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Fractional Retention of Technetium-99m-Sestamibi as an Index of P-Glycoprotein Expression in Untreated Breast Cancer Patients

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The multidrug-resistant phenotype is characterized by the reduced intracellular retention of several structurally and functionally unrelated cytotoxic compounds due to the energy-dependent pump activity of P-glycoprotein (Pgp). Because ^{99m}Tc-sestamibi is a suitable transport substrate of Pgp, we tested whether the time-dependent fractional retention of this tracer could be used as an index of Pgp expression in untreated breast carcinomas. **Methods:** Twenty-seven patients with histologically confirmed breast carcinoma were intravenously injected with 740 MBq (20 mCi) of ^{99m}Tc-sestamibi, and static planar images of the breast were obtained at 10, 60 and 240 min. The fractional retention of ^{99m}Tc-sestamibi was then calculated as the ratios between 60 and 10 min (R60/10) and between 240 and 10 min (R240/10) of decay-corrected counts/pixel registered in the region of interest drawn around the tumor. Surgically excised tumors were then obtained from each patient, and Pgp levels were determined using ^{125}I-labeled MRK16 monoclonal antibody and in vitro quantitative autoradiography. **Results:** The fractional retention of ^{99m}Tc-sestamibi at 60 and 240 min was significantly higher in tumors with low Pgp levels (Group I, n = 18) as compared to that measured in tumors with high Pgp expression (Group II, n = 9) ($p < 0.001$). In particular, R60/10 values were 0.86 and 0.59 in breast carcinomas of Groups I and II, respectively, whereas the values of R240/10 were 0.56 and 0.25 in low- and high-Pgp-expressing tumors, respectively. **Conclusion:** The determination of fractional retention of ^{99m}Tc-sestamibi may be used as a simple functional test for Pgp expression in untreated breast cancer. A preliminary estimate of the sensitivity and the specificity of the test indicates its potential use in clinical practice to identify patients with a high probability of developing multidrug resistance.

Key Words: technetium-99m-sestamibi; multidrug resistance; P-glycoprotein; breast carcinoma

J Nucl Med 1997; 38:1348–1351

The development of the multidrug-resistant phenotype, which is characterized by the ability of tumor cells to survive exposure to different cytotoxic compounds (such as anthracyclines, *Vinca* alkaloids and actinomycin D) (*1*), is a major problem during cancer treatment. One of the mechanisms responsible of such resistance is the reduced intracellular retention of chemotherapeutic agents due to the energy-dependent pump activity of P-glycoprotein (Pgp), a M_r 170,000 transmembrane protein encoded by the *MDR1* gene (*2,3*). Experimental evidence of the association of the multidrug-resistant phenotype with increased levels of Pgp in many cultured tumor cell lines has been well documented (*1–3*). Elevated levels of Pgp also have been found in certain normal tissues (*4,5*), as well as in both treated and untreated human malignant tumors, including renal, colonic, adrenal and hepatocellular carcinoma (*4,6*). Untreated breast carcinomas have been reported to express relatively low levels of *MDR1* mRNA or Pgp as compared to other malignant solid tumors (*7*). However, these low levels of Pgp may reflect the

Received Aug. 1, 1996; revision accepted Feb. 19, 1997.
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presence of a subpopulation of Pgp-expressing cells within the tumor that may have a growth advantage in the course of subsequent chemotherapy (*7*).

In a previous study, Piwnica-Worms et al. (*8*) reported that ^{99m}Tc-sestamibi is a suitable transport substrate for Pgp. Currently used as a myocardial perfusion radiopharmaceutical (*9*), ^{99m}Tc-sestamibi has been recently proposed as a tumor-seeking agent for the diagnostic imaging of a variety of human malignant tumors (*10–12*). In particular, a high and specific ^{99m}Tc-sestamibi uptake has been shown in more than 80% of palpable breast carcinomas (*13–16*). Furthermore, retention of ^{99m}Tc-sestamibi is enhanced in Pgp-expressing cell lines as a result of Pgp-mediated outward transport inhibition by several multidrug resistance-reversing agents, such as verapamil and cyclosporin A (*8,17*). More recently, a close relationship between in vivo efflux rate of ^{99m}Tc-sestamibi and Pgp expression has been demonstrated (*18*).

The aim of this study was to assess whether a simple scintigraphic method, based on an early and delayed ^{99m}Tc-sestamibi scan, could be used to differentiate between tumors with low and high Pgp expression. Therefore, the fractional retention of ^{99m}Tc-sestamibi was determined in patients with untreated breast carcinomas and compared to Pgp levels measured in tumor specimens that were obtained from the same patients.

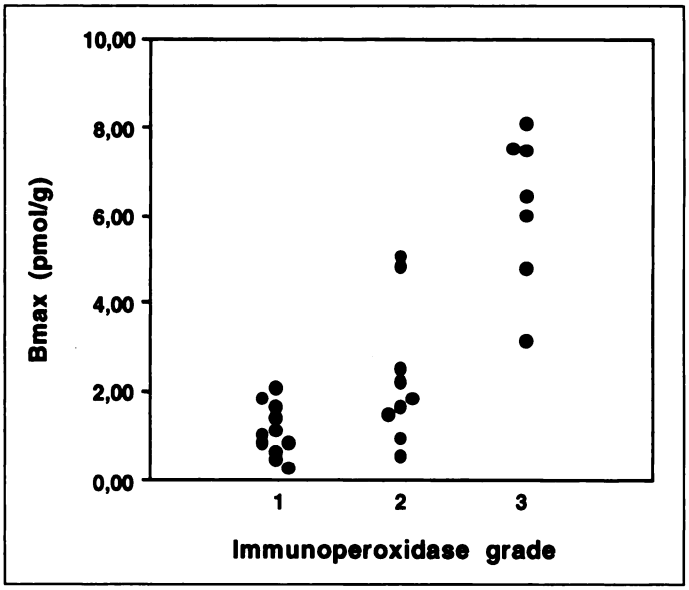

FIGURE 1. P-glycoprotein expression determined by in vitro quantitative autoradiography as the maximal amount of ^{125}I-labeled *MRK16* monoclonal antibody specifically bound to tumor or Bmax and immunoperoxidase grading with the same antibody in 27 samples of untreated breast carcinoma.

MATERIALS AND METHODS

Patients

Technetium-99m-sestamibi retention was determined in 27 patients with histologically confirmed breast carcinoma and unknown Pgp status. Patients were not consecutive because four patients with no initial tumor uptake of ^{99m}Tc-sestamibi could not be included in the analysis of tracer retention. The mean age was 60 ± 11 yr. Tumors were classified according to the WHO nomenclature (*19*) and included 20 ductal, one duct-lobular and six lobular carcinomas. Tumor size ranged from 1.2 to 5 cm. No patient had received previous chemotherapy or preoperative local radiotherapy. The study protocol was approved by the local ethics committee, and all patients gave informed consent.

Technetium-99m-Sestamibi Scintigraphic Study

Patients were intravenously injected with 740 MBq (20 mCi) ^{99m}Tc-sestamibi in the arm contralateral to the lesion. Static planar images (64 × 64 matrix) were obtained at 10, 60 and 240 min after the intravenous injection with the patient in the upright lateral position using a large field-of-view gamma camera equipped with a low-energy, general-purpose collimator and interfaced to a computer system. A preset time of 3 min was used for data acquisition at 10, 60 and 240 min, and at least 200,000 counts were collected in the field of view (range, 200,000–800,000). Patients were carefully repositioned at each imaging time using external markers.

Regions of interest were drawn around each lesion in the image obtained at 10 min and then translated to the images registered at 60 and 240 min. The ratios between 60 and 10 min (R60/10) and between 240 and 10 min (R240/10) of decay-corrected counts/pixel were then obtained from each patient.

P-Glycoprotein Measurement

Tumor biopsy specimens were obtained from each patient 24 hr after the ^{99m}Tc-sestamibi scan and subjected to a saturation assay with radiolabeled *MRK16* monoclonal antibody. The antibody, recognizing an external domain of the class I isoform (*MDR1*) of human Pgp (*20,21*), was radioiodinated with ^{125}I using the iodogen method. The radiolabeled product was purified from unbound iodide by Sephadex G25 chromatography, and the resulting specific activity was 6.8 μCi/μg. The immunoreactive fraction was calculated by the method of Lindmo et al. (*22*), and the mean value obtained from different antibody preparations was 71%.

Consecutive frozen sections were then incubated for 2 hr at 22°C, with increasing concentrations of ^{125}I-labeled *MRK16* (range, 0.5–18 n*M*) in the absence or presence of a 100-fold molar excess of unlabeled antibody. After rinsing in cold PBS, tissue-bound radioactivity was determined by quantitative autoradiography as previously described (*23*).

The experimental binding data from each tumor were fitted with the binding isotherm equation, and the maximal amount of ^{125}I-labeled *MRK16* specifically bound to tissue, or Bmax, was determined using the GraphPAD Inplot program (*24*). The Bmax value represented the tissue concentration of Pgp.

Immunoperoxidase staining was performed with *MRK16* antibody (20 μg/ml) on all tumor specimens using the streptavidin-biotin method as previously described (*25*). The immunoreaction was detected using diaminobenzidine as the chromogen. Samples were scored from 1 to 3 when a positive immunoreaction was observed in occasional single scattered tumor cells (Grade 1), clusters of cells in confined areas of the tumor (Grade 2) or numerous (>20%) malignant epithelial tumor cells (Grade 3).

Statistical Analysis

Values are expressed as mean ± s.d. The fractional retention of ^{99m}Tc-sestamibi and the Bmax values were compared using simple regression analysis and Pearson's coefficient of correlation. Differences in mean values were assessed by Student's t-test. Spearman's rank correlation was used to correlate the immunoperoxidase grading and the Bmax values obtained from quantitative autoradiography. A p value of less than 0.05 was considered significant.

RESULTS

P-Glycoprotein Expression

Tumor biopsy specimens were obtained from each patient, and consecutive sections were assayed for Pgp content by in vitro quantitative autoradiography and immunoperoxidase staining with *MRK16* monoclonal antibody. Figure 1 shows the Bmax values, i.e., the maximal amount of ^{125}I-labeled *MRK16*

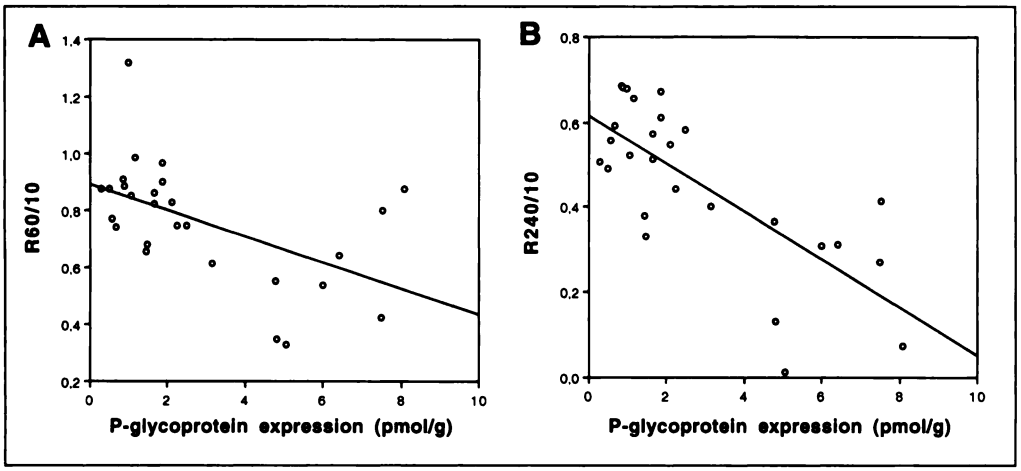

FIGURE 2. Correlation between the fractional retention of ^{99m}Tc-sestamibi determined at 60 (R60/10) and 240 (R240/10) min and Pgp levels (A and B, respectively). R60/10 compared with Pgp levels: $r = -0.53$, $p < 0.01$. R240/10 compared with Pgp levels: $r = -0.74$, $p < 0.001$.

specifically bound to tumor, and the immunoperoxidase grades determined in all breast carcinomas. The Bmax values were 1.12 ± 0.58, 2.34 ± 1.58 and 6.2 ± 1.76 (mean ± s.d.) in tumors with immunoperoxidase Grades 1, 2 and 3, respectively. A positive and significant correlation (Spearman's rank correlation = 0.77; $p < 0.001$) was found between the immunoperoxidase grading and the Bmax values obtained from quantitative autoradiography. In particular, 11 breast carcinomas showed immunoperoxidase Grade 1, and all had Bmax values lower than 3 pmol/g; nine samples had immunoperoxidase Grade 2, and seven showed Bmax values lower than 3 pmol/g; whereas the remaining seven malignant tumors showed immunoperoxidase Grade 3, and all had Bmax values higher than 3 pmol/g.

Fractional Retention of Technetium-99m-Sestamibi

The fractional retention of ^{99m}Tc-sestamibi, determined at 60 and 240 min, was then compared with the Bmax values measured by in vitro quantitative autoradiography in surgically excised tumors. A significant and negative correlation was found between the fractional retention of ^{99m}Tc-sestamibi at 60 min and the Bmax values (Fig. 2A) ($r = -0.53$; $p < 0.01$). An even stronger correlation was found when the tracer retention at 240 min was compared with the Bmax values (Fig. 2B) ($r = -0.74$; $p < 0.001$).

Patients were then classified on the basis of Bmax values (i.e., lower or higher than 3 pmol/g) in two groups: Group I (n = 18), with low or basal Pgp expression, and Group II (n = 9), with high Pgp expression. Patients with basal expression of Pgp in their tumors showed significantly ($p < 0.001$) higher values of both R60/10 and R240/10 than those with high-Pgp-expressing carcinomas (Table 1). The sensitivity and specificity of the scintigraphic study with ^{99m}Tc-sestamibi in the identification of the multidrug-resistant phenotype were estimated using cutoff values of 1 and 2 s.d. below the mean fractional retention found in low-Pgp-expressing tumors. Table 2 shows the values of sensitivity and specificity obtained using the R60/10 and R240/10 ratios.

TABLE 1
Fractional Retention of Technetium-99m-Sestamibi in Untreated Breast Carcinomas with Low and High P-Glycoprotein Expression

	Low Pgp expression (n = 18)	High Pgp expression (n = 9)	p value
R60/10	0.86 ± 0.15	0.59 ± 0.19	0.001
R240/10	0.56 ± 0.10	0.25 ± 0.15	0.001

DISCUSSION

In this study, ^{99m}Tc-sestamibi was used to characterize the expression of Pgp in tumors of patients with untreated breast carcinoma. We found that the fractional retention of ^{99m}Tc-sestamibi, measured as the ratio of counts/pixel between late and early images of the breast (R60/10 and R240/10), is significantly and inversely correlated with the Pgp levels measured in surgically excised tumors. This parameter can be considered as an in vivo index of ^{99m}Tc-sestamibi efflux rate, which has been shown to be related both in vitro (*8*) and in vivo (*18*) to the Pgp expression and can differentiate between tumors with basal and high expression of Pgp. Using in vitro quantitative autoradiography, nine of 27 patients studied had tumors with high levels of Pgp, and in this group of patients, both R60/10 and R240/10 were significantly lower than in the remaining 18 patients. This finding is consistent with previous studies showing faster efflux rate of ^{99m}Tc-sestamibi in the presence of high Pgp levels (*8,18,26*).

The inverse correlation between the fractional retention of ^{99m}Tc-sestamibi and Bmax values was stronger using R240/10 than using R60/10 (Fig. 2), and better values of sensitivity with a substantially unchanged specificity were obtained using R240/10 rather than R60/10. In particular, using the mean fractional retention of ^{99m}Tc-sestamibi in low-Pgp-expressing tumors minus 2 s.d. as a cutoff value, the sensitivity values were 78% and 56% with R240/10 and R60/10, respectively, whereas the specificity values were 95% and 100%, respectively. Using 1 s.d. to define the cutoff value, the sensitivity values were 100% and 78% with R240/10 and R60/10, respectively, whereas the specificity values were 83% and 89%, respectively. These findings indicate that the fractional retention of ^{99m}Tc-sestamibi measured at 240 min should be preferred in clinical practice to the same parameter determined on earlier images.

The diagnostic use of ^{99m}Tc-sestamibi has been recently

TABLE 2
Sensitivity and Specificity Values of R60/10 and R240/10 in the Identification of the Multidrug-Resistant Phenotype Using Cutoff Values of 1 or 2 s.d. Below the Mean Fractional Retention Found in Low-Pgp-Expressing Tumors

	Sensitivity (%)	Specificity (%)
R60/10		
1 sd	78	89
2 sd	56	100
R240/10		
1 sd	100	83
2 sd	78	95

reported in a variety of human malignant tumors, including breast carcinoma (*12*). The high sensitivity and specificity of ^{99m}Tc-sestamibi scan in detecting malignant lesions of the breast allowed the inclusion of this procedure in many diagnostic protocols, in combination with other imaging methods (*13–16*). Although breast carcinomas with high Pgp levels still contain on average 59% of the initial activity at 1 hr from tracer injection, these high Pgp-expressing tumors might be at risk for being interpreted as negative at diagnostic ^{99m}Tc-sestamibi scan. Therefore, it would be advisable to perform the scintigraphic examination immediately after the tracer injection (10 min). The diagnostic accuracy of an early ^{99m}Tc-sestamibi scan would not be substantially affected by the multidrug-resistant phenotype because the initial tracer accumulation is mainly regulated by the transmembrane potentials of tumor cells (*27*), and the kinetics of Pgp-dependent tracer efflux is relatively slow (*18*).

Characterization of Pgp expression in malignant tumors is usually performed by immunohistochemical techniques, which require multiple tissue biopsies. Although positive immunostaining for Pgp has been reported in a considerable percentage of untreated breast carcinomas (*7,28–30*), the clinical relevance of immunohistochemical detection of Pgp in predicting therapeutic response in breast cancer patients is still under investigation (*7,31*) and appears to be limited by several factors, including sampling errors from heterogeneous tumors, specificity of the antibody used, threshold for positivity and functional state of Pgp. Our study reports a simple and noninvasive in vivo functional test for Pgp expression, requiring only an early and delayed ^{99m}Tc-sestamibi breast scan. Moreover, the fractional retention of a Pgp substrate such as ^{99m}Tc-sestamibi is a sensitive and quantitative parameter, determined over the whole lesion, that allows to identify tumors with a high probability to become clinically refractory.

CONCLUSION

The fractional retention of ^{99m}Tc-sestamibi may be used as a simple functional parameter for the phenotypic assessment of multidrug resistance in untreated breast cancer. A preliminary estimate of the sensitivity and the specificity of the test indicates its potential use in clinical practice. Adjuvant or neoadjuvant chemotherapy in breast cancer patients with a tracer retention at 240 min that is lower than 46% of the initial activity might include drugs not affected by the *MDR1* phenotype or the combined use of reversing agents. More studies are needed to evaluate the prognostic value of ^{99m}Tc-sestamibi retention in breast cancer patients.

ACKNOWLEDGMENTS

We thank Dr. S.M. Larson for his valuable help. This work was supported by the Associazione Italiana Ricerca Cancro (AIRC). M. I. P. is a recipient of a fellowship from the AIRC.

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