Detection of *in Vivo* P-Glycoprotein Inhibition by PSC 833 Using Tc-99m Sestamibi

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ABSTRACT

Tc-99m sestamibi is a substrate of P-glycoprotein (Pgp) that has been proposed for use as a functional imaging agent for the multidrug resistance-1 phenotype. *In vitro*, retention of sestamibi by cells that overexpress Pgp can be modified by the presence of Pgp antagonists. In a Phase I trial of the Pgp reversal agent PSC 833, we show that the effects of this reversal agent can also be demonstrated in patients. Nine patients with metastatic renal carcinoma were studied three times: at baseline, approximately 1 day after vinblastine infusion, and while on PSC 833. One patient with metastatic adrenocortical cancer was also studied. Time activity curves and areas under the curve (AUCs) were obtained for tumor, liver, lung, and myocardium, and organ:heart AUC ratios were generated. With PSC 833, tumor visualization was enhanced, and statistically significant increases were found in AUC ratios for tumor and liver compared to baseline. For the liver, significant differences were also found between the vinblastine *versus* PSC 833 scans but not between the baseline *versus* vinblastine scans. This study demonstrates that sestamibi retention by tumor and liver is altered in the presence of the reversal agent PSC 833, presumably reflecting inhibition of Pgp. Thus, sestamibi may be useful *in vivo* as a means of monitoring the effects of this and other reversal agents on various tumors and normal tissues that express Pgp.

INTRODUCTION

Treatment of many tumors is hampered by the presence or development of multidrug resistance. One cause of such resistance is expression of the *MDR-1* gene, which encodes P-glycoprotein, a 170,000 membrane transport protein. This protein, found in many normal as well as malignant tissues, functions as an energy-dependent efflux pump (1) and recognizes a wide variety of chemotherapeutic agents, including the anthracyclines, Vinca alkaloids, paclitaxel, actinomycin D, and etoposide (2). One approach to this problem of multidrug resistance has been to develop antagonists or "reversal agents" that can inhibit Pgp-mediated drug efflux through competitive inhibition (3) or interactions with Pgp at non-drug-binding sites (4). It is hoped that these drugs, when given in conjunction with chemotherapy, will inhibit Pgp and enhance the effectiveness of the chemotherapy (5). To date, limited success has been reported with a variety of reversal agents, primarily in patients with hematological malignancies as opposed to solid tumors (6).

Recently, Piwnica-Worms et al. (7) demonstrated that Tc-99m sestamibi is also a substrate of Pgp and that it can be used as a functional imaging agent for Pgp in tumor xenografts in nude mice. They and others have shown that tumor retention of sestamibi correlates inversely with the degree of Pgp expression and that this can be modified *in vitro* by the presence of Pgp antagonists. In humans, Ciarmiello et al. (8) demonstrated that preoperative washout rates of sestamibi from primary breast tumors correlated with levels of Pgp found in the surgically resected specimens. Others have suggested that sestamibi can serve as an *in vivo* marker for Pgp and that it be used in patients to guide chemotherapy (9, 10).

PSC 833, an analogue of cyclosporine A, is currently under development as a second-generation Pgp antagonist. Cyclosporine A, itself a potent antagonist, is limited in clinical use by nephrotoxicity and immunosuppression. PSC 833, which is neither nephrotoxic nor immunosuppressive, is more potent than cyclosporine and effectively reverses multidrug-resistant cell lines *in vitro* at 100 ng/ml (11–14). Like cyclosporine A, PSC 833 causes a delay in the clearance of concomitantly administered anticancer agents. This delay in clearance has been ascribed to inhibition of Pgp in the biliary canaliculi and to competition for metabolism by the P450 enzymes (15).

Combining sestamibi scanning with a Phase I trial of vinblastine given in conjunction with PSC 833, we explored the possibility that sestamibi could be used as an *in vivo* indicator of the effectiveness of the reversal agent on tumor. If retention of sestamibi by tumor is increased in the presence of the reversal agent, this would be indirect evidence that the reversal agent is also increasing tumor retention of the chemotherapeutic agent, and consequently, that it is acting as hoped to decrease tumor drug resistance.
PATIENTS AND METHODS

Patients. Nine patients with metastatic renal carcinoma and one with metastatic adrenocortical cancer enrolled in a Phase I study combining infusional vinblastine with the P-glycoprotein antagonist PSC 833 ([3'-keto-Bmt]-[Val2]-cy-closporine; Novartis Pharmaceuticals Corporation) and con- sented to undergo serial Tc-99m sestamibi scanning. Patients initially received a 5-day continuous i.v. infusion of vinblastine (1.2 mg/kg/day), followed by an 8-day course of oral PSC 833, during which pharmacokinetic studies were performed. Subsequent cycles involved concomitant administration of the two drugs. Sestamibi scans were performed during the first cycle: at baseline, after vinblastine was discontinued, and while on PSC 833. The patient with adrenocortical cancer underwent only the post-vinblastine and PSC 833 scans.

The nine renal cancer patients ranged from 40 to 58 years old (mean, 48 years) and all were male. One patient with metastatic disease in the ribs and adjacent pleura had not had any previous surgery or therapy, and his primary tumor was present at the time of scanning. Primary tumors had been re-sected in the other eight patients, who had all undergone previous surgery and extensive chemotherapy.

Tumor size was calculated based on the formula for the volume of an ellipse: \( V = \pi da db dc \), where \( a \), \( b \), and \( c \) are dimensions obtained from radiographs or computed tomography. In one patient whose index lesion was located in the deltoid muscle, clinical estimates of dimensions were used.

Measurement of MDR-1 Levels. Adequate biopsy tissue was obtained from three patients. MDR-1 levels were determined by PCR as previously described (16). PCR product was electrophoresed in \( 1 \times \) Tris-borate electrophoresis buffer with 2 mm EDTA on 2% NuSiev/1% agarose gels, after the volume was reduced by evaporation. Gels were then stained with 2 \( \mu \)g/ml ethidium bromide and analyzed using a Foto/ Eclipse image analyzer (Fotodyne, Inc., Hartland, WI). All MDR-1 values were normalized to the level found in SW620 human colon carcinoma cells, an unselected cell line expressing a low but potentially clinically significant level of MDR-1. These cells can be sensitized 2–5-fold to vincristine by the Pgp antagonist verapamil (17). For normalization, the MDR-1 level in unsensitized SW620 cells was arbitrarily assigned a value of 10.

Imaging. Patients were scanned on three occasions: at baseline, at least 1 day (range, 1–3 days) after ending vinblas-tine, and approximately 7 days (range, 5–8 days) after starting PSC 833. PSC 833 scans were generally performed 1–3 h after the patient’s morning dose of PSC 833. Anterior and posterior images were acquired using a 256 \( \times \) 256 matrix, low-energy/high-resolution collimators, and a 20\(^{\circ}\) window centered over the 140-keV photopeak of Tc-99m. Large field of view, dual-headed cameras (ADAC Laboratories, Milpitas, CA) were used. For each individual patient, the same camera was used for all three studies.

Images were obtained as previously described (18). Patients were positioned under the camera, with the heart, liver, and metastatic lesions in the field of view. Immediately following bolus administration of 20 mCi Tc-99m sestamibi, thirty 1-min sequential images were acquired. These were followed by 5-min spot images of the body that were repeated at approximately 1, 2, 3, and 5 h.

Image Analysis. In patients with tumor visualization, an “index lesion” was chosen for analysis. This was the lesion that was visualized best and that had the least overlap with other structures. TACs were generated over index lesions, liver, heart muscle, and normal lung, when possible. For liver, heart, and lung, geometric mean TACs were calculated, whereas for tumor, either the anterior or posterior TAC was used, depending on the location of the lesion. Curves were background and decay corrected and then individually normalized to percentage of maximum counts. Early (1–4 min) frames demonstrating obvi-ous vascular activity from the bolus injection were excluded. Where possible, washout half-lives (\( t_{1/2} \)) were calculated from single exponential fits of the first 30 min of the TACs.

Using the normalized TACs, AUCs were calculated for 0–180 min for each TAC using trapezoidal integration, and organ:heart AUC ratios were generated using the heart as a control. Heart muscle was chosen as a control because it con-

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Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Previous therapy</th>
<th>Index lesion*</th>
<th>Index dimensions (cm)</th>
<th>Index volume (cm(^3))</th>
<th>Sestamibi visualization</th>
<th>Other disease sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>IL2, perf</td>
<td>Mediat.</td>
<td>4.5 ( \times ) 7 ( \times ) 6</td>
<td>98.9</td>
<td>Negative</td>
<td>Bilateral lung, brain</td>
</tr>
<tr>
<td>2</td>
<td>40M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>IFN, IL2</td>
<td>Lung</td>
<td>2.6 ( \times ) 2.9 ( \times ) 3</td>
<td>11.8</td>
<td>Positive</td>
<td>Retroperitoneum</td>
</tr>
<tr>
<td>3</td>
<td>27F</td>
<td>Female</td>
<td>Adrenocortical Ca</td>
<td>C</td>
<td>Lung</td>
<td>2 ( \times ) 2 ( \times ) 2</td>
<td>4.2</td>
<td>Positive</td>
<td>Retroperitoneum</td>
</tr>
<tr>
<td>4</td>
<td>48M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>IL2</td>
<td>Lung</td>
<td>5 ( \times ) 4 ( \times ) 3.5</td>
<td>36.6</td>
<td>Positive</td>
<td>Bilateral lung</td>
</tr>
<tr>
<td>5</td>
<td>51M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>IFN, IL2</td>
<td>Lung</td>
<td>3.8 ( \times ) 2.2 ( \times ) 2.4</td>
<td>10.5</td>
<td>Positive</td>
<td>Retroperitoneum</td>
</tr>
<tr>
<td>6</td>
<td>58M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>IL2</td>
<td>Rib/pleural/lung</td>
<td>4 ( \times ) 4 ( \times ) 5</td>
<td>41.9</td>
<td>Positive</td>
<td>Bilateral lung</td>
</tr>
<tr>
<td>7</td>
<td>48M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>Megace, IFN, IL2</td>
<td>Right deltoid</td>
<td>2 ( \times ) 1 ( \times ) 1</td>
<td>1.0</td>
<td>Positive</td>
<td>Bilateral lung, bone</td>
</tr>
<tr>
<td>8</td>
<td>52M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>None</td>
<td>Rib/pleura</td>
<td>4 ( \times ) 3 ( \times ) 4</td>
<td>25.1</td>
<td>Positive</td>
<td>Bilateral lung</td>
</tr>
<tr>
<td>9</td>
<td>44M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>IFN, IL2, C</td>
<td>Lung</td>
<td>2 ( \times ) 1.4 ( \times ) 1</td>
<td>1.5</td>
<td>Negative</td>
<td>Bilateral lung</td>
</tr>
<tr>
<td>10</td>
<td>46M</td>
<td>Male</td>
<td>Renal Ca</td>
<td>IL2, perf</td>
<td>Lung</td>
<td>2 ( \times ) 2 ( \times ) 1.25</td>
<td>2.6</td>
<td>Positive</td>
<td>Bilateral lung</td>
</tr>
</tbody>
</table>

* Parentheses indicate not visualized.

# M, male; F, female; Ca, carcinoma; IL2, interleukin 2; C, chemotherapy; perf, isolated lung perfusion.
Pharmacokinetic data showed that all patients achieved peak blood PSC 833 concentrations above 1000 ng/ml, a level well above that shown to antagonize P-glycoprotein in vitro (12, 13). Two formulations of PSC 833 were used during the study. The first formulation, an oral drink solution using labrifil and corn oil as diluents, was given every 12 h. This was later replaced by a soft gel capsule formulation with increased bioavailability given at 6 or 8 h intervals. As shown in Table 2, both formulations achieved peak blood levels above 1000 ng/ml, with higher trough levels noted in patients receiving more frequent dosing.

Heart and liver TACs were generated in all patients. Normal lung could be identified only in seven patients. Seven of the nine renal cancer patients’ tumors had sestamibi uptake adequate to allow visualization and generation of TACs. Visualized index lesions ranged from 1.0 to 41.9 cm³ in size. In addition, a previously unsuspected rib metastasis was discovered in patient 8.

When the PSC 833 scan was compared with those performed at baseline and after vinblastine, the most dramatic visual changes were seen in liver retention of sestamibi. Tumor visualization was also enhanced by the presence of PSC 833 (Figs. 1–3); in two patients (patients 2 and 10), definite tumor visualization was only appreciated on the PSC study. No obvious visual changes were seen in lung or heart uptake on either the vinblastine or PSC 833 scans compared to baseline.

In the renal cancer patients, tumor, heart, and lung activity peaked approximately 3–4 min postinjection (range, 2.5–6.5 min) on all scans. Neither vinblastine exposure nor PSC 833 appeared to affect uptake of sestamibi in those tissues. On the other hand, peak liver uptake was markedly delayed by the presence of PSC 833 compared to the other scans, and accurate times to peak uptake could not be determined because this always occurred after the 30-min period of continuous imaging. In the presence of PSC 833, peak liver uptake occurred between 30 and 60 min in one patient, between 60 and 140 min in one patient, between 120 and 180 min in two patients, between 130 and 220 min in two patients, and sometime after 200 min in three patients. This is compared to the baseline and post-vinblastine studies, in which peak liver uptake occurred at means of 12 min (range, 8.5–16.5 min) and 16 min (range, 11.5–18.5 min), respectively.

AUC ratios are provided in Table 3, including those of the patient with adrenocortical cancer. In general, tumor and liver ratios increased from baseline to vinblastine to PSC 833, whereas this did not occur with normal lung. For both tumor: heart and liver:heart ratios, this increase was significant when comparing baseline and PSC 833 (p2 < 0.05). Average tumor: heart ratios increased 20% (range, −7 to +65%), and average liver:heart ratios increased 79% (range, 25–210%). PSC 833 also caused a significant increase in liver:heart ratios compared to the vinblastine study, but not between the vinblastine and baseline scans. With tumor, baseline versus vinblastine and vinblastine versus PSC 833 AUC ratios did not show any statistically significant differences.

Analysis of individual TACs and the t1/2 values derived from them during the first 30 min failed to demonstrate any statistically significant differences between the baseline, vinblastine, and PSC 833 scans for tumor, heart, or lung. Washout half-life values were not obtained for the liver because of the prolonged uptake phase present on the PSC 833 scans.

**Table 2** PSC 833 dosing and peak/trough levels

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>PSC formulationa</th>
<th>PSC dose (mg/kg)</th>
<th>Dosing interval</th>
<th>Peak PSC (ng/ml)</th>
<th>Trough PSC (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>12.5</td>
<td>12 h</td>
<td>2229</td>
<td>1133</td>
</tr>
<tr>
<td>2</td>
<td>L</td>
<td>12.5</td>
<td>12 h</td>
<td>2967</td>
<td>2432</td>
</tr>
<tr>
<td>3</td>
<td>L</td>
<td>15</td>
<td>12 h</td>
<td>1146</td>
<td>165</td>
</tr>
<tr>
<td>4</td>
<td>L</td>
<td>15</td>
<td>12 h</td>
<td>2376</td>
<td>1620</td>
</tr>
<tr>
<td>5</td>
<td>G</td>
<td>4</td>
<td>8 h</td>
<td>1068</td>
<td>606</td>
</tr>
<tr>
<td>6</td>
<td>G</td>
<td>5</td>
<td>8 h</td>
<td>3042</td>
<td>1831</td>
</tr>
<tr>
<td>7</td>
<td>G</td>
<td>5</td>
<td>8 h</td>
<td>4550</td>
<td>2150</td>
</tr>
<tr>
<td>8</td>
<td>G</td>
<td>5</td>
<td>8 h</td>
<td>4710</td>
<td>3420</td>
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<tr>
<td>9</td>
<td>G</td>
<td>5</td>
<td>8 h</td>
<td>5710</td>
<td>3050</td>
</tr>
<tr>
<td>10</td>
<td>G</td>
<td>4</td>
<td>6 h</td>
<td>3860</td>
<td>2420</td>
</tr>
</tbody>
</table>

a L, oral drink solution; G, soft gel capsule.

RESULTS

Pharmacokinetic data showed that all patients achieved peak blood PSC 833 concentrations above 1000 ng/ml, a level well above that shown to antagonize P-glycoprotein in vitro (12, 13). Two formulations of PSC 833 were used during the study. The first formulation, an oral drink solution using labrifil and corn oil as diluents, was given every 12 h. This was later replaced by a soft gel capsule formulation with increased bioavailability given at 6 or 8 h intervals. As shown in Table 2, both formulations achieved peak blood levels above 1000 ng/ml, with higher trough levels noted in patients receiving more frequent dosing.

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Analysis of individual TACs and the t1/2 values derived from them during the first 30 min failed to demonstrate any statistically significant differences between the baseline, vinblastine, and PSC 833 scans for tumor, heart, or lung. Washout half-life values were not obtained for the liver because of the prolonged uptake phase present on the PSC 833 scans.

**MDR-1** levels were obtained by quantitative PCR from biopsy specimens in three patients (patients 3, 6, and 8) and normalized to a level of 10 for SW620 cells, as described above. In these three patients, normalized values of 23.9, 21.2, and 81.0 were obtained, respectively. In two cases (patients 6 and 8),...
Fig. 2 Three-hour images obtained on the patient imaged in Fig. 1. The deltoid (arrow) and some lung lesions are still visible on the PSC 833 scan (P50 but not on the baseline (PRE) or post-vinblastine (VIN) scans. Marked retention of activity by the liver is seen with PSC 833 as compared to the baseline and post-vinblastine studies. (Relative intensity scale different from Fig. 1).

Fig. 3 Patient 7: Baseline (PRE), post-vinblastine (VIN), and PSC 833 (PSC) images obtained 5 and 30 min after injection of sestamibi. Note improved and prolonged visualization of tumor mass in the right deltoid (arrows) with PSC 833. Other tumor masses are seen in the right lung. A right-sided thyroid nodule appears as a cold defect on the PRE and VIN studies at 30 min but as a “hot” nodule on the PSC 833 study (arrowheads). All images are displayed at the same intensity scale.

these relatively high MDR-1 levels correlated with increases in tumor:heart AUC ratios of 28 and 17%, respectively, with PSC 833 as compared to baseline. In a third patient (patient 3) with adrenocortical cancer, the tumor: heart AUC ratio increased 25% with PSC 833 as compared to the post-vinblastine study (Table 3). For comparison, MDR-1 levels were also measured in four normal liver samples from unrelated patients. These showed a mean MDR-1 level of 21.9 ± 10.4 (range, 8.2–32.7).

In two patients (patients 1 and 9), both of whom had multiple pulmonary metastases, no tumor uptake of sestamibi was seen under any of the three conditions. In these patients, the largest lesions not otherwise obscured by sestamibi uptake in other structures measured 98.9 and 1.5 cm³, respectively (Table 1). However, a brain metastasis was visualized in patient 1.

Lastly, a marked increase in sestamibi uptake and retention was seen in a thyroid nodule in patient 7 in the presence of PSC 833 (Figs. 1 and 3). Prior to sestamibi scanning, this nodule had been undetected. On the baseline and vinblastine scans, this area appeared as a cold defect. On ultrasound, a solid, heterogeneous nodule measuring 31 × 23 × 26 mm was found. Interestingly, ultrasound found a similar nodule measuring 17 × 13 × 12 mm in the left lobe of the thyroid, although this was not apparent on the sestamibi studies. After 3 months, repeat ultrasound showed that both nodules had increased in size. Needle aspirations were performed, and bloody fluid aspirated from each side, but cytology was indeterminate. The patient subsequently died, but no autopsy was performed. Therefore, the exact nature of the two thyroid nodules remains unclear; possibilities include thyroid adenomas, malignancies, and renal cell carcinoma metastases to the thyroid gland.

DISCUSSION

For several years, successful tumor imaging with Tc-99m sestamibi has been reported in various tumor types, including those of lung (20–22), breast (23, 24), thyroid (25, 26), bone (27), and lymphoma (28, 29). This agent has been recommended for tumor detection, as an indicator of invasiveness (30), and to differentiate between benign and malignant disease (24, 31). The discovery that sestamibi acts as a substrate of Pgp and can be used as a functional imaging agent to detect multidrug resistance has also raised the possibility that it could be used to guide choice of chemotherapeutic agents (7, 9, 10).

In this study, we show, in vivo, that tumor retention of sestamibi is increased in the presence of the Pgp antagonist PSC 833. This was demonstrated visually in two patients in whom tumor could be seen only on the PSC 833 scan, while in others, sestamibi uptake was visually enhanced by the PSC 833, presumably due to its blockage of sestamibi efflux. Quantitatively, this was demonstrated by changes in tumor:heart AUC ratios, which increased significantly in the presence of PSC 833 as compared to baseline and post-vinblastine. These increases are not likely to be due to increases in availability of the agent (i.e., increased input function due to alterations in sestamibi pharmacokinetics; Ref. 15), because AUC ratios using the heart as a
Fig. 3  Corresponding 0–30 min TACs for patient illustrated in Figs. 1 and 2. Curves have been normalized to percentage of maximum counts. Insets in liver and lung diagrams show curves for 0–400 min and demonstrate hepatic retention in the presence of PSC 833 over 400 min, whereas sestamibi is continuously cleared from the lung. Note changes in tumor and right thyroid nodule curves in the presence of PSC 833. Left thyroid curves demonstrate activity in a region of interest drawn over a normal-appearing left thyroid, although ultrasound revealed the presence of a thyroid nodule in this lobe too. PRE, baseline; VIN, post-vinblastine; PSC, PSC 833.

Table 3  AUC ratios, 0–180 min

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Tumor:heart</th>
<th>Liver:heart</th>
<th>Lung:heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre*</td>
<td>Vin</td>
<td>PSC</td>
</tr>
<tr>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
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<td>0.619</td>
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<td>3b</td>
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<td>0.586</td>
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<td>0.741</td>
<td>0.811</td>
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<tr>
<td>5</td>
<td>0.54</td>
<td>0.535</td>
<td>0.616</td>
</tr>
<tr>
<td>6</td>
<td>0.336</td>
<td>0.446</td>
<td>0.393</td>
</tr>
<tr>
<td>7</td>
<td>0.29</td>
<td>0.371</td>
<td>0.478</td>
</tr>
<tr>
<td>8</td>
<td>0.396</td>
<td>0.401</td>
<td>0.506</td>
</tr>
<tr>
<td>9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>0.361</td>
<td>0.374</td>
<td>0.387</td>
</tr>
</tbody>
</table>

* Pre, baseline scan; Vin, vinblastine scan; PSC, PSC 833 scan; NA, not available.

b  Patient with adrenocortical cancer, not included in statistical analysis. All other patients had renal cancer.

control were used. Any changes in availability of sestamibi would also be reflected in changes in uptake by the heart, which expresses little Pgp (18, 19). Therefore, we believe that the increases in tumor:heart and liver:heart AUC ratios with PSC 833 seen in this study represent Pgp reversal in those tissues and not increased sestamibi availability. Further evidence of this is supplied by the demonstration that no significant increases were observed in lung, another tissue without significant Pgp expression.

Similar to the effects of PSC 833 on sestamibi retention, other substrates of Pgp, such as vinblastine, could potentially increase tumor retention of sestamibi. This may explain why
tumor:heart AUC ratios in this study also tended to increase on the post-vinblastine scans as compared to baseline. Continuous-infusion vinblastine has a plasma half-life of approximately 19 h (32), but this can be quite variable (33, 34). Therefore, although the post-vinblastine scans were performed at least 24 h after the infusion was discontinued, residual vinblastine may still have been present in some patients’ tumors. The presence of this residual vinblastine may have acted to block Pgp-mediated efflux of sestamibi from tumor, causing a small, statistically insignificant increase in the tumor:heart AUC ratios as compared to baseline.

In humans, Tc-99m sestamibi is reported to be excreted without evidence of metabolism (35), the major routes of excretion being through the renal and hepatobiliary systems. Approximately 37% of an injected dose is recoverable in the feces by 48 h (36). In normal human tissue, Pgp is usually found along the apical membranes of cells facing ducts and other excretory lumens, where it is thought to play a role in excretion of physiological substances and toxins. Examples include the bileary canalicular surfaces of hepatocytes and in biliary ductules, brush borders of proximal tubular cells in the kidney, and adrenal glands (18, 37). One can postulate that PSC 833 increases hepatic retention of sestamibi by blocking Pgp-mediated efflux from hepatocytes responsible for sestamibi excretion into bile. This observation is consistent with the delay in etoposide clearance seen by Boote et al. (38) in patients changes in a Phase I study of etoposide with i.v. PSC 833. However, because the exact mechanism(s) involved in sestamibi excretion by the liver is unknown, the possibility that other liver transport mechanisms are being effected by PSC 833 and causing sestamibi retention cannot be ruled out.

As for why the effects of PSC 833 on sestamibi retention by the liver appeared so much more significant than its effects on tumor, one explanation is that Pgp expression levels in tumor samples greatly exceed the levels in liver. However, our data show that MDR-1 levels in liver are comparable to those found in the tumor samples. Therefore, several other potentially coexisting explanations seem more likely. First, it may be that delivery of PSC 833 is better to the liver than to sites of tumor. Delivery of other agents, both diagnostic and therapeutic, into tumor is often heterogeneous and may be related to poor tumor vascularity or penetration. Second, the smaller tumor mass with the overlapping background could mask changes in retention induced by PSC 833. Third, assuming that blockage of Pgp in the liver is responsible for the increased retention of sestamibi, one could postulate that the high PSC 833 levels achieved in these patients were adequate to antagonize the high expression of Pgp along the biliary surface of hepatocytes and fully inhibit sestamibi excretion from the liver. If sestamibi were trapped in the liver and did not reenter the bloodstream, there would be a major increase in hepatic visualization, without an increase in availability to other tissues. Similar observations have been made by Luker et al. (39). Lastly, if sestamibi is being handled by liver transport mechanisms other than Pgp, the possibility that these other mechanisms are being affected by PSC 833 cannot be excluded. This could also explain the marked changes seen in the liver compared to those in other organs.

In vitro studies have demonstrated that in unselected human colon carcinoma cells, PSC 833 is able to reverse Pgp-mediated resistance and increase vinblastine accumulation 3-fold (13). Compared to that in vitro data, the changes seen on our scans with PSC 833 are less impressive. However, uptake of sestamibi by tumor is likely to have been underestimated in our studies because we relied on planar images that include significant background activity from overlapping structures and that are also subject to problems of attenuation.

In this study, tumor visualization was considered on a per patient basis rather than on a per lesion basis, due to the overlapping nature and numbers of lesions in some patients with lung metastases, which were occasionally so numerous that no areas of “normal” lung could be identified on planar imaging. Thus, 8 of 10 patients demonstrated tumor visualization with sestamibi, whereas 2 did not. Possible reasons for tumor non-visualization include: (a) small size of tumors; (b) overlap of tumors with activity in normal organs; (c) tumor necrosis or poor tumor vascularity preventing uptake and/or delivery of sestamibi to a lesion; and (d) tumor expression of high levels of Pgp causing such rapid efflux of tracer that tumor is never visualized.

In this series, both patients without tumor visualization had pulmonary metastases; in one case (patient 9), lack of tumor visualization was probably due to the fact that the largest lesion unobscured by other structures was only 1.5 cm³. It is more difficult to explain the lack of visualization in the second patient (patient 1), in whom metastases of up to 99 cm³ were missed. Biopsy tissue was not obtained in these patients, so that correlation with MDR-1 levels was not performed.

One drawback of our study is the small number of patients included. To address this and other problems, we are currently planning to study more patients enrolled in an upcoming Phase II study of PSC 833 and vinblastine. Not only will blood samples to measure sestamibi clearance be obtained, but a greater effort to obtain biopsy material for correlation with in vitro Pgp levels will be made.

It has been suggested that sestamibi be used to guide chemotherapy (7–10). A more urgent need, however, is to aid in determining the success of Pgp antagonism in clinical trials. Numerous clinical studies have been completed, incorporating a wide range of Pgp antagonists and chemotherapeutic agents (40). Most studies have used antagonists of less potency than those now in clinical development. Overall, little convincing evidence for clinical benefit has been amassed. Many explanations can be offered, including the presence of high levels of Pgp that cannot be inhibited, insufficient levels of the antagonist, and other mechanisms of intrinsic or acquired resistance. In our study, we believe that other mechanisms of resistance must have prevailed when treatment failure occurred despite sestamibi scans that demonstrated increased uptake in tumor after PSC 833 administration.

In this study, we have shown that sestamibi can be used in vivo to indicate inhibition of drug efflux by the Pgp antagonist PSC 833. As such, sestamibi may prove most useful in interpreting the results of studies with this and other Pgp antagonists, as well as other strategies aimed at inhibition of Pgp-mediated drug efflux.
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