Annals of Nuclear Medicine Vol. 12, No. 6, 307–312, 1998

Preoperative evaluation of the chemosensitivity of breast cancer by means of double phase ^{99m}Tc-MIBI scintimammography

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The chemosensitivity of breast cancer is important for its management, but it is difficult to evaluate preoperatively. Tc-99m hexakis-2-methoxyisobutylisonitrile (MIBI) scintimammography has been reported to indicate the expression of P-glycoprotein, which is one factor concerned with multidrug resistance. We developed a chemosensitivity assay by using surgical specimens to investigate whether ^{99m}Tc-MIBI scintimammography findings before the operation are related to chemosensitivity according to our assay. Fifteen patients with primary breast cancer were enrolled into the study. Early and delayed images were obtained at 10 and 120 minutes after intravenous injection of ^{99m}Tc-MIBI, respectively. Regions of interest were placed on the tumors and the contralateral healthy breasts in each patient to estimate ^{99m}Tc-MIBI uptake in the tumor, and retention indices were then calculated to assess the washout of ^{99m}Tc-MIBI. Chemosensitivity assay was performed by incubating surgical specimens with anticancer agents such as doxorubicin, epirubicin, pinorubicin, mitomycin C, cisplatin and 5-fluorouracil. ^{99m}Tc-MIBI washout on scintimammography was successfully related to inhibition ratios on chemosensitivity tests when compared with ^{99m}Tc-MIBI uptake by the tumor. In particular, high correlation coefficients were obtained between the retention index of ^{99m}Tc-MIBI and the inhibition ratios of doxorubicin ($r = 0.75$), epirubicin ($r = 0.60$) and pinorubicin ($r = 0.62$), but poor correlation was found for mitomycin C ($r = 0.44$) and cisplatin ($r = 0.31$). Our results indicate that the retention index of ^{99m}Tc-MIBI is closely correlated to chemosensitivity to anthracyclines, suggesting that double-phase scintimammography allows preoperative prediction of chemosensitivity of breast cancer.

Key words: breast cancer, technetium-99m methoxyisobutylisonitrile, scintimammography, anthracycline

INTRODUCTION

MANY COMBINATION chemotherapy regimens including anthracyclines have improved the outcome of patients with advanced breast cancer.[1] Some cancers, however, have shown an insufficient response because of resistance to chemotherapy. Because chemotherapy of resistant tumors gives rise to serious side effects without any benefits, it is important to estimate chemosensitivity before starting treatment.

We have developed an *in vitro* chemosensitivity test for surgical specimens[2] and shown that a single-cell suspension assay by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method can predict the clinical effect of chemotherapy.[3]

On the other hand, a recent study has indicated that accumulation of Tc-99m hexakis-2-methoxyisobutylisonitrile (MIBI) in breast tumor cells varies with the level of expression of P-glycoprotein,[4] which is involved in the resistance to anthracycline antibiotics.[5]

This is a study to assess whether ^{99m}Tc-MIBI scintimammography findings are related to the chemosensitivity of breast cancer predicted by our assay methods.

Received May 19, 1998, revision accepted July 2, 1998.

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MATERIALS AND METHODS

Subjects (Table 1)

Fifteen female patients aged 30–66 years, diagnosed with breast cancer by mammography, ultrasonography or biopsy, were examined. Pt. 1, Pt. 6 and Pt. 13 received adjuvant chemotherapy before surgery, but no cancer chemotherapy was added between the scintimammography and the surgery. All pathological diagnoses were confirmed by using surgical specimens.

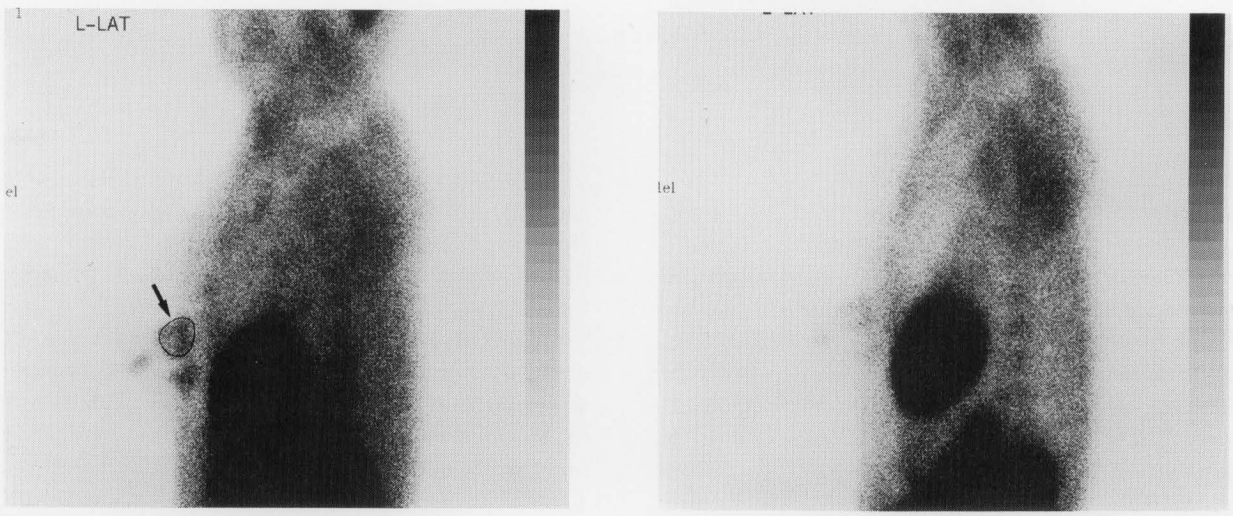
^{99m}Tc-MIBI scintimammography

^{99m}Tc-MIBI, 600 MBq, was injected into the cubital vein of the side opposite to the breast with the cancer lesion. Scintigraphy was performed with a GCA-7200A/DI (Toshiba, Tokyo) with a low-energy, high-resolution collimator. Lateral views of both breasts and an anterior view of the chest were acquired in the prone position. When the lateral view of the breast was acquired, the breast was suspended from the edge of the table and the contralateral breast was compressed onto the bed and a lead block was put between both breasts to avoid the influence of activity

Table 1 Characteristics of the patients

Pt.	Age	Location & Size (cm)	TNM clinical classification	Pathology	Response to Chemotherapy
1.	47	lt 5.8 × 5.8	T3N4M1	scirrhous	NC
2.	47	lt 5.5 × 3.7	T3N1M0	mucinous	
3.	51	lt 3.5 × 3.5	T2N1M0	solid-tubular	
4.	66	lt 4.9 × 3.9	T2N0M0	papillotubular	
5.	48	lt 4.5 × 3.8	T2N0M0	solid-tubular	
6.	48	rt 4.8 × 5.2	T3N0M0	scirrhous	NC
7.	45	lt 6.5 × 6.5	T3N1M0	scirrhous	
8.	38	rt 4.0 × 3.3	T2N2M0	solid-tubular	
9.	62	lt 5.5 × 4.0	T3N3M0	scirrhous	
10.	41	rt 7.5 × 6.9	T4N0M0	scirrhous	
11.	30	rt 5.0 × 5.0	T3N1M0	solid-tubular	
12.	50	lt 3.6 × 3.0	T2N1M0	scirrhous	
13.	56	lt 5.0 × 4.7	T4N1M0	solid-tubular	PD
14.	46	lt 4.0 × 3.6	T2N1M0	scirrhous	
15.	49	rt 5.7 × 5.2	T4N1M0	invasive lobular	

NC: no response, which means that the decrease in tumor size is lower than 50%.
PD: progressive disease
The chemotherapy for Pt. 1, 6 and 13 consisted of 50 mg/m^2 of FAM and 200 mg/m^2 of cyclophosphamide.



a b

Fig. 1 Scintimammograms of Pt. 1. On the early image (a), strong ^{99m}Tc accumulation was noted in the tumor (➡) in the left breast, and faint activity remained on the delayed image (b). The ROI was shown on the early image.

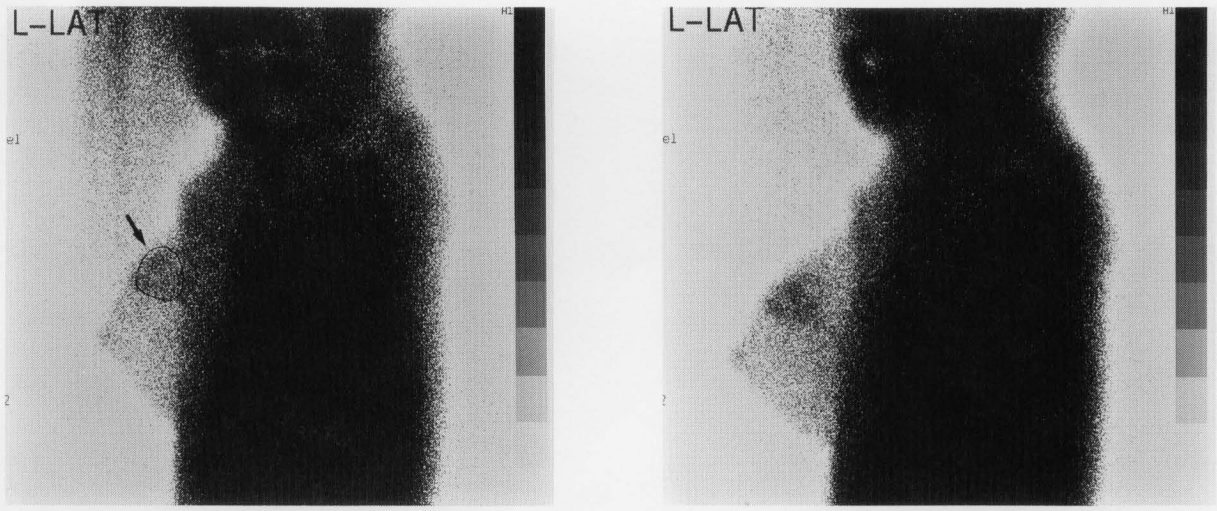


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Fig. 2 Scintimammograms of Pt. 2. High activity was seen in the left breast mass (➡) in both the early (a) and the delayed (b) images. The ROI was shown on the early image.

Table 2 Results of scintimammography and chemosensitivity test

Pt.	Retention index	Early ratio	Delayed ratio	Inhibition ratio (%)					
				DOX	FAM	PINO	MMC	CDDP	5-FU
1.	−0.30	5.65	3.95	0	0	0	25	25	21
2.	+0.11	2.19	2.42	34	36	39	16	17	34
3.	−0.08	4.54	4.17	0	0	0	4	0	0
4.	+0.11	3.36	3.74	50	43	47	52	58	30
5.	−0.37	4.76	2.98	2	0	0	9	3	0
6.	−0.18	6.09	4.99	10	6	6	7	0	0
7.	−0.27	4.49	3.24	18	34	33	21	29	0
8.	+0.02	4.66	4.73	84	84	86	88	15	33
9.	−0.22	7.50	5.88	0	31	32	27	49	12
10.	+0.02	2.00	2.05	55	58	71	63	49	37
11.	−0.13	3.15	2.75	50	59	55	62	56	47
12.	−0.20	3.95	3.15	18	35	34	36	30	15
13.	−0.34	3.07	2.03	1	25	32	47	0	17
14.	+0.01	1.59	1.61	65	75	78	80	80	49
15.	−0.08	1.51	1.39	35	*	*	45	56	31

*: not measured

from the opposite side. Early and delayed images were obtained at 10 and 120 minutes after injection, respectively. The acquisition time of the early images was 7 minutes, and of the delayed images, 9 minutes, in consideration of the physical attenuation of Tc-99m. Accumulation of ^{99m}Tc-MIBI in the tumor was evaluated by placing the regions of interest (ROIs) on lateral scintimammography images. Breast tumors were imaged more clearly on lateral images than on anterior images because the lateral images were less affected by the activity of surrounding organs such as the heart and liver. A ROI was drawn over a tumor (L: lesion) so that it covered the whole tumor and a ROI with the same pixel size was placed on the contralateral healthy breast (N: normal). The retention index was calculated by the following equation: retention index = (delayed L/N ratio − early L/N ratio)/early L/N ratio.

In vitro *chemosensitivity test*

The sensitivity of surgical specimens to anticancer agents was assayed by the method previously reported.[6] Briefly, sterile surgical specimens from primary breast tumors were minced quickly with scissors in cold Hanks' balanced salt solution, added to an enzyme cocktail containing 0.2 mg/m*l* collagenase, 0.2 mg/m*l* DNase and 0.5 mg/m*l* pronase, and stirred in a water bath at 37°C for 30 min. The solution was then filtered and centrifuged at 1,000 rpm for 5 min, the supernatant was removed, and RPMI1640 containing 10% fetal calf serum (FCS) was added to the pellet. The resulting single-cell suspension was plated in 96-well plates with anticancer agents. The

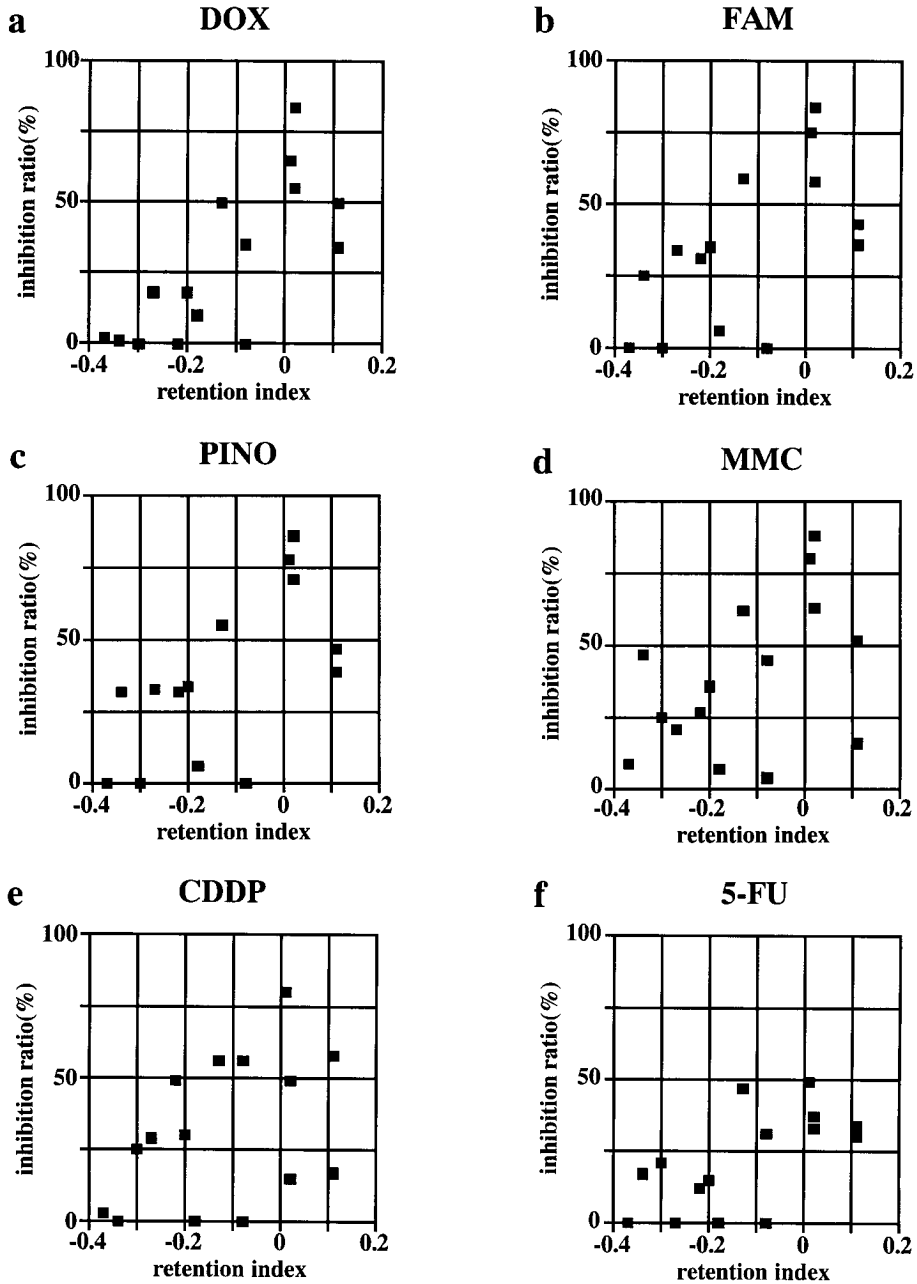

Fig. 3. Correlations between the retention indices and inhibition ratios of anti-cancer agents: (a) DOX, (b) FAM, (c) PINO, (d) MMC, (e) CDDP, (f) 5-FU.

anticancer agents examined were doxorubicin (DOX) 10 μg/ml, epirubicin (FAM) 10 μg/ml, pinorubicin (PINO) 10 μg/ml, mitomycin C (MMC) 10 μg/ml, cisplatin (CDDP) 25 μg/ml, and 5-fluorouracil (5-FU) 50 μg/ml. After incubating the plate at 37°C in a humidified atmosphere containing 5% CO_2 for 48 hours, MTT (5 mg/ml in PBS) 10 μl/well, and sodium succinate (0.1 M PBS) 10 μl/well were added. After an additional 4 hours of incubation, dimethyl sulfoxide, 150 μl/well, was added to dissolve the formazan salt. The absorbance of each well was read at 530–640 nm. The inhibition rate compared with the absorbance of drug-free control wells was calculated as follows:

inhibition rate (%) = (1− (absorbance of treated wells/ absorbance of control wells)) × 100.

Statistical analysis

Values are shown as the means ± s.d. Statistical comparisons were made by a Mann-Whitney U-test. A p value of < 0.05 was considered statistically significant.

RESULTS

Scintimammography

The cases are summarized in Table 1. All of the tumors were invasive carcinomas and consisted of thirteen invasive ductal carcinomas, one mucinous carcinoma and one invasive lobular carcinoma. Seven of the 13 patients with invasive ductal carcinomas showed a scirrhous pattern. The primary tumor in all the patients was greater than 2 cm in diameter, and the primary tumors were classified as T2 or a higher grade according to the TNM clinical classification proposed by UICC in 1987. LN metastases in the axillary and parasternal regions were detected in eleven patients by ultrasonographic studies or palpation, and some of them were smaller than 1 cm in diameter. Pt. 1 had cervical node metastases.

The primary breast tumors showed high ^{99m}Tc-MIBI accumulation on the early images in all 15 cases, and the ROIs could be placed on the tumors without any problem. Some tumors showed high washout of ^{99m}Tc, and with faint activity noted in the primary tumors on the delayed images. There were no cases in which ^{99m}Tc was completely washed out, and we were therefore able to place the ROIs on the primary tumors in both early and delayed images.

Clear abnormal ^{99m}Tc accumulation was detected in axillary node metastases in 6 of 11 node-positive patients, weak activity in 4, and no visible activity in 1 patient. No accumulation of ^{99m}Tc-MIBI in the node metastases was evaluated.

Typical scintimammograms are shown in Figures 1 and 2. Pt. 1 did not respond to FAM, and her scintimammogram (Fig. 1) showed strong ^{99m}Tc accumulation in the tumor on the early image, with faint activity on the delayed image, indicating the rapid washout of ^{99m}Tc. Figure 2, on the other hand, shows that ^{99m}Tc had accumulated in her tumor on the early image and the retention of ^{99m}Tc was observed on the delayed image.

Quantitative analysis

The scintimammography indices and results of the chemosensitivity tests are summarized in Table 2. No statistically significant differences between scirrhous and non-scirrhous carcinomas were observed in the early L/N ratio (4.47 ± 2.16 versus 3.41 ± 1.19, $p = 0.35$), delayed L/N ratio (3.55 ± 1.52 versus 3.03 ± 1.12, $p = 0.42$) or retention index (-0.16 ± 0.13 versus -0.10 ± 0.18, $p = 0.39$). The correlation between retention indices and inhibition ratios of anti-cancer agents are shown in Figure 3. The best positive correlation was shown between retention indices and inhibition ratios of DOX ($r = 0.75$, $p = 0.008$) (Fig. 3-a), followed by FAM ($r = 0.60$, $p = 0.02$), PINO ($r = 0.62$, $p = 0.02$) (Fig. 3-b, c) and 5-FU ($r = 0.63$, $p = 0.01$), although the effect of 5-FU was inferior to that of the other drugs (Fig. 3-f). A poor correlation between retention indices and inhibition ratios was noted for MMC ($r = 0.44$, $p = 0.10$) and CDDP ($r = 0.31$, $p = 0.11$) (Fig. 3-d, e). The correlation coefficients of the early and delayed L/N ratios and the inhibition ratios for DOX were -0.53 and -0.28, respectively, without statistically significant correlation.

DISCUSSION

In this study, all of the breast cancers examined had primary tumors greater than 2 cm in diameter, and all of them could be detected by both early and delayed scintimammographies. Some metastatic nodal lesions, however, were small and not visualized well enough to put ROIs on the lesions, and therefore we did not evaluate node metastases in this study.

We used early L/N- and delayed-ratios and retention indices to evaluate tumor uptake and washout quantitatively, and retention indices were devised to evaluate the activity of lung cancer to enable differentiation between benign and malignant lesions.[7] No significant differences were noted between scirrhous and non-scirrhous carcinoma in the early L/N ratios, delayed ratios or retention indices, suggesting that these indices are independent of the pathological findings of breast tumors. A similar observation was described by Del Vecchio et al.[8]

The response of breast cancer to drugs should be evaluated by the reduction in the measurable tumor size, but this clinical correlation was not observed in the present study because of small cases treated with preoperative chemotherapy. As a result, *in vitro* chemosensitivity testing was applied to compare the chemosensitivity of the breast tumors and ^{99m}Tc retention. Our chemosensitivity test has mainly been used for tumors of the digestive system, and the results have been shown to predict the clinical response to chemotherapy.[9] We have also reported that our chemosensitivity test is effective in predicting the outcome of breast cancer.[10] When 6 agents, DOX, FAM, PINO, MMC, CDDP and 5-FU were examined, a good positive correlation was observed between the retention indices and inhibition ratios of all three anthracycline antibiotics, DOX, FAM and PINO, whereas MMC and CDDP did not yield good correlations between retention indices and inhibition ratios. It is important that the preoperative scintimammography predict the chemosensitivity of breast cancer to DOX, FAM, and PINO, which are often used as the main anticancer agents for breast cancer.

While the predictability of chemosensitivity was lower for MMC and CDDP, these drugs are not usually used for the initial treatment of breast cancer. Although 5-FU is often used for breast cancer and a strongly positive correlation between the retention index and inhibition ratio has been shown, the inhibition ratio of 5-FU was low. Because 5-FU inhibits thymidylate synthetase, tumor cells should be incubated for a long period to evaluate the anticancer effect of 5-FU. But the tumor cells from the

surgical specimens did not grow well during the incubation period of the MTT method in the present study, which may explain the low inhibition ratio of 5-FU.

Although previous studies have reported a positive correlation between P-glycoprotein expression and the Tc-99m MIBI washout ratio, many factors besides P-glycoprotein are involved in multidrug resistance, complicating the mechanism. In this study we suggested that the washout of Tc-99m MIBI from breast cancers might predict overall chemosensitivity.

The coefficients for the correlations between the inhibition ratios and the early or delayed L/N ratios were lower than the coefficients for the correlations between the inhibition ratios and retention indices in breast cancer. The retention index, i.e., ^{99m}Tc-MIBI wash-out, is a better parameter than the early or delayed L/N ratios, i.e., the absolute uptake of ^{99m}Tc-MIBI, for predicting the response to chemotherapy.

In conclusion, a good correlation was observed between the *in vitro* chemosensitivity of anthracycline anticancer agents and the retention indices on the ^{99m}Tc-MIBI scintimammograms. Our results suggest that double-phase ^{99m}Tc-MIBI scintimammography can predict the sensitivity of breast cancer to chemotherapy in the preoperative stage.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid for Scientific Research (Grants-in-Aid for Encouragement of Young Scientists 09770716) from the Ministry of Education, Science, Sports and Culture.

We wish to thank radiological technologists, Koshi Okabe and Toshikazu Sanmiya for their technical assistance in imaging and quantification.

We are grateful to International Medical Information Center for their linguistic review.

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