treatment.

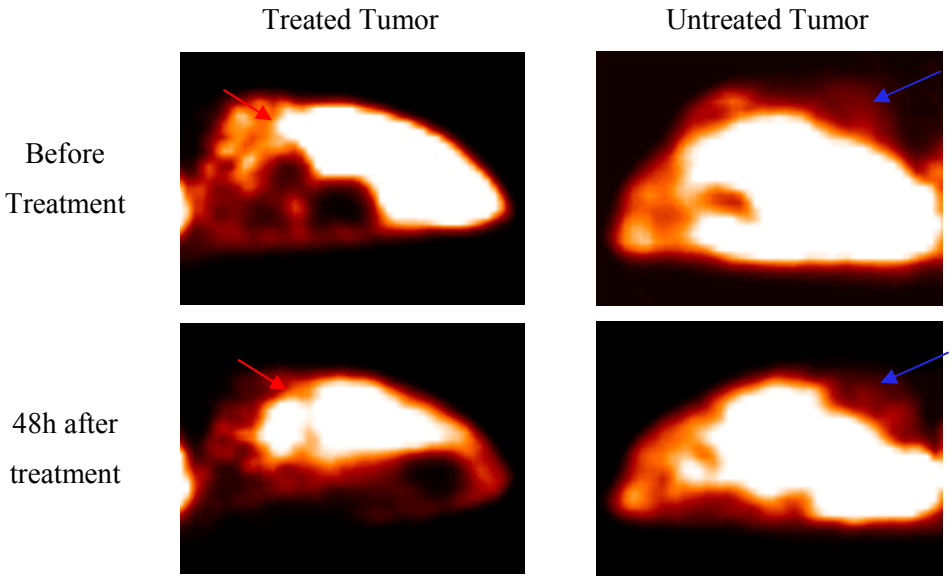

Fig. 2. PET images of the treated and untreated tumors before and 48 h after PDT. The first column is PET image of the treated tumor (arrow) before and 48 h after PDT showing the decrease of the choline uptake. The second column is the PET images of the untreated tumor before and 48 h after PDT and there is no apparent change after treatment.

Normalized time-activity curves of ^{11}C-choline uptake were computed for PC-3 tumors before and 48 h after PDT. Fig. 3 showed the time activity curves before and 48h after PDT of treated and untreated tumors. A decrease in choline uptake was observed in all treated tumors 48 h after therapy. On the histologic image, there are massive areas of inflammation and damages within the treated tumor. In contrast, ^{11}C-choline uptake by the control tumors was increased at the 48 h time point. The increase in the choline uptake may have been caused by tumor growth, as verified by the tumor sizes. Histologic image shows that the untreated tumor was not damaged and the cells were intact.

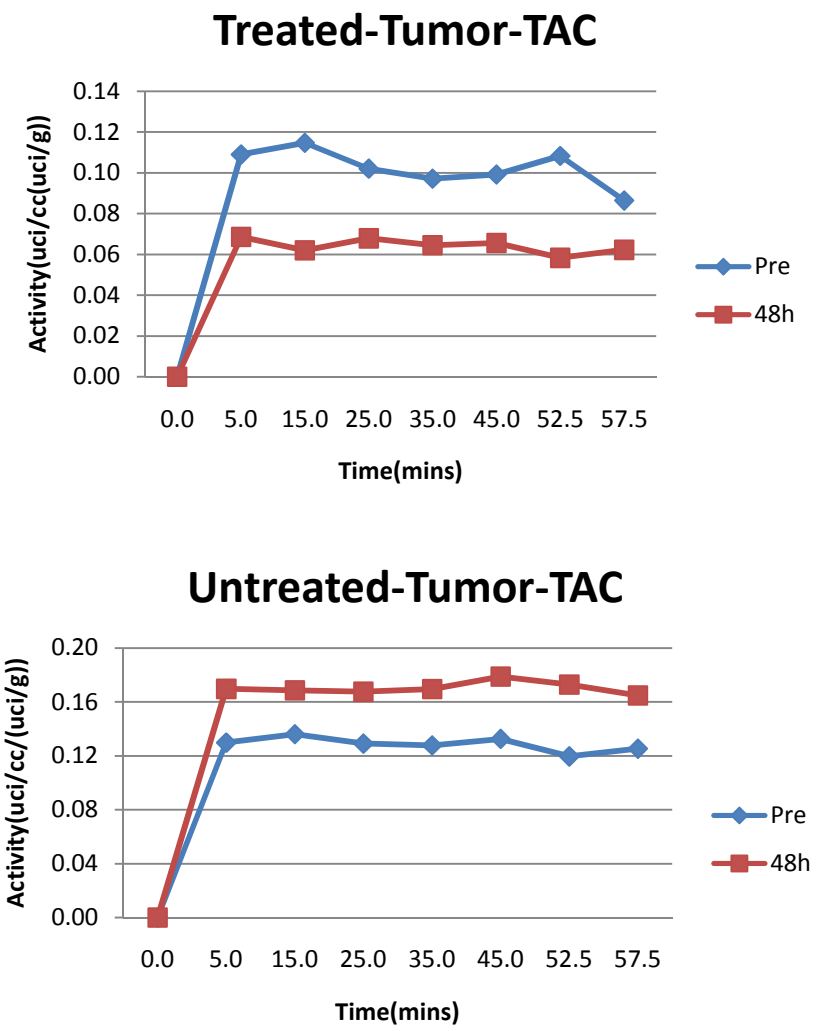

Fig. 3. Time activity curves of the treated (top) and untreated (bottom) tumors. The treated tumor shows a decreased activity 48 h after PDT but the untreated tumor shows an increased activity 48 h after PDT.

DISCUSSION AND CONCLUSION

We evaluated microPET with ^{11}C-choline as a noninvasive imaging marker for monitoring tumor response to PDT in mice. PET images are able to reveal PDT-induced changes in choline uptake of tumors 48 h after therapy. Treated tumors demonstrated markedly decrease of choline uptake after treatment, whereas increases in choline uptake were observed in the contralateral untreated tumor at the same time. Histologic images verified the therapeutic effect on the treated tumors. PET imaging with radiolabeled choline may provide a noninvasive tool for monitoring early tumor response to photodynamic therapy, for evaluating new PDT drugs, and for optimizing the therapy and assessing its efficacy.

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