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Imaging recognition of inhibition of multidrug resistance in human breast cancer xenografts using ^{99m}Tc-labeled sestamibi and tetrofosmin

Zhonglin Liu*, Gail D. Stevenson, Harrison H. Barrett, Lars R. Furenlid, Donald W. Wilson, George A. Kastis, Michael Bettan, James M. Woolfenden

Department of Radiology, The University of Arizona, Tucson, AZ 85724-5067, USA

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Abstract

Background: ^{99m}Tc-sestamibi (MIBI) and ^{99m}Tc-tetrofosmin (TF) are avid transport substrates recognized by the multidrug resistance (MDR) P-glycoprotein (Pgp). This study was designed to compare the properties of MIBI and TF in assessing the inhibition of Pgp by PSC833 in severe combined immunodeficient mice bearing MCF7 human breast tumors using SPECT imaging.

Methods: Animals with drug-sensitive (MCF/WT) and drug-resistant (MCF7/AdrR) tumors were treated by PSC833 and by carrier vehicle 1 h before imaging, respectively. Dynamic images were acquired for 30 min after intravenous injection of MIBI/TF using a SPECT system, FastSPECT. The biodistribution of MIBI and TF was determined at the end of the imaging session.

Results: MCF7/WT in the absence and presence of PSC833 could be visualized by MIBI and TF imaging within 5 min and remained detectable for 30 min postinjection. MCF7/AdrR could be visualized only 2–5 min without PSC833 treatment but could be detected for 30 min with PSC833, very similar to MCF7/WT. MCF7/AdrR without PSC833 showed significantly greater radioactive washout than MCF7/WT and MCF7/AdrR with PSC833 treatment. PSC833 increased the accumulation (%ID/g) in MCF7/AdrR 3.0-fold (1.62 ± 0.15 vs. 0.55 ± 0.05, $P<.05$) for TF and 1.9-fold (1.21 ± 0.04 vs. 0.64 ± 0.05, $P<.05$) for MIBI but did not affect MCF7/WT.

Conclusions: The feasibility of MIBI and TF for assessment of MDR expression and inhibition was demonstrated in mice through FastSPECT imaging. The results indicate that TF may be at least comparable with MIBI in recognizing Pgp expression and modulation.

Keywords: ^{99m}Tc-sestamibi; ^{99m}Tc-tetrofosmin; Multidrug resistance; P-glycoprotein modulation; Human breast cancer; SPECT

1. Introduction

Chemotherapy failure linked to multidrug resistance (MDR) plays an important role in breast cancer death in women. In these cases, malignant tumors with MDR expression are resistant to chemotherapy such that the effectiveness of doxorubicin and other chemotherapeutic drugs is hindered [1–3]. The MDR gene (*MDR1*) encodes a transmembrane P-glycoprotein (Pgp) that generates a broad pattern of resistance to a number of structurally and functionally unrelated drugs. Pgp acts as a membrane-efflux transporter that pumps xenobiotic drugs out of the cancer cells and reduces intracellular drug concentrations dramatically. Pgp is a 170-kDa protein that can be found in the cytoplasm as a storage pool able to maintain a steady-state level of membrane Pgp [4,5]. Overexpression of Pgp can be present in breast tumors at the time of initial diagnosis or can develop following treatment with chemotherapeutic agents associated with MDR. Pgp substrates are generally lipophilic cationic compounds. Along with the drugs, many surrogate markers of Pgp function in vivo can also be expelled from cells by energy-dependent drug-efflux pump. Another transport protein that causes resistance in tumor cells is the MDR-associated protein (MRP). MRP is a 190-kDa protein that transports various natural product oncolytics such as vinca alkaloids, epipodophyllotoxins, anthracyclines and camptothecins [6], most of which are also substrates for Pgp transport. Pgp and MRP can be overexpressed at the same time in drug-resistant cells [7].

MDR modulators or chemosensitizers have been introduced to block the function of Pgp and increase intracellular concentrations of chemotherapeutic drugs in tumors. Examples of modulators include verapamil, cyclosporin A,

* Corresponding author. Tel.: +1 520 626 4248; fax: +1 520 626 2892.
E-mail address: zliu@radiology.arizona.edu (Z. Liu).

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GG918 (elacridar) and PSC833 (valspodar). PSC833 is a cyclosporine derivative that lacks immunosuppressive and nephrotoxic effects and has been reported to be a powerful MDR modulator in animal studies and clinical trials [8–14]. It was also conceived that PSC833 could inhibit the development of cancer by compromising Pgp function [15]. In tumor cells with Pgp expression, PSC833 acts as a competitive Pgp blocker and interferes with the ceramide glycosylation pathways [16–18]. In addition, PSC833 may act on the membrane physical status, inducing a moderate effect on intracellular drug concentration independent of its Pgp-blocking activity [19].

^{99m}Technetium-labeled sestamibi [^{99m}Tc-sestamibi (MIBI)] and tetrofosmin [^{99m}Tc-tetrofosmin (TF)] are lipophilic monocationic radiotracers that are widely used for myocardial perfusion imaging [20]. Sestamibi (Cardiolite) is an isonitrile, while tetrofosmin (Myoview) is a diphosphine. They share the properties of lipophilicity, small molecular size and monocationic charge. MIBI and TF are transport substrates for both Pgp and MRP, and both agents have been used to assess Pgp/MRP-mediated drug resistance in a variety of tumors [21–27]. It has been suggested that cellular accumulation of the cationic agents is inversely proportional to the level of Pgp expression and that enhancement of radioactivity in tumor cells is observed after use of MDR modulators or chemosensitizers [11,21,23,28].

The utility of chemosensitizers in reversing Pgp function and increasing intracellular accumulation of the ^{99m}Tc-labeled cationic agents has not been well studied. Most of the available results of the two lipophilic cationic agents in recognizing MDR and its modulation in tumors are from in vitro studies. There are few data that directly compare properties of TF and MIBI in recognizing MDR modulation by in vivo imaging. Our present study is designed to compare the effect of the Pgp modulator PSC833 on MIBI and TF accumulation in drug-sensitive (MCF7/WT) and drug-resistant (MCF7/AdrR) human breast cancer xenografts in severe combined immunodeficient (SCID) mice. We used a small-animal SPECT system with fast dynamic acquisition capability to quantify the uptake and efflux of the two agents.

2. Materials and methods

2.1. Cell lines

The MCF7/WT cell line was originally obtained from the American Type Culture Collection (#HTB-22, Rockville, MD, USA). The cells are parental, doxorubicin-sensitive human breast carcinoma cells. The doxorubicin-resistant variant MCF7/AdrR was generated in vitro by successive culturing of parental MCF7/WT cells in slowly increasing concentrations of doxorubicin in a multiple-step procedure [29]. Fresh drug was added when the medium was changed three times a week. The concentration of doxorubicin was increased from initially 1×10^{-8} to 7×10^{-8} M over a period of 19 months. An additional 12 months was required to reach the final concentration of 4×10^{-7} M. The resistance to doxorubicin of the MCF7/AdrR variants was 40-fold relative to MCF7/WT cells. Overexpression of Pgp in MCF7/AdrR and the absence of Pgp expression in MCF7/WT were determined by Western blotting using the C219 mouse monoclonal antibody (IgG) and ^{125}I-labeled rabbit anti-mouse IgG as the secondary antibody. The cells were maintained in a drug-free medium for 1 week prior to experiments.

2.2. Establishment of tumor models

Tumor cells were grown in RPMI-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin streptomycin and were maintained in a humidified atmosphere of air containing 5% CO_2 at 37°C. When the cell cultures were grown to 95–100% confluence, the cells were harvested by incubation with 1 ml of trypsin solution/5 ml of Hanks balanced salt solution. The cells (90–100% viability) were counted and resuspended in sterile saline. A total volume of 200 μl with 9×10^6 cells was injected into the right thigh of SCID mice (18–22 g) to establish subcutaneous breast adenocarcinomas. Mice were obtained from the SCID mouse core facility of the University of Arizona Comprehensive Cancer Center and were housed under pathogen-free conditions in microisolator cages with laboratory chow and water available. The volume of tumor was monitored every other day. After 14–20 days, tumors reached a size of 300–500 mm^3, appropriate for imaging.

2.3. Reagents

Tetrofosmin (Myoview) kits were gifts from Amersham Health. Tetrofosmin was radiolabeled according to the manufacturer's instructions. One milliliter of sodium pertechnetate-99m (no less than 60 mCi, 2.22 GBq) was added to the vial and allowed to react at room temperature for 15 min. TF radiochemical purity was more than 95% as determined by instant thin-layer chromatography with silica gel 10×1 cm strip and 35:65 acetone/dichloromethane solvent. MIBI was prepared with a Cardiolite kit (Bristol Myers Squibb) provided by Cardinal Health. The radiochemical purity was greater than 95%. PSC833 was a gift from Novartis (Basel, Switzerland). A stock solution of PSC833 was prepared in DMSO and diluted with saline for intraperitoneal injection at 50 mg/kg 60 min prior to radiotracer administration. The dose and injection schedule were applied according to the methods previously described in the literature and our pilot studies [30].

2.4. Experimental groups

Group I–MCF7/WT control group (MIBI: $n=5$; TF: $n=5$): SCID mice with MCF7/WT tumor xenografts were studied in the absence of PSC833 treatment.