A New in Vivo Method to Study P-Glycoprotein Transport in Tumors and the Blood-Brain Barrier


ABSTRACT

Drug resistance is a major cause of chemotherapy failure in cancer treatment. One reason is the overexpression of the drug efflux pump P-glycoprotein (P-gp), involved in multidrug resistance (MDR). In vivo pharmacokinetic analysis of P-gp transport might identify the capacity of modulation by P-gp substrate modulators, such as cyclosporin A. Therefore, P-gp function was measured in vivo with positron emission tomography (PET) and [14C]verapamil as radiolabeled P-gp substrate.

Studies were performed in rats bearing tumors bilaterally, a P-gp-negative small cell lung carcinoma (GLC4) and its P-gp-overexpressing subline (GLC4/P-gp). For validation, in vitro and biodistribution studies with [13C]daunorubicin and [14C]verapamil were performed.

[14C]Daunorubicin and [14C]verapamil accumulation were higher in GLC4 than in GLC4/P-gp cells. These levels were increased after modulation with cyclosporin A in GLC4/P-gp cells. Biodistribution studies showed 159% and 185% higher levels of [14C]daunorubicin and [14C]verapamil, respectively, in GLC4 than in GLC4/P-gp tumors. After cyclosporin A, [14C]daunorubicin and [14C]verapamil content in the GLC4/P-gp tumor was raised to the level of GLC4 tumors. PET measurements demonstrated a lower [14C]verapamil content in GLC4/P-gp tumors compared with GLC4 tumors. Pretreatment with cyclosporin A increased [14C]verapamil levels in GLC4/P-gp tumors (184%) and in brains (1280%). This pharmacokinetic effect was clearly visualized with PET.

These results show the feasibility of in vivo P-gp function measurement under basal conditions and after modulation in solid tumors and in the brain. Therefore, PET and radiolabeled P-gp substrates may be useful as a clinical tool to select patients who might benefit from the addition of a P-gp modulator to MDR drugs.

INTRODUCTION

Resistance of tumors to chemotherapeutic drugs is a major problem in the treatment of cancer patients. Important anticancer drugs, such as anthracyclines (daunorubicin and doxorubicin), Vinca alkaloids (vincristine, vinblastine), epipodophyllotoxins (etoposide), and taxanes (paclitaxel) are involved in the so-called MDR1 (1–5). Several mechanisms are responsible for MDR. One of them is the overexpression of the MDR1 gene, resulting in increased levels of the ATP-dependent M, 170,000 P-gp drug efflux pump (2, 6–8). Apart from the above-mentioned anticancer drugs, several other nonchemotherapeutic drugs are substrates for this pump (9–19). For instance, for several HIV-1 protease inhibitors such as indinavir, nelfinavir, and saquinavir, it is known that P-gp plays a role in the transport function of these HIV-1 protease inhibitors (19). Also cyclosporin A (an immunosuppressive agent), domperidone (antiemetic agent), and loperamide (antidiarrheal drug) are transported by P-gp (18). To overcome MDR, several drugs have been identified that can inhibit transport by P-gp in P-gp-expressing tissues. Modulation with relatively nontoxic reversal agents such as cyclosporin A and its immunosuppressive analogue PSC833 might increase the pharmacological effects of MDR involved chemotherapeutic drugs in different normal tissues (20, 21) and in P-gp-overexpressing solid tumors. However, until now, clinical studies in solid tumors combining reversal agents with MDR chemotherapeutic drugs are relatively disappointing (10, 22, 23). However, in these studies the P-gp functionality in the tumors is unknown. In these tumors P-gp may be present but does not have to be functional. Then modulation will be disappointing. A technique that would allow us to visualize blockade by modulators of the P-gp drug efflux pump might help to select the proper patients for treatment of MDR involved chemotherapeutic drugs in combination with a modulator. Until now, several studies have been described with 99mTc-labeled substrates and single photon emission computed tomography to visualize a decreased uptake of 99mTc-sestamibi and 99mTc-Q-complexes in solid tumors and in the blood-brain barrier due to P-gp expression (24–28). However, we and others observed for example that 99mTc-sestamibi is apart from P-gp, also a substrate for MRP (29, 30). In the present study, the pharmacokinetics of [14C]verapamil is investigated with PET in tumor-bearing rats. For these studies, rats were inoculated with a human P-gp-negative small cell lung carcinoma cell line GLC4 and its MDR1 gene-transfected, P-gp-overexpressing subline GLC4/P-gp. The potential of imaging P-gp reversal effect by cyclosporin A on the tumor pharmacokinetics of [14C]verapamil was analyzed by PET and validated with biodistribution studies with [13C]daunorubicin and [14C]verapamil. Namely, when [13C]verapamil is combined with cyclosporin A, both compounds are actively transported by P-gp. Because of competition for P-gp, the amount of transported [14C]verapamil will be diminished, resulting in increased radioactivity in the tumor.

MATERIALS AND METHODS

Chemicals. RPMI 1640 and FCS were purchased from Life Technologies, Inc. (Paisley, United Kingdom) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide from Sigma Chemical Co. (St. Louis, MO). Ketamine (Ketalar; 50 mg/ml) was obtained from Parke-Davis (Münich, Germany), xylazine (Rompun solution 2%) was from Bayer (Leverkusen, Germany), cyclosporin A (50 mg/ml) in Cremophor EL (650 mg/ml; Sandimmune) was from Sandoz (Basel, Switzerland), Matrigel was from Becton Dickinson (Bedford, MA), and C219 was from Thaner Diagnostica (Uithoorn, the Netherlands). N-Nor-methyl verapamil was purchased from RBI (Natick, MA), and vincristine was from Eli Lilly (Indianapolis, IN). 9-Formyl trifluoroacetyl daunorubicin was a kind gift from Farmitalia Carlo Erba (Dr. Stauro, Milano, Italy).

Preparation of [14C]Daunorubicin and [14C]Verapamil. [14C]Daunorubicin and [14C]verapamil ([t]1/2 = 20.4 min) were prepared as described by Elingsa et al. (31). The specific activity of [14C]daunorubicin was >370 GBq/mmol and [14C]verapamil was 555 GBq/mmol at the end of the synthesis. The [14C]-labeled radiopharmaceuticals were purified by high-pres-
growth phase, 3,750 and 10,000 per well for GLC4 and GLC4/P-gp, respectively. Cell survival test, the microculture tetrazolium assay (33). Cells in logarithmic phase were pooled (32). The virus titer was 1 × 10^4 particles per ml, the retrovirus-producing packaging cell line GP1 (50 nM) twice a week. Daunorubicin resistance was tested by exposure of GLC4/P-gp cell line was assayed by culturing this line in the presence of vincristine (50 nM). Thereafter, the GLC4/P-gp subline was obtained after infection of GLC4 cells with a retrovirus gene-carrying retrovirus. The P-gp expression in cell lines and tumor tissue was tested with the P-gp-directed monoclonal antibody C219. To check whether the MRPs expression was induced, MRPs was stained with monoclonal antibody (kindly provided by Dr. R. Schepers, Free University, Amsterdam, the Netherlands) on cytopsin and tumor tissue (34).

**Cellular Accumulation of [11C]Daunorubicin and [11C]Verapamil in Tumor Cells.** A total of 2 × 10^6 GLC4 and GLC4/P-gp cells were incubated in polystyrene tubes for 60 min at 37°C with 5 μM [11C]daunorubicin or 5 μM [11C]verapamil in 5 ml of RPMI 1640/10% FCS. To study modulating effects, GLC4 and GLC4/P-gp cell lines were incubated simultaneously with cyclosporin A (50 μM) and daunorubicin (5 μM) or verapamil (5 μM). Thereafter, the cells were washed with 5 ml of ice-cold PBS (0.14 M NaCl, 2.7 M KCl, 6.4 mM Na₂HPO₄, and 1.5 mM KH₂PO₄, pH 7.4), followed by centrifugation (5 min of 180 g; 4°C). The supernatant was removed, and the cellular radioactivity was measured in water in a gamma counter (KLB Wallac, Turku, Finland). Correction for the extracellular adhesion of radioactivity to polystyrene tubes was performed by subtracting the results obtained after incubation with [11C]daunorubicin or [11C]verapamil for 60 min at 4°C. The cellular accumulation is expressed as % dose/10^6 cells (mean ± SD).

**Conventional Biodistribution of [11C]Daunorubicin and [11C]Verapamil in Nude Rats.** Tumor rats were anesthetized with ketamine:xylazine (2:1; 1 ml/kg). Thereafter, 0.3 ml [11C]daunorubicin (10 mg/kg) or [11C]verapamil (0.1 mg/kg) was injected into the tail vein. After 60 min, the rats were sacrificed by extirpation of the heart, and several tissues were dissected. Heparin-plasma was obtained from the collected blood by centrifugation (3 min; 1000 g). Radioactivity was measured with a gamma counter. Modulation of [11C]daunorubicin and [11C]verapamil kinetics with cyclosporin A was tested in the nude rats. Cyclosporin A was injected into the peritoneal cavity (50 mg/kg). After 30 min, [11C]verapamil or [11C]daunorubicin was administered into the tail vein. Sixty min after the injection of [11C]verapamil or [11C]daunorubicin, the biodistribution of [11C]-labeled radioactivity was measured as described above. Results are expressed as ng/g tumor for [11C]daunorubicin and as μg/g tumor for [11C]verapamil.

**Tumor Histology.** To allow histological data of GLC4 and GLC4/P-gp tumors, the 1–2 cm-sized tumors were excised and frozen in liquid nitrogen. From the frozen tumor tissue, 4-μm slices were cut. The cell viability and the cell survival by 50% (IC50) were determined as the mean of test samples/mean of untreated samples. Controls consisted of media without cells (background extinction) and cells incubated with medium instead of the drug. Three independent experiments were performed, each in quadruplicate. Results were expressed as mean ± SD. From the survival curves, the daunorubicin concentrations were determined that inhibited cell survival by 50% (IC50).

**Animal Model.** Male nude rats (HSD Ham RNU nu; Harlan, Horst, the Netherlands; 280–300 g; age, 6 weeks) were inoculated subcutaneously in the left and the right sides, respectively, of the nude rat. Ten to 14 days after injection of the cells, solid tumor nodules of −1 cm diameter were grown. Thereafter, the tumors were removed, and pieces of GLC4 and GLC4/P-gp tumors (−2–3 mm in diameter) were transplanted in the left and the right sides, respectively, in other male nude rats (HSD Ham RNU nu; Harlan). Fourteen days after transplantation of these GLC4 and GLC4/P-gp tumor pieces, tumors of 1–2 cm in diameter were developed. These rats were used various experimental settings. All experiments were carried out in compliance with the local guidelines.

**Fig. 1.** Cell survival after 1 h incubation with daunorubicin in GLC4 (●) and GLC4/P-gp (○). At all concentrations, survival of GLC4 was significantly reduced compared with GLC4/P-gp. Each point represents the mean of three independent experiments, each performed in quadruplicate; bars, SD.

**Fig. 2.** [11C]Daunorubicin and [11C]verapamil accumulation (y-axis) in GLC4 and GLC4/P-gp tumor cells (x-axis) after 1 h incubation plus (○) or minus (●) 50 μM cyclosporin A. Data are expressed as means of three independent experiments, each performed in duplicate; bars, SD.
Radioactivity Clearance from Plasma. Rats were anesthetized as described above, and the carotid artery was cannulated. Arterial blood samples (100–200 μl) were drawn at various time points from 0–60 min after injection of the 11C-labeled radioactivity. Plasma and RBCs were separated by centrifugation (3 min; 3000 g). Plasma (50 μl) samples were counted in a calibrated gamma counter. Results were expressed as mean ± SD.

Analysis of [11C]Verapamil Metabolism. For analysis of [11C]verapamil, 1 h after injection of radioactivity in the penile vein, the rats were sacrificed by exsanguination. In the brain, the plasma and both tumors, metabolism of [11C]verapamil was studied. These tissues were homogenized in acetonitrile (2 ml), using a Heidolph D70 600 apparatus (Salm and Kipp b.v., Breukelen, the Netherlands). Thereafter, the extracts were centrifuged (3 min; 3000 g), and acetonitrile was evaporated under reduced pressure at 50°C. To the extracts of the brain and the tumors, 180 μl of sterile water, 20 μl of sodium hydroxide (0.1 M), and 1 ml of diethyl-ether was added. The mixtures were vortexed for 1 min and centrifuged (5 min; 3000 g). The organic phase was separated and acidified with 300 μl of sulfuric acid (0.5 M). Subsequently, the mixtures were vortexed for 1 min and centrifuged (5 min; 3000 g). To the supernatant, 350 μl of sterile water was added, and this was applied onto a HPLC column [Novapack C18 4 μm, 150 × 3.9 mm; mobile phase, KH2PO4 (0.1 M): acetonitrile = 70:30; pH 2.5 by addition of ortho-phosphoric acid; flow rate, 1.0 ml/min]. Fractions (0.5 ml) of the eluate were collected during 10 min and counted in a calibrated gamma counter (LKB Wallac, Turku, Finland). The radioactive recovery was >80%. The recovery was calculated as follows. Before injection of radioactivity on HPLC, the radioactivity was counted in a calibrated gamma counter. After separation of fractions on a HPLC column, all of the fractions were counted, and the eluted percentage of radioactivity over the HPLC column was calculated. Results are expressed as percentage of parent compound (mean ± SD).

PET Studies in Tumor-bearing Rats. The pharmacokinetics of [11C]verapamil in rats were studied with a positron camera. After anesthesia as described above, the long axis of the rat was positioned in the PET camera (ECAT 951/31 positron camera; Siemens/CTI, Knoxville, TN) parallel to the transaxial plane of the tomograph to obtain sagittal sections. A transmission scan to correct for attenuation of photons by the body tissues was obtained immediately before the emission scan. Because of homogeneous diffusion of [15O]H2O over the rat body, a PET-[15O]H2O study is useful for a good anatomical localization of the brain and the tumors. Therefore, [15O]H2O (37 MBq) dissolved in 0.3 ml of saline was injected into the tail vein. The following frames were acquired: 4 consecutive frames of 5 s, 1 frame of 10 s, 2 consecutive frames of 30 s, and 1 frame of 120 s. Twenty min after injection of [15O]H2O, [11C]verapamil (37 MBq, 0.1 mg/kg) was injected into the tail vein. The following frames were acquired: 12 consecutive frames of 10 s, 6 of 30 s, 5 of 60 s, 5 of 120 s, and 8 of 300 s. After finishing [11C]verapamil kinetics, cyclosporin A (50 mg/kg, i.v.) was injected into the penile vein. Thirty min after injection of cyclosporin A, [11C]verapamil (0.1 mg/kg) was injected in the tail vein, and an emission scan was obtained as described above. Therefore, in this experimental setting, the rats were their own controls when modulating effects of cyclosporin A on [11C]verapamil kinetics in GLC4 and GLC4/P-gp tumors were investigated. In addition, to avoid carryover of the first [11C]verapamil injection, there was a time interval of at least 100 min between both injections. After 100 min, only ±3% of [11C] radioactivity could be present when [11C]verapamil was injected for the second time. Radioactive decay was corrected automatically, and data analysis was performed using the Siemens ECAT software (V6.3 D) on a Sun workstation. Results are expressed as percentage of injected dose/ml tumor.

Statistical Analysis. Differences in 11C accumulation in GLC4 and GLC4/P-gp tumors and differences in 11C accumulation with and without cyclosporin A modulation were analyzed using Wilcoxon’s test (two-sided). P < 0.05 were considered significant.

RESULTS

Cytotoxicity Assay. Cell survival curves of the GLC4 and GLC4/P-gp cell lines after exposure to daunorubicin are shown in Fig. 1. GLC4 is 12.2-fold more sensitive for daunorubicin than GLC4/P-gp. The IC50s for daunorubicin are 36 nm for GLC4 cell line and 440 nm for the GLC4/P-gp cell line.

Cellular Accumulation of [11C]Daunorubicin and [11C]Verapamil. Cellular accumulation of [11C]daunorubicin and [11C]verapamil were 3.3-fold (P < 0.001) and 2.3-fold (P < 0.001) higher, respectively, in GLC4 compared with GLC4/P-gp. Coincubation with cyclosporin A increased the cellular content of [11C]daunorubicin and [11C]verapamil in GLC4/P-gp 3.2-fold (P < 0.005) and 2.0-fold (P < 0.02), respectively. Cyclosporin A did not affect the cellular content of [11C]daunorubicin and [11C]verapamil in GLC4 cells (Fig. 2). In addition, although the ratio of [11C]daunorubicin accumulation in GLC4 and GLC4/P-gp is higher than the [11C]verapamil ratio, only camera studies in vivo with [11C]verapamil were performed. The reason for this was that the fact that [11C]verapamil production could be scaled up so that sufficiently high enough radioactivity allowed extensive in vivo studies in rats using the positron camera.

Tumor Histology. A semiquantitative assessment demonstrated that >80–90% of the cells in both tumor types were viable. The GLC4 and the GLC4/P-gp tumors consisted of tumor cells, blood vessels, and rat stroma. Histology revealed that in GLC4 tumors, the cell density was lower than in GLC4/P-gp tumors (P = 0.0008; n = 3). The cell density in GLC4 tumors was 8.7 × 109 ± 0.7 × 109 cells/ml tumor and in GLC4/P-gp tumors, 13 × 109 ± 0.4 × 109 cells/ml tumor. GLC4 tumor tissue showed no P-gp expression, and GLC4/P-gp stained clearly for P-gp. Furthermore, the GLC4 and GLC4/P-gp tumors showed an equally weak MRp staining (data not shown).

Conventional Biodistribution of [11C]Daunorubicin and [11C]Verapamil in Tumor Tissue of Tumor-bearing Nude Rats. One h after injection, the [11C]daunorubicin content was 159 ± 28% higher (n = 4; P < 0.05) in GLC4 tumors than in GLC4/P-gp tumors. Results for individual animals with their paired tumors are shown in Fig. 3A. Modulation with cyclosporin A (50 mg/kg) increased the [11C]daunorubicin levels in the GLC4/P-gp tumor to the same levels as compared with GLC4 tumors (Fig. 3B). In addition, after modulation with cyclosporin A, [11C]daunorubicin levels were not significantly increased in any other tissue than the GLC4/P-gp tumor (data not shown). After injection of [11C]verapamil, the verapamil content was 184 ± 53% higher (n = 5; P < 0.05) in GLC4 tumors than in GLC4/P-gp tumors (Fig. 4A). The tissue levels obtained after injection of [11C]verapamil are summarized in Table 1. After modulation with cyclosporin A (50 mg/kg), the [11C]verapamil content in the GLC4/P-gp tumor was raised to the level of the GLC4 tumor (Fig. 4B). In addition, the results of the [11C]verapamil content observed in other tissues are shown in Table 1. One h after injection of [11C]verapamil, [11C]verapamil was present as parent compound for 90 ± 7% (n = 3) in GLC4 and GLC4/P-gp tumor tissue, in plasma for 83 ± 5% (n = 5), and in the brain for 93 ± 5% (n = 3).

Plasma Clearance in Tumor-bearing Nude Rats. Plasma clearance of 11C was rapid after [11C]daunorubicin injection (10 mg/kg; data not shown). The curve showed a biphasic pattern. The first phase had a half-life of 0.70 min, and the second phase had a half-life of 69.3 min. After [11C]verapamil injection (0.1 mg/kg), a biphasic plasma curve was shown (data not shown). The first phase had a half-life of 0.50 min, and the second phase had a half-life of 69.3 min. Furthermore, no significant differences in [11C]daunorubicin and [11C]verapamil arterial plasma kinetics were observed after modulation with cyclosporin A (data not shown).

PET of [15O]H2O in the Brain and the Tumors of the Rat. Experiments were performed with [15O]H2O. PET-H2O images enable separate localization of the tumor and the head region (data not shown).

PET of [11C]Verapamil in the Brain and the Tumors of the Rat. Time activity curves after [11C]verapamil injection (0.1 mg/kg) were obtained from PET images (Fig. 5) in three different rats. Fig. 6a demonstrates different pharmacokinetics in GLC4 and GLC4/P-gp.
Cyclosporin A (50 mg/kg), the accumulation of [11C]verapamil was significantly increased in GLC4 tumors compared with GLC4/P-gp tumors. Pretreatment with cyclosporin A increased the levels of [11C]daunorubicin and [11C]verapamil in GLC4/P-gp tumors to the level in the GLC4 tumors. The effects of cyclosporin A in the GLC4/P-gp tumors were most likely due to blockade of the P-gp drug efflux by cyclosporin A. This is underscored by the fact that cyclosporin A had no effect on plasma kinetics of [11C]daunorubicin and [11C]verapamil in the rat.

In the blood-brain barrier, P-gp is abundantly expressed (35), and because in this study [11C]verapamil levels were increased after pretreatment with cyclosporin A, we were able to show P-gp function and its reversal effects ex vivo and in vivo. This important role of P-gp in the blood-brain barrier was also shown earlier in the mouse model. In biodistribution studies in the brain of mdrla(−/−) P-gp knock-out mice, drug levels of several drugs, such as verapamil, digoxin, and cyclosporin A, were found to be increased compared with mdrla(+/+) wild-type mice (16, 17). Furthermore, in wild-type mice, the brain levels of digoxin were increased after blockade of P-gp in the blood-brain barrier by PSC 833 (20). The [11C]daunorubicin and [11C]verapamil biodistribution data are in agreement with results obtained with the radiolabeled P-gp substrate colchicine, confirming that it is possible to distinguish P-gp-expressing tumors from sensitive tumors in vivo by [11C]-radiolabeled drugs (36–38). In contrast to verapamil, the daunorubicin levels in the brain were not increased after modulation with cyclosporin A. Daunorubicin is a substrate for P-gp as well as MRP1. Because of expression of MRP1, in many tissues in the body, including the blood-brain barrier (39), it might be present in GLC4 and GLC4/P-gp tumors 1 h after exposure to [11C]daunorubicin (10 mg/kg) in the absence (A) and presence (B) of cyclosporin A (50 mg/kg). The content was expressed as ng [11C]daunorubicin/g tumor. Each paired set of points represents the radioactivity level in a two-sided tumor-bearing rat. In each animal, the [11C]daunorubicin content was significantly increased in GLC4 tumors compared with GLC4/P-gp tumors (P < 0.05). After modulation with cyclosporin A, there was no significant difference between [11C]daunorubicin levels in GLC4 tumors and GLC4/P-gp tumors.

**Table 1** Tissue levels of radioactivity in tumor-bearing nude rats 1 h after i.v. injection of [11C]verapamil (0.1 mg/kg) (A) and effects on [11C]verapamil content after modulation with cyclosporin A (50 mg/kg) (B).

<table>
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<th>Tissue</th>
<th>A (n = 3–5)</th>
<th>B (n = 3–4)</th>
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<tr>
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*NS, not significant.
possible that after modulation with cyclosporin A, daunorubicin is still transported by the MRP1 drug efflux pump.

Ex vivo data demonstrated the usefulness of P-gp-mediated [11C]verapamil kinetics in the P-gp-expressing tumor and in the central nervous system. For [11C]verapamil production, it was possible to scale up so that sufficiently high enough radioactivity was available for in vivo studies in rats and potentially for humans with a positron camera. Therefore, in vivo studies were performed with [11C]verapamil. After injection of [11C]verapamil in the rat, a fast radioactivity transport was observed in the P-gp-overexpressing tumor, and 10 min after injection, a steady-state level was reached (Fig. 6). Possibly, due to the high lipophilicity, verapamil may be bound in the tumor, preventing transport from the tumor back into the blood. Furthermore, it might be possible that other routes such as passive efflux may play an important role in the cellular transport of verapamil. It is speculated that the time-activity curve of [11C]verapamil might be the result of partly passive efflux of verapamil over tumor membranes and partly by P-gp-mediated pharmacokinetics. This is supported by the observed rapid cellular verapamil passive diffusion of 50% within 5 min for P-gp-negative and P-gp-positive ovarian carcinoma cell lines (40, 41).

Several experiments with 99mTc-sestamibi and other 99mTc-labeled substrates, such as 99mTc-Q-complexes and 99mTc-tetrofosmin, demonstrated in rats and in patients an increased efflux of 99mTc-sestamibi in P-gp-expressing tumor cells and modulation effects with reversal agents (24–28, 42–44). However, with single photon emission computed tomography, quantitative information about pharmacokinetics in the tumor is less accurate than with PET. Recently, we have shown that 99mTc-sestamibi is also a substrate for MRP1 (29, 30). In contrast to 99mTc-sestamibi, [11C]verapamil is a relatively poor substrate for MRP1. This makes [11C]verapamil a more specific tracer for P-gp function than 99mTc-sestamibi. Comparison of sensitivity between 99mTc-sestamibi and [11C]verapamil demonstrates that 99mTc-sestamibi has the ability to image P-gp in a tumor that is 2–3-fold more resistant for doxorubicin compared with the P-gp-negative tumor (25). In contrast, [11C]verapamil showed 2-fold less radioactivity in a P-gp-expressing tumor, which is 12-fold resistant for doxorubicin compared with a P-gp-negative tumor. Because of high lipophilicity of [11C]verapamil, this compound most likely binds aspecifically to cellular membranes. Additional studies in cancer patients have to be performed to elucidate whether it is possible to measure low resistance factors with this PET approach in human tumors.

In conclusion, P-gp kinetics and its reversal can be visualized in vivo with a positron camera. This can be a new clinical tool to study the P-gp function noninvasively and to predict the effect of modulators on treatment with MDR involved chemotherapeutic drugs.
REFERENCES


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