Clinical Validation of the Influence of P-Glycoprotein on Technetium-99m-Sestamibi Uptake in Malignant Tumors

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We prospectively studied 48 patients with either breast cancer (30 patients) or lung cancer (18 patients) to ascertain the relationship between the degree of accumulation of ^{99m}Tc-sestamibi and the expression of p-glycoprotein in tumor tissues. **Methods:** During initial presentation (37 patients) or post-therapy evaluation (11 patients), the patients underwent contemporaneous ^{99m}Tc-sestamibi imaging and biopsy (30 patients) or surgery (18 patients). The interval between surgery/biopsy and imaging varied between 3 and 15 days. All patients had radiologically detectable tumors. Immunohistochemical studies were performed on paraffin sections using a monoclonal antibody, JSB-1, developed against the internal epitope of p-glycoprotein. Tumor-to-background ratios were correlated with the level of p-glycoprotein expression determined by immunohistochemical studies. **Results:** Our results showed an inverse correlation between the tumor-to-background ratios of ^{99m}Tc-sestamibi and the density of p-glycoprotein expression in tumor tissues. The values for the tumor-to-background ratios were significantly lower for those tumors expressing p-glycoprotein at high levels than those with scattered and no expression ($p < 0.01$ and $p < 0.001$, respectively). **Conclusion:** Although our results warrant further studies at the molecular level using PCR techniques after the extraction of mRNA, our data strongly suggest that ^{99m}Tc-sestamibi imaging is useful to noninvasively determine the presence of multidrug resistance in patients with malignant tumors.

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

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Drug resistance is one of the major unsolved problems in the treatment of patients with cancer. Although multidrug resistance (MDR) is a highly complex and multifaceted phenomenon, one specific form of MDR is defined as the ability of cells exposed to a single drug to develop resistance to a broad range of structurally unrelated drugs due to enhanced outward transport of drugs mediated by p-glycoprotein, a 170 kD transmembrane glycoprotein that is encoded by multidrug-resistance gene (MDR1). This specific protein functions as an energy-dependent extrusion pump that efficiently transports cationic and lipophilic chemotherapeutic agents as well as some toxins, thereby causing a concomitant decrease in accumulation and retention of these agents (*1,2*). Recent evidence has also shown that an organotechnetium complex, ^{99m}Tc-sestamibi (MIBI), a lipophilic cationic radiopharmaceutical, is a suitable transport substrate for p-glycoprotein (*3,4*). Accumulation rates of MIBI are in fact driven by negative mitochondrial inner matrix and plasma membrane potentials, thereby concentrating the agent as a lipophilic, cationic probe of membrane potential (*5,6*). The same mechanism responsible for the outward transport of all drugs was found to transport MIBI outside the cell rendering its intracellular accumulation suboptimal. The potential advantage of MIBI would lie in its superiority to noninvasively diagnose the presence of p-glycoprotein overexpression in vivo. In this context, the information derived from MIBI images might prove quite useful in patient management since information regarding p-glycoprotein expression could be primarily used in the design of most effective therapy protocols.

We sought to determine the association of enhanced MIBI efflux, a potential noninvasive p-glycoprotein probe, with overexpression of p-glycoprotein in the clinical setting because the noninvasive detection of p-glycoprotein-dependent MDR would aid in tailoring chemotherapy protocols and developing new drugs targeted to inhibit p-glycoprotein expression.

MATERIALS AND METHODS

Patients

Forty-eight patients (34 women, 14 men; age range 31–71 yr; mean age 48 ± 16) were included in the study. Thirty patients had breast carcinoma and 18 patients had lung carcinoma. Of the 30 patients with breast carcinoma, 28 patients had infiltrating ductal and two had medullary carcinoma of the breast. Of the 18 patients with lung carcinoma, 11 had epidermoid cell, and seven had small-cell lung carcinoma (SCLC). All patients with lung cancer and 19 patients with breast cancer were evaluated before therapy. Eleven of 30 patients (37%) with breast cancer had been previously treated with various chemotherapy regimens consisting of cisplatin, methotrexate, 5-fluorouracil, doxorubicin, taxol and etoposide. The presence of tumors was confirmed by radiological modalities such as CT/MRI (23 patients), mammography and/or ultrasound (25 patients). Thirty patients had biopsy before and 18 patients had surgery after MIBI imaging. All tumor specimens were obtained within 2 wk of imaging studies for immunohistochemical analysis.

Imaging

Images were obtained 30 min after the injection of 740 MBq ^{99m}Tc-sestamibi. Late images could not be acquired due to the time constraints related to the scheduling of surgical procedures. A dual-head ADAC Genesys camera with a LEHR collimator interfaced with an ADAC 3300 computer was used for image acquisition. Whole-body and planar spot images for involved areas were acquired. SPECT was performed in all patients, and images were acquired immediately after the planar images using a matrix size of 64 × 64 × 64 for 64 projections and an imaging time of 30 sec per projection. Tomographic images were reconstructed using a Butterworth filter with a cutoff frequency of 0.35 and an order of 6. Attenuation correction was applied to all frames. Quantitative

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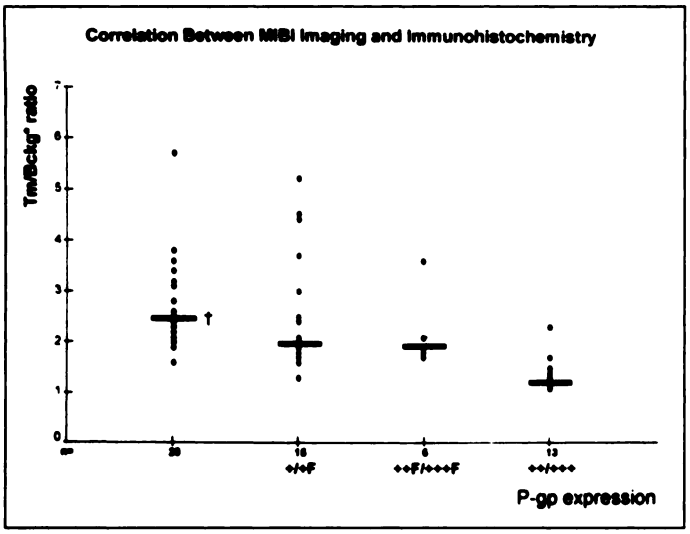

FIGURE 1. Inverse correlation between the tumor-to-background ratios from MIBI imaging and the density of p-glycoprotein expression in tumor tissues are depicted with all the values represented in solid dots. The values for tumor-to-background ratios were significantly lower for those tumors expressing p-glycoprotein at high levels than those with scattered and no expression ($p < 0.01$ and $p < 0.001$, respectively).

analyses were done using consecutive transverse sections of the SPECT in all patients. The uptake ratios were taken in the regions of interests (ROIs) drawn over the tumor and the contralateral site. In only four patients, an ROI was drawn adjacent to the tumor due to the cardiac activity in the contralateral site.

MIBI scans were interpreted by two nuclear medicine physicians blinded to the patients' clinical information and immunohistochemistry findings. All imaging findings were correlated with histological findings.

Immunohistochemistry

Five-micrometer-thick, formalin fixed, paraffin-embedded tissue samples were placed on poly-L-lysine coated slides. The avidin-biotin-peroxidase procedure was used for immunostaining. After deparaffinization and rehydration, the sections were treated with 0.1% methanol-hydrogen peroxide to block endogenous peroxidase activity, incubated with normal horse serum for 30 min at 37°C and incubated with a primary antibody, JSB-1, overnight in a moist chamber at 4°C at a dilution of 1:20. The tissue sections were incubated with secondary biotinylated horse antimouse antibody and with avidin-biotin-peroxidase complex. The final reaction product was revealed by exposure to 0.03% diaminobenzidine, and the nuclei were counter-stained with Mayer's hematoxylin. A negative control was obtained by staining the sample with secondary antibody and a positive control by inclusion of a tumor section with known positivity for p-glycoprotein. The results of p-glycoprotein immunostaining were independently interpreted by two pathologists who had no knowledge of the imaging studies. The tumors were classified under four groups according to the distribution of p-glycoprotein expression and the degree of immunostaining as follows:

Group 1. Tumors strongly positive for p-glycoprotein. Diffuse positivity (>10% of the specimen) and weak staining were scored as ++, and diffuse positivity (>10% of the specimen) and strong staining were scored as +++ (Fig. 1).

Group 2. Tumors weakly positive for p-glycoprotein. Scattered positive cells (involvement of <10% of the specimen) and weak staining were scored as +.

Group 3. Tumors completely negative for p-glycoprotein. Tumor samples were scored as negative when there was complete absence of staining for p-glycoprotein.

Group 4. Tumors strongly but focally positive for p-glycoprotein. Focal positivity (focal expression in areas <10% of the specimen) and strong staining were evaluated as a separate group.

Statistical Analysis

The correlation between p-glycoprotein expression levels and tumor-to-background ratios obtained from MIBI imaging was determined using the Spearman correlation test. The pairwise comparisons of these ratios in different p-glycoprotein levels were performed using Dunn's test as a post hoc analysis after detecting significance with Kruscal-Wallis test. P-glycoprotein expressions in breast tumors versus other tumors were analyzed with the Mann-Whitney test for those patients who were evaluated before treatment.

RESULTS

Immunohistochemistry

The results according to the previously described classification of p-glycoprotein distribution were as follows:

Group 1. Strongly positive for p-glycoprotein, excluding those with only focal expression (12 patients). P-glycoprotein immunostaining was diffuse, although weak in intensity (++) in four patients (Table 1, Patients 10–12 and Table 2, Patient 1), and diffuse and strong (+++) in eight patients (Table 1, Patients 1–8). These 12 tumors were evaluated together as one group. In these specimens, both membrane and cytoplasmic immunoreactivity was observed.

Group 2. Weakly positive for p-glycoprotein (+) (15 patients).

Group 3. Completely negative for p-glycoprotein (17 patients).

Group 4. Strongly but focally positive for p-glycoprotein. When correlating immunohistochemistry with MIBI findings, these four patients were evaluated separately (Table 1, Patient 9 and Table 2, Patients 2–4). On the other hand, those tumors with weak and focal p-glycoprotein expression (four patients) (Table 1, Patients 17, 18, 22 and 23) were evaluated in the same group as those with scattered p-glycoprotein expression. There were two tumors with accompanying stromal p-glycoprotein expression. (Table 1, Patients 2, 22).

In our study group, the patients with breast cancer had higher p-glycoprotein expression in their tumor sections than the patients with lung cancer ($p = 0.099$; Mann-Whitney test). Eleven of 30 (36.6%) patients with breast cancer were evaluated after completion of chemotherapy consisting of chemotherapeutic regimens generally implicated in p-glycoprotein-dependent MDR (etoposide, doxorubicin, taxol). Immunostaining for p-glycoprotein was positive at varying levels more frequently (81%) in these 11 patients as compared with all other patients who were included in the study before the induction of chemotherapy.

The difference in the incidence of p-glycoprotein positivity could not be evaluated according to histologic subgroups because of the homogeneity of the distribution of tumor subtypes (Table 1).

Correlation Between MIBI Imaging and Immunohistochemistry

The correlation between MIBI imaging and immunohistochemistry findings is summarized in Tables 1 and 2. Our results showed an inverse correlation between the tumor-to-background ratios of MIBI imaging and the density of p-glycoprotein expression in tumor tissues ($r = -0.64$, $p < 0.001$;

TABLE 1
Correlation Between Tumor-to-Background Ratios and P-gp Expression in Breast Carcinoma

Patient no.	Therapy	Tumor-to-background	P-gp status	Size (cm)	Site	Type	Comment
1	Pre	1.2	+++	1.0	Breast	Inf Duc*	Tm*
2	Pre	1.2	+++	2.0	Breast	Inf Duc	St†
3	Post	1.1	+++	2.5	Axilla	Inf Duc	Tm
4	Pre	1.2	+++	1.0	Axilla	Inf Duc	Tm
5	Pre	1.1	+++	2.0	Breast	Inf Duc	Tm
6	Pre	1.8	+++	1.5	Breast	Inf Duc	Tm
7	Pre	1.2	+++	0.7	Breast	Inf Duc	Tm
8	Pre	1.3	+++	5.0	Axilla	Inf Duc	Tm
9	Post	1.8	+++F	2.0	SCL	Inf Duc	Tm
10	Pre	1.3	++	4.0	Breast	Inf Duc	Tm
11	Post	1.2	++	1.5	Breast	Inf Duc	Tm
12	Pre	2.3	++	7.0	Breast	Inf Duc	Tm
13	Post	2.4	+	1.5	Axilla	Inf Duc	Tm
14	Pre	1.3	+	2.0	Breast	Inf Duc	Tm
15	Pre	1.8	+	5.0	Breast	Inf Duc	Tm
16	Post	3.0	+	2.2	Axilla	Inf Duc	Tm
17	Post	1.9	+ F	4.0	Breast	Inf Duc	Tm
18	Post	4.3	+ F	4.0	Breast	Inf Duc	Tm
19	Pre	1.3	+	1.5	SCL	Inf Duc	Tm
20	Pre	5.2	+	7.0	Breast	Inf Duc	Tm
21	Post	1.8	+	3.0	Chest wall	Inf Duc	Tm
22	Post	2.5	+ F	1.5	Breast	Inf Duc	St
23	Pre	1.8	+ F	1.0	Breast	Inf Duc	Tm
24	Pre	3.6	+	3.5	Breast	Medullary	Tm
25	Pre	2.0	−	2.0	Breast	Inf Duc	
26	Post	3.8	−	10	Breast	Inf Duc	
27	Pre	2.3	−	2.8	SCL	Inf Duc	
28	Pre	3.3	−	3.0	Axilla	Inf Duc	
29	Post	2.8	−	2.0	Axilla	Medullary	
30	Pre	2.6	−	4.5	Breast	Inf Duc	

*Expression in the tumor only.
†Stromal expression in addition to tumoral expression.
Inf Duc = infiltrating ductal carcinoma; F = focal expression of P-gp; SCL = supraclavicular lymph node; P-gp = p-glycoprotein.

Spearman correlation) (Fig. 1). We correlated MIBI results with immunostaining in four main groups:

Group 1. Tumors strongly positive for p-glycoprotein (++ and +++). Since there were only four tumors expressing p-glycoprotein with a score of ++, these two groups were lumped together for statistical analysis, with the mean ratio being 1.37 ± 0.32, excluding those with strong but focal p-glycoprotein expression.
Group 2. Tumors weakly positive for P-p (+). These tumors had a mean ratio of 2.63 ± 1.22, range 1.3–5.2.
Group 3. Tumors completely negative for p-glycoprotein. These tumors had a mean ratio of 2.62 ± 0.59, range 1.9–3.8.
Group 4. Tumors strongly but focally positive for p-glycoprotein. These tumors had a mean ratio of 2.35 ± 0.84, range 1.8–3.6 (Table 1, Patient 9; Table 2, Patients 2–4).

The following indications were observed for the above four groups: (a) There was statistically significant difference in the tumor-to-background ratios between Group 1 (strong p-glycoprotein expression) and Group 2 (weak p-glycoprotein expression) or Group 3 (no expression) (mean ratios 1.37 versus 2.63 or 2.62, $p < 0.01$, 0.001, respectively, Dunn's test) (Fig. 2), (Table 3); (b) statistically significant difference between Groups 1 (strong p-glycoprotein expression) and 4 (strong but focal expression) (mean ratios 1.37 versus 2.35, $p < 0.01$, Dunn's test) (Table 3); (c) no difference between Groups 2

TABLE 2
Correlation Between Tumor-to-Background Ratios and P-gp Expression in Lung Carcinoma

Patient no.	Therapy	Tumor-to-Background	P-gp status	Size (cm)	Type
1	Pre	1.5	++	2.5	SCLC
2	Pre	2.1	++ F	5.0	Epidermoid ca.
3	Pre	3.6	++ F	4.5	Epidermoid ca.
4	Pre	1.9	++ F	2.4	SCLC
5	Pre	2.1	+	1.6	Epidermoid ca.
6	Pre	4.5	+	3.5	Epidermoid ca.
7	Pre	2.0	+	2.5	Epidermoid ca.
8	Pre	2.2	−	2.7	Epidermoid ca.
9	Pre	3.1	−	3.5	Epidermoid ca.
10	Pre	3.6	−	3.0	Epidermoid ca.
11	Pre	1.9	−	3.0	SCLC
12	Pre	2.6	−	4.0	SCLC
13	Pre	2.1	−	3.6	SCLC
14	Pre	2.2	−	2.0	SCLC
15	Pre	2.0	−	4.5	Epidermoid ca.
16	Pre	3.2	−	5.0	Epidermoid ca.
17	Pre	2.5	−	2.5	Epidermoid ca.
18	Pre	2.3	−	1.7	SCLC

SCLC = small-cell lung cancer; F = focal expression P-gp; ca. = carcinoma; P-gp = p-glycoprotein.

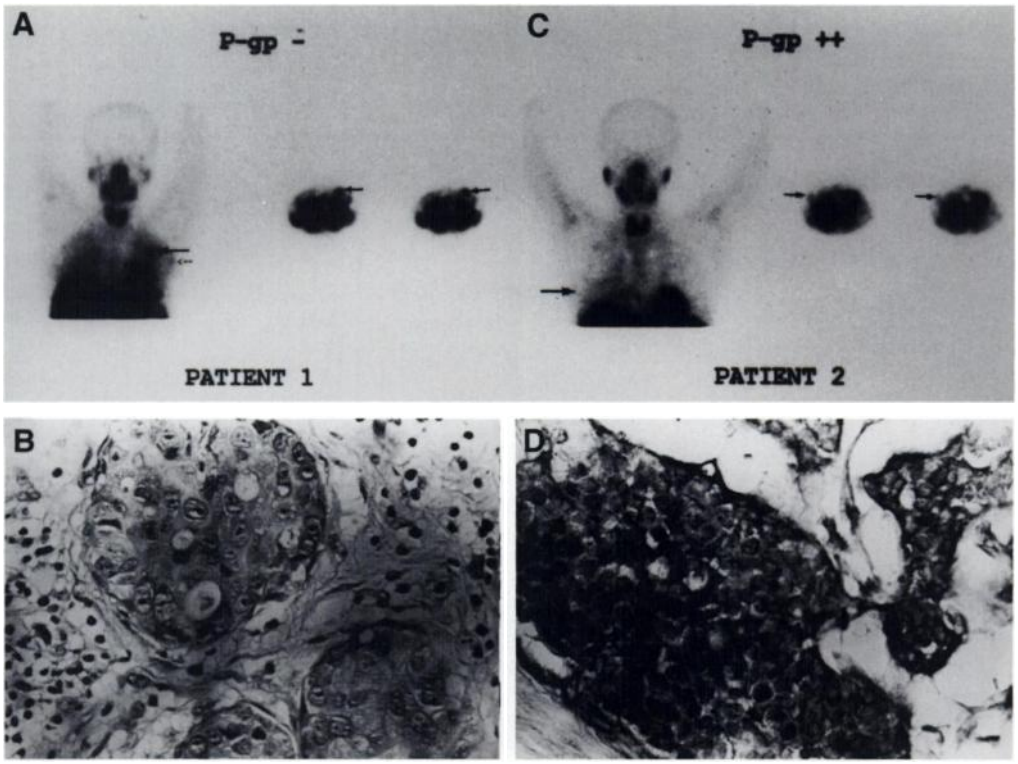

FIGURE 2. (A) A 45-yr-old woman with breast carcinoma underwent MIBI imaging and subsequent modified radical mastectomy (Patient 1). Anterior spot view and transverse slices of the SPECT scan demonstrate an area of intense radiotracer uptake in the left upper quadrant of the breast in the region of the tumor (arrows). Tumor-to-background ratio 2.8. (B) Immunohistochemistry (460 ×) reveals no staining for p-glycoprotein (Group 3). The dark stain in the sections are the nuclei of the tumor cells. (C) A 48-yr-old woman with breast carcinoma underwent MIBI imaging and subsequent modified radical mastectomy (Patient 2). Anterior spot view of the chest and transverse slices of the SPECT scan demonstrate an area of faint radiotracer uptake in the right upper quadrant corresponding to the tumor site (arrows). Tumor-to-background ratio 1.3. (D) Immunohistochemistry (460 ×) reveals strong and diffuse (+++) staining for p-glycoprotein (Group 1).

(weak p-glycoprotein expression) and 3 (no expression) (mean ratios 2.63 versus 2.62, $p > 0.05$; Dunn's test) (Table 3); (d) no difference in the ratios between Groups 4 (strong but focal expression) and 2 (weak p-glycoprotein expression) or Group 3 (no expression) (mean ratios 2.35 versus 2.63 or 2.62, $p > 0.05$, Dunn's test) (Fig. 3) (Table 3).

There was no visually conceivable necrosis in the tumors except in Patient 22 (Table 1). In some tumors, particularly in one patient with a lung tumor with strong p-glycoprotein positivity, there was no discernable difference on qualitative evaluation (Fig. 4), although the tumor-to-background ratios were strikingly different.

Exceptions to Overall Findings

The data on five tumors contrasted with the overall statistical findings. There were two tumors with strong p-glycoprotein positivity but higher ratios (Table 1, Patients 6 and 12) than the mean value for this group (2.0 versus 1.37). On the other hand, there were three patients with either weak or no p-glycoprotein expression with ratios of 1.3 and 1.3, respectively (Table 1, Patients 14 and 19), all of which were below the mean value for this group (1.4 versus 2.63 or 2.62).

TABLE 3
Statistical Correlation Between Groups with Different P-gp Levels*

	P-gp −	P-gp +	P-gp strong and focal†
P-gp ++ or +++	Different $p < 0.001$	Different $p < 0.01$	Different $p < 0.01$
P-gp +	No different $p < 0.05$		No different $p > 0.05$
P-gp −		No different $p > 0.05$	No different $p > 0.05$

*Statistical evaluation was performed using Dunn's test.
†Strong but focal expression.
P-gp = p-glycoprotein.

As there were no patients with mere stromal expression, the importance of the presence of p-glycoprotein in the stroma could not be ascertained.

The size of the tumors for the entire patient population ranged from 0.7 cm to 15 cm (mean 3.33 ± 2.33). The tumor-to-background ratios were not dependent on the size, site or the histologic type of the tumors.

DISCUSSION

Failure of therapy due to cellular resistance to multiple chemotherapeutic agents remains a major clinical problem in the treatment of cancer. In a large survey, frequent expression of MDR1 gene was found in many intrinsically resistant human tumor types (*7*). Since early identification of the presence of MDR could allow poor-prognostic patients to receive alternative therapy with MDR modifiers, determination of drug resistance mechanisms is critical to the development of rational therapeutic strategies to prevent drug resistance. Characterization of p-glycoprotein by current techniques such as immunohistochemistry, polymerase chain reaction (PCR) and flow cytometry requires serial tissue biopsies (*8–12*). Moreover, the usefulness of these approaches has been limited by sampling errors, the high sensitivity required of RNA and antibody probes, labor intensity and invasiveness of the procedures (*12*). Immunohistochemical staining of tissue sections has been used for semiquantitative determination of antigen concentration (*13*). These readings, however, are operator-dependent visual interpretations and therefore require both judgment and experience of the observer, which renders reproducibility suboptimal. Nevertheless, densitometry evaluation of tissue staining may provide better information, but the quantity of antibody binding to tissue cannot be determined without a system of standards. Although negative controls are generally used for standard immunohistochemistry, "specific binding" actually represents the "total binding" of the antibody to the tissue (*13*). On the other hand, nonspecific binding cannot be measured directly. Considering all these drawbacks, there is still a great demand for a noninvasive, highly sensitive MDR screening

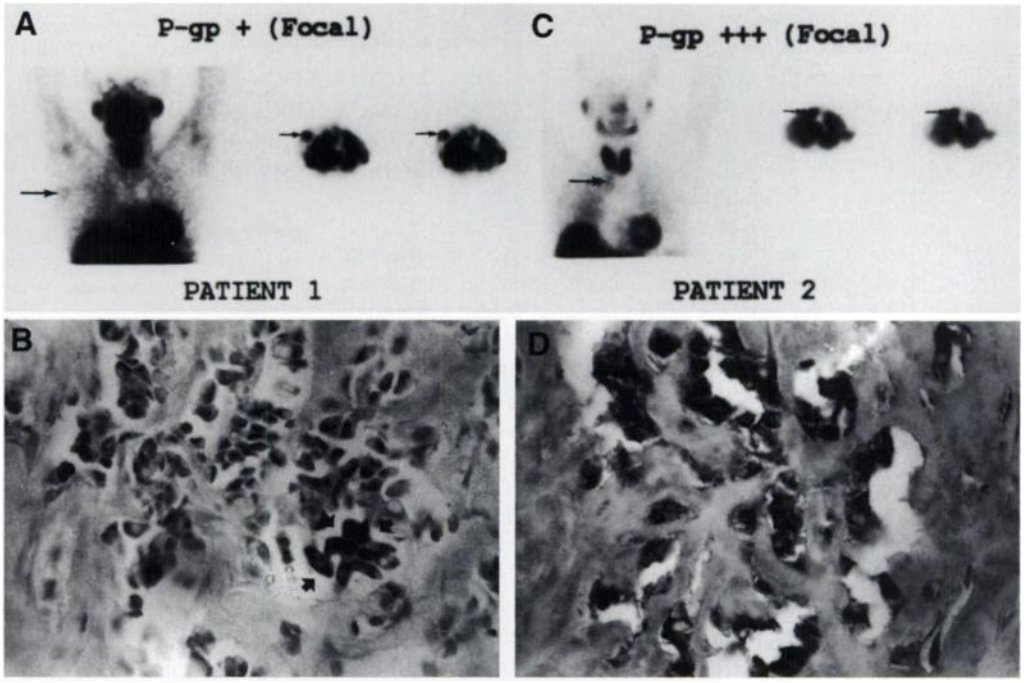

FIGURE 3. (A) A 57-yr-old woman with untreated breast carcinoma had an incisional biopsy 15 days before MIBI imaging (Patient 1). Anterior spot view and transverse slices of the SPECT scan demonstrate focally increased uptake in the right upper quadrant consistent with tumor (arrows). Tumor-to-background ratio 2.5. (B) Immunohistochemistry (460×) reveals weak (+) and focal staining for p-glycoprotein (arrows) (Group 2). (C) A 36-yr-old woman with treated carcinoma of the breast underwent MIBI imaging and a subsequent excision of the right infraclavicular lymph node (Patient 2). Anterior spot view of the chest and transverse slices of the SPECT scan demonstrate an area of intense radiotracer uptake in the corresponding region (arrows). Tumor-to-background ratio 1.8. (D) Immunohistochemistry (460×) reveals strong (+++) but focal staining for p-glycoprotein (Group 4).

technique to detect p-glycoprotein activity in vivo. In this context, recent data have demonstrated that MIBI, a tumor imaging agent (*14,15*), is recognized as a transport substrate by the human p-glycoprotein efflux pump, and overexpression of p-glycoprotein in cell lines correlated well with reduced MIBI accumulation (*3,4,16*). However, despite all the extensive in vitro work performed thus far, its clinical relevance has not been clarified yet.

Our clinical data revealed that the tumor-to-background ratios obtained from MIBI imaging were inversely proportional with the density of p-glycoprotein expression (Figs. 1, 2). There was statistically significant difference in tumor-to-background ratios between Groups 1 (strong p-glycoprotein expression) and 2 (weak p-glycoprotein expression) or 3 (no expression) (Fig. 2). These in vivo findings were in complete agreement with the previous in vitro work performed on cell lines (*3,4,16*). On the other hand, the data on five tumors contrasted with the overall statistical findings. This discrepancy could be ascribed to the heterogeneity of the tumors, subsets of cells expressing varying and multiple resistance mechanisms that may well co-exist. Also, the different washout rates of MIBI driven out of the cell by p-glycoprotein efflux pump, depending on the p-glycoprotein density as well as the ATP content of the cells, might give rise to varying p-glycoprotein function capacities and washout rates. As the transport of any radiotracer into the tumor is also governed by biological properties of the diffusing molecules, the necrotic component of the tumor and the reduced volume of blood flow to the tumor could be held accountable for poor penetration of MIBI in patients with either scattered or no p-glycoprotein expression and unexpectedly low tumor-to-background ratios. In such patients, a tumor perfusion agent such as ^{201}Tl whose initial tissue distribution has been well documented to correlate with regional perfusion could enable one to perform dual-isotope imaging to assess tissue perfusion status.

Our data suggest that strong but focal expression of p-

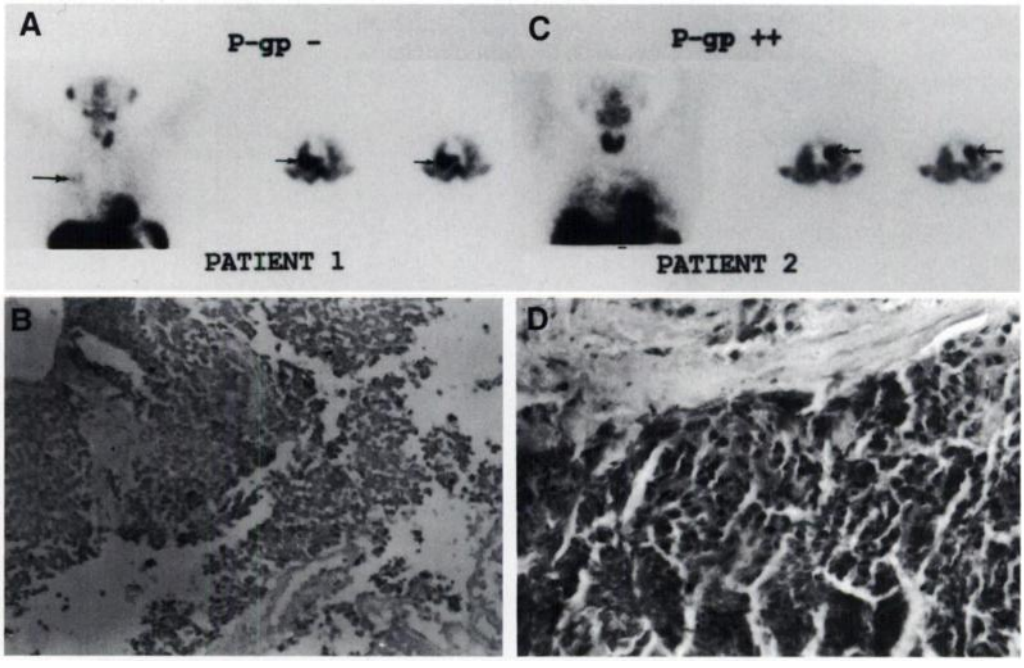

FIGURE 4. (A) A 67-yr-old man with epidermoid lung carcinoma had bronchoscopic biopsy 10 days before MIBI imaging (Patient 1). Anterior spot views of the chest and transverse sections of the SPECT scan (arrows) demonstrate an area of focal increased uptake in the right upper lung fields consistent with tumor, tumor-to-background ratio 2.5. (B) Immunohistochemistry (115×) reveals no staining for p-glycoprotein (Group 3). (C) A 45-yr-old woman with SCLC located in the left midlung fields underwent MIBI imaging and subsequent bronchoscopic biopsy. Anterior spot view of the chest and transverse slices of the SPECT scan demonstrate an area of intense radiotracer uptake in the corresponding region (arrows). Tumor-to-background ratio 1.5. Although the difference in p-glycoprotein expression levels are not visually conceivable, the ratios were different. (D) Immunohistochemistry (460×) reveals strong (++) staining for p-glycoprotein (Group 1).

glycoprotein has little effect on the MIBI uptake (Fig. 3). There was no difference in the ratios between Groups 2, 3 or 4. One could infer that in the clinical setting, a particular threshold level should be reached for p-glycoprotein expression to be therapeutically significant. To determine therapeutically significant p-glycoprotein density, further clinical studies correlating MIBI imaging with quantitative mRNA assays as well as clinical outcome need to be performed (*17,18*).

MDR1 gene expression is an inherent characteristic of tumor cells in breast cancers since it has been observed at varying levels in 53% of the patients with untreated breast carcinoma (*19,20*). Similarly, our data show that in patients with breast cancer, both the post- and pretherapy groups had higher p-glycoprotein expression than those with lung cancer. Despite the fact that SCLC is associated with progressive unresponsiveness to chemotherapy, the patients with SCLC in our study did not demonstrate exceptionally higher levels of p-glycoprotein as compared with patients with epidermoid cancer. Nevertheless, an extensive study by Lai et al. (*21*) revealed only low levels of MDR1 mRNA expression in patients with SCLC. We did not, however, have sufficient numbers of patients with SCLC to arrive at a statistically meaningful conclusion. In other reported studies, conflicting results have been obtained due to the differences in the nature of tissues tested (fixed or frozen), type of antibodies used, analysis techniques, threshold used for positivity and whether studies were performed at the mRNA or p-glycoprotein level (*18,22*). Our data should be supported by further investigations at the mRNA level using other techniques such as PCR (*22*).

Limitations

MIBI uptake is primarily a cell metabolism-dependent process, and optimal perfusion of the tissues is the initial step in MIBI accumulation. Due to the time constraints, tumor perfusion status and MIBI washout rates could not be assessed in this study. In our study group, there were only two patients with low tumor-to-background ratios associated with weak p-glycoprotein positivity (Table 1, Patients 14, 19). Although the tumor sizes were within a range (2.0 cm and 1.5 cm, respectively) in which necrosis would not be expected, poor MIBI penetration could be attributable to poor tumor perfusion. Therefore, in prospective studies, a perfusion agent such as ^{201}Tl could be used to eliminate the possibility of poor penetration instead of overexpression of p-glycoprotein. Although it is a controversial issue, lower net uptake of MIBI could be a result of enhanced extrusion of the agent by the p-glycoprotein pump, therefore, the washout rates of MIBI in relation to the level of p-glycoprotein expression remains to be ascertained.

CONCLUSION

This clinical study further extended our understanding of functional imaging with MIBI in patients with malignant tumors by correctly identifying the presence of varying levels of p-glycoprotein expression in tumors. MIBI imaging as a non-invasive test could help guide treatment by allowing the inclusion of agents capable of modulating MDR. However, the accuracy of this scintigraphic method is yet to be verified with additional techniques at the molecular level.

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