Usefulness of $^{99}$mTc-Sestamibi Scintigraphy in Suggesting the Therapeutic Effect of Chemotherapy against Gastric Cancer

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ABSTRACT

Purpose: Imaging with $^{99}$mTc-sestamibi ($^{99}$mTc-MIBI) has been used to assess 170-kDa P-glycoprotein (P-gp) expression and predict chemotherapy responses in several types of malignancy, such as breast and lung cancers. The purpose of this study was to evaluate the relationship between $^{99}$mTc-MIBI accumulation in tumors and sensitivity to chemotherapy in gastric cancer patients.

Experimental Design: Thirty-six patients with advanced gastric cancer underwent $^{99}$mTc-MIBI scintigraphy before chemotherapy. Patients also underwent endoscopic biopsy, and the expression of P-gp or multidrug resistance-associated protein was analyzed by immunohistochemical staining. The relationship between the accumulation of $^{99}$mTc-MIBI in tumors and responses to chemotherapy with 5-fluorouracil/cis-diaminedichloroplatinum(II) or epirubicin was examined.

Results: Higher accumulation of $^{99}$mTc-MIBI in tumors was observed in 25 and 23 of 36 gastric cancer patients at the early (30 min) and delayed (120 min) imaging, respectively. Accelerated accumulation of $^{99}$mTc-MIBI was observed in 25 and 23 of 36 gastric cancer patients at the early (30 min) and delayed (120 min) imaging. The purpose of this study was to evaluate the relationship between $^{99}$mTc-MIBI accumulation in tumors and sensitivity to chemotherapy in gastric cancer patients.

Conclusions: $^{99}$mTc-MIBI scintigraphy is useful to suggest the responses to chemotherapy of patients with advanced gastric cancer.

INTRODUCTION

Multidrug resistance is the major barrier to efficient chemotherapy of cancer. Causes of chemotherapy failure are multifactorial, including the physical inability of drugs to reach malignant cells because of poor tumor vascularization and diverse cellular mechanisms lowering intracellular drug concentration or altering the ability of the drugs to affect their targets (1). One of the cellular mechanisms of multidrug resistance involves 170-kDa P-glycoprotein (P-gp), which acts as an ATP-dependent efflux pump for several anticancer agents (2).

$^{99}$mTc-Sestamibi ($^{99}$mTc-MIBI), a lipophilic cation originally developed for scintigraphic evaluation of coronary blood flow (3, 4), has been used for imaging of tumors originating from multiple organs, such as the parathyroid gland (5), brain (6), lung (7, 8), breast (9), and thyroid gland (10). Because $^{99}$mTc-MIBI is passively taken up into the mitochondria in metabolically active tumor cells and exported from cells by P-gp (11, 12), this imaging procedure has been also applied to detect functional P-gp expression and to predict sensitivity of cancer cells to chemotherapy in some types of cancers (13–19). Significant correlations have been reported between $^{99}$mTc-MIBI scintigraphy and P-gp immunohistochemistry, in vitro cytotoxicity assay, chemotherapy response, and/or patient outcome in several types of malignancy, including breast cancer (13), lung cancer (14–16), hepatocellular carcinoma (17), malignant lymphoma (18), soft tissue sarcoma, and bone sarcoma (19). Although P-gp expression can be evaluated by other methods, such as immunohistochemistry and Western or Northern blotting, in tumor tissues available from diagnostic biopsy, the results from these methods may not represent entire tumors but only small portions of large and heterogeneous tumors. In contrast, imaging with $^{99}$mTc-MIBI can evaluate entire tumors and may provide a number of advantages to assess P-gp expression and predict chemotherapy responses. Of note, it can be performed noninvasively, allowing for sequential evaluation of P-gp expression and function through repeated assessment at different time points.

Although some investigators have reported the usefulness of $^{99}$mTc-MIBI imaging in several types of cancers (13–19), others have suggested conflicting results that $^{99}$mTc-MIBI imaging did not correlate with measures of P-gp expression or chemotherapy responses in some tumors (20–22). Because the relationship between the extent of $^{99}$mTc-MIBI accumulation and chemotherapy responses has not been addressed in gastric cancer, we performed $^{99}$mTc-MIBI scintigraphy in patients with advanced gastric cancer. In the present...
**PATIENTS AND METHODS**

**Patient Population.** Thirty-six patients with advanced gastric cancer who underwent $^{99m}$Tc-MIBI scintigraphy before chemotherapy were studied (Table 1). No patients had ischemic heart disease. Patients consisted of 25 men and 11 women (age, 24–79 years; mean age, 64.5 years). Clinical examination revealed intestinal-type gastric cancer in 17 patients and diffuse-type gastric cancer in 19 patients. Clinical staging before chemotherapy showed that 11 patients had stage III disease, and 25 patients had stage IV disease. The patients were divided into two groups by the treatment given. Twenty-four patients received three cycles of a combined 5-fluorouracil (5-FU)/cis-diamminedichloroplatinum(II) (CDDP) therapy, and each cycle consisted of a continuous infusion of 5-FU at 500 mg/m$^2$/day for 7 days and a bolus infusion of CDDP at 7.5 mg/m$^2$/day for 5 days in a row. Twelve patients were given a bolus infusion of epirubicin at 30 mg/m$^2$/week, two or three times.

**$^{99m}$Tc-MIBI Scintigraphy.** The instrument used was Starcom 4000 (General Electric Company) connected with a low-energy high-resolution collimator (collection energy, 140 KeV; window width, 20%). Early and delayed images were acquired at 30 and 120 min after i.v. injection of 600 MBq $^{99m}$Tc-MIBI, respectively. A representative case is shown in Fig. 1. For quantitative evaluation, the regions of interest were placed over the gastric lesion and the heart. The count per pixel for each region of interest was determined, and then early uptake ratio (ER) and delayed uptake ratio (DR) were calculated by dividing the lesion count by the heart count obtained from the early and delayed images, respectively. Accumulation of $^{99m}$Tc-MIBI was considered high when the patients showed either high ER ($>1.13$) or high DR ($>0.75$) and low when patients showed both low ER ($<1.00$) and low DR ($<0.75$).

**Immunohistochemical Analysis.** All patients underwent endoscopic biopsy before chemotherapy. The expression of P-gp or multidrug resistance-associated protein (MRP) was analyzed in paraffin-embedded specimens by immunohistochemical staining with anti-P-gp monoclonal antibody (UIC2; Beckman Instruments Inc., Fullerton, CA) or anti-MRP monoclonal antibody (MRPm6; MONOSAN, Uden, the Netherlands). Tumors were classified as positive when $>50\%$ of cells were stained and negative when $<50\%$ of cells were stained (Fig. 2).

**Evaluation of Therapeutic Effects and Statistical Analysis.** Therapeutic effects were evaluated according to the clinical and histological criteria described in Japanese Classification of Gastric Carcinoma (23). Values are expressed as mean ± SD. Statistical analysis was performed with the Fisher’s exact test for nominal data and Mann-Whitney test for numerical data, using a statistical analysis program (StatView 5.0 software; Abacus Concepts, Inc., Berkeley, CA), and differences at $P < 0.05$ were considered as significant.

**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>24–79 (mean, 64.5)</th>
</tr>
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<tbody>
<tr>
<td>Gender</td>
<td>Male 25, Female 11</td>
</tr>
<tr>
<td>Type</td>
<td>Intestinal 17, Diffuse 19</td>
</tr>
<tr>
<td>Stage</td>
<td>III 11, IV 25</td>
</tr>
<tr>
<td>Chemotherapy regimen</td>
<td>5-FU/CDDP 24, Epirubicin 12</td>
</tr>
</tbody>
</table>

* 5-FU, 5-fluorouracil; CDDP, cis-diammine dichloroplatinum(II).

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**Fig. 1** $^{99m}$Tc-Sestamibi ($^{99m}$Tc-MIBI) scintigraphy in a gastric cancer patient. Endoscopy (A), upper gastrointestinal series (B), and $^{99m}$Tc-MIBI scintigraphy (C) in a gastric cancer patient are shown. Arrowheads represent the $^{99m}$Tc-MIBI accumulation in the gastric lesion (closed arrowhead) and the heart (open arrowhead).
RESULTS

99mTc-MIBI Accumulation in Gastric Cancer. There have been an increasing number of studies describing 99mTc-MIBI accumulation in tumors, including tumors of the parathyroid gland (5), brain (6), lung (7, 8), breast (9), and thyroid gland (10). To know whether 99mTc-MIBI can also accumulate in gastric cancer tissues, we quantitatively evaluated the ER and DR from the early (30 min) and delayed (120 min) images after i.v. injection of 99mTc-MIBI in 36 gastric cancer patients. As shown in Fig. 1, 99mTc-MIBI was strongly accumulated in the gastric cancer tissue in some of patients. According to the quantitative evaluation, higher accumulation of 99mTc-MIBI in tumors was observed in 25 and 23 of 36 gastric cancer patients in the early (30 min) and delayed (120 min) images, respectively.

Higher Uptake of 99mTc-MIBI Correlates with the Decrease in P-gp Expression in Gastric Cancer Tissues. 99mTc-MIBI has been known to be a suitable transport substrate of P-gp and/or MRP (21). Decrease in P-gp expression in gastric cancer tissues may be dependent, at least in part, on the expression level of P-gp. In contrast, the expression of MRP was positive in 11 patients and negative in 17 patients, but there was no significant correlation between expression of MRP and 99mTc-MIBI accumulation in gastric cancer tissues (Table 2). Although the data are not shown, there was no significant correlation between histology and 99mTc-MIBI accumulation; ER was 0.97 ± 0.35 in intestinal type (n = 17) versus 1.11 ± 0.55 in diffuse type (n = 19); and DR was 0.82 ± 0.29 in intestinal type (n = 17) versus 0.89 ± 0.60 in diffuse type (n = 19).

5-FU/CDDP Chemotherapy Was Effective in Patients with Lower 99mTc-MIBI Accumulation. The response rate was 33.3% in 24 patients who underwent conventional chemotherapy with 5-FU and CDDP: complete response in 0 patients; partial response in 8 patients; no change (NC) in 16 patients; and progressive disease partial response in 0 patients. We evaluated the relationship between 99mTc-MIBI accumulation in tumors and responses to this chemotherapeutic regimen in these patients. As shown in Table 3, the response rate to 5-FU/CDDP chemotherapy in patients with high 99mTc-MIBI accumulation who showed either high ER (≥1.00) or high DR (≥0.75) was much lower than that in patients with low 99mTc-MIBI accumulation who showed both low ER (<1.00) and low DR (<0.75) [15.4% versus 54.5%]. In particular, the response rate of the patients whose ER was >1.20 was 0% (NC in 11 patients). In addition, both ER and DR tend to be lower in 8 cases with partial response than in 16 cases with NC (ER = 0.79 ± 0.31 in partial response cases versus 1.26 ± 0.77 in cases with NC (P = 0.22); DR = 0.66 ± 0.22 in cases with partial response versus 0.88 ± 0.54 in cases with NC (P =

Table 2 Relationship between 99mTc-MIBI accumulation and P-gp or MRP expression

<table>
<thead>
<tr>
<th></th>
<th>ER</th>
<th>DR</th>
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</thead>
<tbody>
<tr>
<td>P-gp (–) (n = 19)</td>
<td>1.41 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01 ± 0.43</td>
</tr>
<tr>
<td>P-gp (+) (n = 9)</td>
<td>0.62 ± 0.18</td>
<td>0.48 ± 0.43</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MRP (–) (n = 17)</td>
<td>1.05 ± 0.37</td>
<td>1.01 ± 0.43</td>
</tr>
<tr>
<td>MRP (+) (n = 11)</td>
<td>1.32 ± 0.83</td>
<td>0.94 ± 0.65</td>
</tr>
<tr>
<td>P&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.42</td>
<td>0.62</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values (except for P values) are mean ± SD.

<sup>b</sup>P-gp, 170-kDa P-glycoprotein; MRP, multidrug resistance-associated protein; ER, early uptake ratio; DR, delayed uptake ratio.

<sup>c</sup>P-gp (–) versus P-gp (+).

<sup>d</sup>MRP (–) versus MRP (+).

Table 3 99mTc-MIBI<sup>a</sup> accumulation and therapeutic responses to chemotherapy with combined 5-FU/CDDP or epirubicin in advanced gastric cancer patients

<table>
<thead>
<tr>
<th></th>
<th>Low 5-FU/CDDP</th>
<th>High 5-FU/CDDP</th>
<th>High epirubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>0.45 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55 ± 0.60</td>
<td>1.35 ± 0.22</td>
</tr>
<tr>
<td>DR</td>
<td>0.42 ± 0.13</td>
<td>1.07 ± 0.46</td>
<td>0.98 ± 0.12</td>
</tr>
<tr>
<td>Response rate</td>
<td>54.5% (6/11)</td>
<td>15.4% (2/13)</td>
<td>41.7% (5/12)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values for ER and DR are mean ± SD.

<sup>c</sup>99mTc-MIBI, 99mTc-sestamibi; 5-FU, 5-fluorouracil; CDDP, cis-diammine dichloroplatinum(II); ER, early uptake ratio; DR, delayed uptake ratio.

<sup>d</sup>5-FU/CDDP, chemotherapy with combined 5-FU/CDDP or epirubicin.

*Fig. 2* Immunohistochemical analysis of the expression of 170-kDa P-glycoprotein in gastric cancer tissues. The expression of 170-kDa P-glycoprotein was analyzed immunohistochemically. Tumors were classified as positive when >50% of cells were stained (A) and negative when <50% were stained (B).
0.32), although the differences are not statistically significant (Table 4). These results suggested that patients with lower 99mTc-MIBI accumulation show higher sensitivity to chemotherapy with 5-FU/CDDP, compared with patients with higher 99mTc-MIBI accumulation.

Chemotherapy with Epirubicin Was Effective in Patients with Higher 99mTc-MIBI Accumulation. As shown in Table 2, the accelerated accumulation of 99mTc-MIBI in gastric cancer tissues might be associated with a lower expression or absence of P-gp, which has been shown to export several anticancer drugs, such as anthracyclines, Vinca alkaloids, and actinomycin D, out of cells (2). Because patients with higher 99mTc-MIBI accumulation were not sensitive to the combined 5-FU/CDDP therapy, we tried another regimen with epirubicin, which is a substrate of P-gp, in 12 patients who showed either high ER (≥1.00) or DR (≤0.75). As shown in Table 3, the response rate to chemotherapy with epirubicin (41.7%) was much higher than that to the conventional chemotherapy with 5-FU/CDDP (15.4%) in patients with high accumulation of 99mTc-MIBI.

Fig. 3 shows the case of a 30-year-old male patient with high accumulation of 99mTc-MIBI, who was successfully treated with epirubicin. He was diagnosed with a diffuse type of gastric cancer spreading from the cardia to the angle of the stomach by a series of examinations including upper gastrointestinal series (Fig. 3A), endoscopy (Fig. 3B), and computed tomography (Fig. 3C). Because 99mTc-MIBI scintigraphy showed high accumulation in the gastric tumor (Fig. 3D), he was treated with a bolus infusion of epirubicin (40 mg/m²/week) three times. After chemotherapy with epirubicin, the gastric tumor was remarkably reduced in size, and both mucosal irregularity and restricted wall distension were drastically improved (Fig. 3E–G). The 99mTc-MIBI scintigraphy performed again after chemotherapy showed a remarkable reduction in 99mTc-MIBI accumulation in the gastric lesion (Fig. 3H).

**DISCUSSION**

It is well known that tumor cells that have become resistant to one type of anticancer drug acquire cross-resistance to other anticancer drugs with completely different structures. This phenomenon is called multidrug resistance, which is a major obstacle to treatment of cancer patients in the clinic. One of the important mechanisms resulting in multidrug resistance is the increased expression and/or function of cellular membrane proteins, such as P-gp and MRP, which can export anticancer drugs from cancer cells (1). Different types of drugs, including doxorubicin, epirubicin, actinomycin D, mitomycin C, and vinblastine, have been known to be substrates of a cell surface P-gp

<table>
<thead>
<tr>
<th>Partial response</th>
<th>No change</th>
</tr>
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<tbody>
<tr>
<td>(n = 8)</td>
<td>(n = 16)</td>
</tr>
<tr>
<td>ER</td>
<td>0.79 ± 0.31*</td>
</tr>
<tr>
<td>DR</td>
<td>0.66 ± 0.22</td>
</tr>
</tbody>
</table>

* Values for ER and DR are mean ± SD.

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**Fig. 3** Epirubicin was effective in an advanced gastric cancer patient with high 99mTc-sestamibi accumulation. Upper gastrointestinal series (A and E), endoscopy (B and F), computed tomography (C and G), and 99mTc-sestamibi scintigraphy (D and H) are shown in a patient with highly advanced gastric cancer before (A–D) and after (E–H) chemotherapy with epirubicin.
transporter (2). Previous studies have reported a significant correlations between $^{99m}$Tc-MIBI accumulation and P-gp expression in immunohistochemistry in several types of cancer (13–19). In addition, Piwnica-Worms et al. (24) have demonstrated that the uptake of $^{99m}$Tc-MIBI is increased about 10 times in cells with low expression of P-gp and about 200 times in those with abundant P-gp after administration of drugs that can inhibit the activity of P-gp, such as verapamil and cyclosporin. These findings strongly indicate that $^{99m}$Tc-MIBI is a suitable substrate of P-gp. In the present study, we also showed a significant correlation between P-gp expression and the extent of $^{99m}$Tc-MIBI accumulation determined by both ER and DR in gastric tumors. Consistent with studies in other types of cancers (13–19), our finding suggests that the accumulation of $^{99m}$Tc-MIBI in gastric cancer tissues may be dependent, at least in part, on the expression level of P-gp. Several methods, such as Northern or Western blotting and immunohistochemistry, have been used to evaluate P-gp expression in human tissues. However, the results of these methods cannot always represent all tumor characteristics because most specimens are obtained from small parts of heterogeneous and large tumors and may not be good representatives for evaluating tumor characteristics. In contrast, $^{99m}$Tc-MIBI imaging can evaluate the entire tumor noninvasively, allowing for repeated assessments, and might have a number of theoretical advantages for the assessment of P-gp expression.

It has been suggested that MRP also affects the accumulation of $^{99m}$Tc-MIBI in some types of cancer (25–27), but our study could not demonstrate any correlation between MRP expression and ER or DR in $^{99m}$Tc-MIBI imaging in gastric cancer. Similar to our finding, previous studies reported that an increased expression of P-gp, but not of MRP, correlates with low accumulation of $^{99m}$Tc-MIBI in some types of malignancy, such as lung cancer (28) and hepatocellular carcinoma (29). Moretti et al. (26) have shown that MRP-dependent $^{99m}$Tc-MIBI efflux from cancer cells is highly dependent on intracellular glutathione concentration in cancer cell lines. Although further study is needed, intracellular glutathione or unknown other factors may be involved in the mechanism of $^{99m}$Tc-MIBI export by MRP in gastric cancer cells.

In terms of the correlation between the chemotherapeutic effect and $^{99m}$Tc-MIBI accumulation, several studies have shown that chemotherapy with doxorubicin, one of the substrates of P-gp, is more effective in patients with higher $^{99m}$Tc-MIBI accumulation in lung cancer (14–16), breast cancer (13), and bone and soft tissue tumors (19). Consistent with these previous reports, the present study has demonstrated that gastric cancer patients with accelerated $^{99m}$Tc-MIBI accumulation show a higher sensitivity to epirubicin, another anthracycline derivative structurally similar to doxorubicin. Of note, we have also shown that gastric cancer patients with lower $^{99m}$Tc-MIBI accumulation have a higher sensitivity to 5-FU/CDDP therapy, although we do not know the molecular mechanism behind this unexpected result. Besides P-gp or MRP expression in tumor tissues, $^{99m}$Tc-MIBI accumulation in tumors has been reported to be affected by a number of factors, including blood flow, tissue viability, vascular permeability, tumor necrosis, metabolic demand, and mitochondrial activity of the tumor (30). Thus, some factors may be responsible for higher chemotherapeutic responses to 5-FU/CDDP therapy in patients with lower $^{99m}$Tc-MIBI accumulation.

Although we need additional studies with an extended number of patients to confirm the accuracy of our findings, the present study suggests the usefulness of the determination of $^{99m}$Tc-MIBI accumulation in tumors to predict the therapeutic effect of chemotherapy in gastric cancer patients. In our pilot study, the response rates to epirubicin and 5-FU/CDDP in patients with high and low $^{99m}$Tc-MIBI accumulation were 41.7% and 54.5%, respectively. These response rates are very close to those reported in the most promising and standard chemotherapy regimen currently available in advanced gastric cancer, a combination of epirubicin, CDDP, and 5-FU, which causes more severe adverse effects (31, 32). Therefore, $^{99m}$Tc-MIBI imaging seems to be beneficial for selecting a more effective and less toxic chemotherapy protocol for individual patients by suggesting response or resistance to each of chemotherapeutic drug. It might also have diagnostic utility and potential therapeutic implications to help determine whether the accumulation of $^{99m}$Tc-MIBI can be modulated in tumors through administration of drugs to inhibit P-gp activity, such as PSC833 (33, 34). In addition, evaluations at different time points or changes in the scans obtained for sequential evaluation may be useful for selecting the most appropriate chemotherapy protocol because the sensitivity of tumors to chemotherapeutic drugs may change frequently during treatment.

REFERENCES


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