Non-Small Cell Lung Cancer Functional Imaging: Increased Hexakis-2-methoxy-isobutyl-isonitrile Tumor Clearance Correlates with Resistance to Cytotoxic Treatment

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ABSTRACT

Detection of P-glycoprotein and other multidrug resistance protein activity is currently under investigation to identify subgroups of cancer patients with tumors resistant to chemotherapy. Application of a test that reliably evaluates the phenomenon in vivo would not only serve as a predictor for responses to chemotherapy but would also be of use in testing the efficacy of multidrug resistance reversers in humans. Tc-99m radiolabeled hexakis-2-methoxy-isobutyl-isonitrile (Tc-sestamibi) has been recently shown to be extruded from cells through P-glycoprotein activity. In the present study, we examined the uptake and extrusion rate of the radiotracer in 25 patients with advanced non-small cell lung cancer undergoing chest radiotherapy, using a novel scintigraphic technique based on simulation-guided pinhole imaging. Five-min tumor images were taken 10, 60, and 120 min postinjection of 20 mCi of Tc-sestamibi. Six of 25 (24%) of tumors showed a 1.3-1.7 times higher extrusion rate as compared to that of normal lung tissue. Increased tumor clearance of Tc-sestamibi significantly correlated with resistance to radiotherapy (P = 0.05) as well as the existence of distant metastasis (P = 0.008). Patients with known resistance to chemotherapy had a higher extrusion rate as compared to chemotherapy-naive patients (P = 0.01). Moreover, increased Tc-sestamibi tumor capture was seen in patients with distant metastasis (P = 0.09). We concluded that functional imaging of lung cancer with Tc-sestamibi may have a role in predicting responses to cytotoxic treatment and in identifying tumors with aggressive behavior. Additional clinicopathological trials are required to investigate whether Tc-sestamibi kinetics correlates with P-glycoprotein expression, intratumoral angiogenesis, or other mechanisms.

INTRODUCTION

The role of P-glycoprotein in the development of cancer cell resistance to chemotherapy is of recent interest. This transmembrane protein is a 170-kDa product of the MDR1 gene (1). A variety of drugs such as anthracyclines, Vinca alkaloids, and taxanes have been shown to be extruded from cancer and normal cells through the ATP-dependent P-glycoprotein efflux pump (2, 3).

Immunohistochemical detection of P-glycoprotein expression is considered one of the most sensitive methods to assess MDR1 activity (4). Blot analysis, hybridization techniques, or reverse transcription-PCR are probably more sensitive quantitative techniques; however, P-glycoprotein also expressed in connective tissue elements may compromise their efficacy as compared to immunohistochemistry (5).

In vivo methods for P-glycoprotein activity assessment would not only allow a more reliable estimation of the phenomenon but would also permit the testing of the efficacy of different MDR1 reversal agents before chemotherapy administration (6, 7). Sestamibi is a lipophilic monovalent cation that was initially used for myocardial perfusion imaging (8). Because of its affinity to highly anaplastic tissues, it was subsequently used for tumor scintigraphy (9, 10). Radiolabeled sestamibi has been recognized to have a lower accumulation in cell lines expressing high levels of P-glycoprotein (11, 12). In the present study, we evaluated the accumulation of Tc-sestamibi and the ability of tumor and normal lung tissue to extrude Tc-sestamibi in patients with non-small cell lung cancer. A novel, previously described technique was used to enhance image quality and to allow more accurate measurements of tumor and normal lung radioactivity (13). Tumor and patient parameters were also assessed for correlation with Tc-sestamibi-related kinetics.

MATERIALS AND METHODS

Twenty-five of 32 patients with locally advanced and/or metastatic non-small cell lung cancer (stage IIIb/IV) referred for radiotherapy between January 1996 and April 1996 underwent functional pinhole scintigraphy of the tumor. Three of 32 patients refused the test, and 4 patients with obstructive symptoms or hemoptysis who were urgently treated with local radiotherapy were excluded from the prospective study. Thirteen of 25 patients had disease resistant to 2–3 different chemotherapy combinations that included etoposide, Adriamycin, Taxol, or taxo-
750 Tc-sestamibi Clearance in Lung Cancer

In a previous paper, we described and tested the efficacy of a novel technique using pinhole collimation (121-pixel area) provided with a 128 × 121-pixel matrix able to scan an area 4 cm in diameter. Observers were blinded to the clinical data. Standard regular regions of interest (121-pixel area) were drawn to the hottest area of the tumor and the surrounding normal lung areas. Total counts and t/nl ratio were calculated. Thereafter, all count values obtained at 60 and 120 min were corrected using the decay coefficients for Tc-99m. All analysis was done considering the decay-corrected values for both normal lung tissue and tumors.

The Tc-sestamibi E values were calculated as follows:

\[
E = \frac{[\text{t/nl}]_{(10 \text{ min})}}{[\text{t/nl}]_{(120 \text{ min})}}
\]

in which [t/nl]_{(10 min)} is the t/nl ratio calculated at 10 min postinjection, and [t/nl]_{(120 min)} is the one calculated at 120 min postinjection. t/nl values give an estimate of the ability of tumor cells to accumulate the radionuclide, whereas E values show the differences in the ability of tumor tissue to extrude the radionuclide as compared to that of normal lung tissue.

E values and t/nl ratio were analyzed within different groups of patients to assess possible correlation with tumor dimensions, histology, resistance to cytotoxic treatment, or metastatic phenotype.

Table 1 Correlation of E values with tumor and patient parameters

<table>
<thead>
<tr>
<th>Tumor dimensions</th>
<th>No. of patients</th>
<th>Mean E value</th>
<th>Range SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;65 cm²</td>
<td>10</td>
<td>1.14</td>
<td>0.93–1.58</td>
<td>0.06 0.37</td>
</tr>
<tr>
<td>&gt;64 cm²</td>
<td>15</td>
<td>1.21</td>
<td>1.00–1.62</td>
<td>0.04</td>
</tr>
<tr>
<td>M stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>11</td>
<td>1.07</td>
<td>0.93–1.24</td>
<td>0.03 0.008</td>
</tr>
<tr>
<td>M1</td>
<td>14</td>
<td>1.26</td>
<td>0.95–1.62</td>
<td>0.05</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Squamous</td>
<td>15</td>
<td>1.16</td>
<td>0.90–1.62</td>
<td>0.05 0.65</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>10</td>
<td>1.20</td>
<td>1.02–1.58</td>
<td>0.05</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;54</td>
<td>17</td>
<td>1.18</td>
<td>0.92–1.62</td>
<td>0.04 0.96</td>
</tr>
<tr>
<td>&lt;55</td>
<td>8</td>
<td>1.18</td>
<td>1.04–1.58</td>
<td>0.06</td>
</tr>
<tr>
<td>Previous chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>12</td>
<td>1.09</td>
<td>0.93–1.49</td>
<td>0.04 0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>1.26</td>
<td>1.03–1.62</td>
<td>0.05 0.05</td>
</tr>
<tr>
<td>Radiotherapy outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NR/MR</td>
<td>7</td>
<td>1.26</td>
<td>1.04–1.62</td>
<td>0.07 0.05</td>
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<tr>
<td>PR</td>
<td>12</td>
<td>1.19</td>
<td>0.95–1.58</td>
<td>0.05</td>
</tr>
<tr>
<td>CR</td>
<td>6</td>
<td>1.05</td>
<td>0.93–1.18</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Product of three dimensions measured by computed tomography scan.
† Tumors unresponsive to multiple chemotherapy agents.
§ NR, tumor reduction of <25%; MR, reduction of 25–49%; PR, reduction of 50–90%; CR, reduction of >90%.

Statistical Analysis. We used the Graphpad Prism 2.01 for graphics and statistics. Groups were compared by using unpaired two-tailed t tests as appropriate. Linear regression analysis was used to assess the radionuclide excretion rate from tumors and normal lung tissue. P < 0.05 was considered to be statistically significant.

RESULTS

Tumor and Lung Tissue Accumulation of Tc-sestamibi. The tumor and normal lung tissue accumulation was invariably higher than that of the surrounding normal lung tissue. The mean counts collected within 5 min from a 121 × 121-pixel region at 10 min postinjection were 11,820 (SD = 3,434) for the tumor area and 3,636 (SD = 703) for the surrounding normal lung tissue. The t/nl ratio ranged between 1.85 and 5.02, showing a wide range of tumor Tc-sestamibi affinity.

Tumor and normal lung counts dropped to 8990 (SD = 2978) and 3146 (SD = 636) 60 min postinjection and to 8108 (SD = 2902) and 2902 (SD = 644) 120 min postinjection, respectively. Mean values of the counts obtained for tumor and normal lung tissue at 10, 60, and 120 min postinjection underwent regression analysis (Fig. 3). Overall, tumor tissue showed a higher reduction rate of radioactivity as compared to that of normal lung tissue (P = 0.09).

The t/nl ratio did not correlate with tumor dimensions, histology, or resistance to chemotherapy or radiotherapy (data not shown). There was a trend of high t/nl values observed in cases with distant metastases (mean t/nl value = 3.5 and SD = 0.97 versus mean t/nl value = 2.9 and SD = 0.42; P = 0.09; Fig. 2a).

E Analysis. E for 25 lung cancer cases was calculated and plotted in Fig. 4. Six of 25 (24%) cases had an E value of 1.3–1.7, showing a substantial ability of these tumors to extrude Tc-sestamibi as compared to that of normal lung tissue. Of
Fig. 1  a and b, pinhole scintigraphy of a large squamous cancer around the right main bronchus 10 min postinjection of 20 mCi of Tc-sestamibi (a) and at 120 min (b). A high efflux rate was observed (1.62). c and d, pinhole scintigraphy of a squamous cell lung cancer of the left upper lobe 10 min postinjection of 20 mCi of Tc-sestamibi (c) and at 120 min (d). A low efflux rate was observed (1.05). A, a 121-pixel region within the hottest part of the tumor mass; B, a 121-pixel region over normal lung tissue.

interest, five of these cases had a known resistance to chemotherapy. Ten of 25 (40%) cases had a 1.1–1.3 \( E \) value, showing a moderate higher tumor clearance of the radionuclide, and for 9 of 25 (36%) cases, no difference could be confirmed (\( E \) value = 0.9–1.1).

In Table 1, \( E \) is compared with tumor, patient, and treatment parameters. A significantly higher mean \( E \) value was observed for patients with tumors not responsive to chemotherapy (1.26 versus 1.09; \( P = 0.01 \)) as compared to the group of patients that had never received chemotherapy (Fig. 5a). The response rate to radiotherapy significantly correlated with \( E \) values. Complete responders had tumors with lower mean \( E \) values as compared with those of other patients (\( P = 0.05 \); Fig. 5b). The \( E \) of the primary tumors of patients with known distant metastases was significantly higher than the one of patients with an apparent nonmetastatic phenotype (\( P = 0.008 \); Fig. 2b). No correlation was found with the tumor dimensions, histology, or age of patients.

DISCUSSION

Inoperable non-small cell lung cancer is a disease with poor curability. Five-year survival is achieved in less than 10% of patients, approaching 0% for patients with distant metastasis (14). High-dose radiotherapy for early local stages results in about a 30% 5-year overall survival rate (14, 15). Although surgery is considered to be the only curative treatment for stage I/II cases, the 5-year survival rate does not exceed 60%. Still, for those cases with high tumor vasculature and/or expression of oncogenes such as c-erbB-2 or loss of apoptosis-regulating genes, the 5-year survival rate after surgery is less than 25% (16–18). It is therefore necessary to search for nonsurgical management that would increase postoperative results and allow an effective treatment of inoperable stages. Chemotherapy usefulness in the treatment of non-small cell lung cancer local or metastatic disease remains controversial (19). In a large analysis from the European Lung Cancer Working Party (20), objective response to chemotherapy was found to be of prognostic significance.

Up to now, no tests exist that would reliably predict response to chemotherapy or radiotherapy. The expression of P-glycoprotein in tumors is considered important in determining the resistance of tumors to a variety of cytotoxic drugs (2–4, 21). Immunohistochemical detection of P-glycoprotein in non-small cell lung cancer has been reported (22). Recently, two
more proteins with activity to that of P-glycoprotein have been identified. The MRP and the lung resistance protein were coexpressed with P-glycoprotein in 64% of the cell lines examined (23). MRP was abundantly expressed in about 50% of adenocarcinomas and in 20% of squamous cell and large cell carcinomas (24). A clinical role of both P-glycoprotein and MRP expression in non-small cell lung carcinoma has also been reported (22, 25).

Sestamibi is a lipophilic monovalent cation currently used for in vivo tumor scintigraphy (8). Recent data show P-glycoprotein involvement in Tc-sestamibi extrusion from normal and tumor cells (11, 12). It is well known that although MRP has a role distinct from that of P-glycoprotein, MRP transfectants display increased resistance to a variety of lipophilic drugs (26). Whether MRP and lung resistance protein are also involved in the extrusion of the sestamibi lipophilic molecule is not known. However, the structural similarity of the MRP and P-glycoprotein, together with the above-mentioned observations, suggests that functional imaging of tumors using this radiotracer could be of value for the detection of the activity of a large group of proteins involved in multidrug resistance (27).

Uptake of Tc-sestamibi from lung cancer and normal lung tissue has been studied with planar parallel-hole scintigraphy by Hassan et al. (28). The t/nl ratio was found to be 1.59 + 0.36, and a rather increased radionuclide clearance from tumor tissue was also observed. In our study, the t/nl ratio was found to be substantially higher and showed a wider range of values (1.85–5.02). This difference is a consequence of the different functional imaging technique used to assess tissue uptake, an issue analyzed in a previous study in which we compared planar parallel-hole and pinhole scintigraphy (13). This technique, which enables a more reliable grading of relative radionuclide capture by tumors, could be of importance in identifying tumors with increased extrusion ability for Tc-sestamibi. Indeed, with simulator-aided pinhole scintigraphy, we were able to identify a group of tumors with marked ability to extrude the radionuclide as compared to normal lung tissue. Twenty-four percent of our cases showed a relative efflux ratio as high as 1.3–1.7. Tumors with known resistance to a variety of P-glycoprotein-dependent cytotoxic drugs had significantly higher E values as compared with those of chemotherapy-naïve cases (Fig. 5). Although chemotherapy-responsive tumors should be present in the chemotherapy-naïve group of patients, this could not be confirmed. In that way, it is not clear whether the difference between groups is a result of different sensitivities to chemotherapy or other events. It may well be that exposure to previous chemotherapy alters the Tc-sestamibi tumor clearance by modulating other than P-glycoprotein mechanisms. A prospective trial is necessary to answer the problem. Complete response to radiotherapy was seen in patients with low E values. This later observation should be viewed with caution, because tumors refractory to or preexposed to chemotherapy might be less responsive to radiotherapy no matter what the P-glycoprotein status is. To our
Patients unresponsive to multidrug chemotherapy had statistically significant higher E values (a). Lower E values were associated with tumors responsive to radiotherapy (b).

knowledge, no data exist on multidrug resistance correlation with resistance to radiotherapy.

In a previous study of 19 breast cancer cases, Scopinaro et al. found a correlation between pathologically assessed high microvessel counting and Tc-sestamibi uptake (29). In an immunohistochemical study, we also found a correlation between pathologically assessed high microvessel counting and nodal metastasis and with clinical outcome (16). In the present study, the only known parameter of clinically aggressive behavior was the presence of distant metastases. Indeed, patients with distant metastases had tumors with a higher ability to intrude the radionuclide, but this was not statistically significant. Of interest, high extrusion rate was significantly associated (P = 0.008) with distant metastases, implying possible implications in defining groups of patients with tumors bearing aggressive behavior. High tumor vascularization should rather be associated to radionuclide cell capture and not to the extrusion rate out of cells implied in our study. However, what we actually measured was the radionuclide clearance from the tumor mass, not that from independent cells. Increased blood flow may well have accelerated radionuclide removal away from the tumor mass after its extrusion into the extracellular matrix and tumor stroma. Still, recent data also imply a direct association of multidrug resistance phenotype with aggressive behavior and metastatic phenotype that further supports our clinical findings (30). It may be that at least two mechanisms, angiogenesis and multidrug resistance, contribute to the results of the Tc-sestamibi functional scintigraphy. Still, confirmation of our findings in a large study may be of value in defining high-risk groups of operable patients that would benefit from an adjuvant treatment.

Our study reports a novel method for lung cancer functional imaging and analysis of Tc-sestamibi kinetics. Moreover, we provide evidence of direct correlation of tumor ability to extrude the radionuclide with resistance to cytotoxic treatment as well as with an increased metastatic potential. Whether the phenomenon is directly related to multidrug resistance, angiogenesis, or other intrinsic tumor factors is not clear from the present study. However, substantial experimental work on P-glycoprotein-mediated cellular extrusion of sestamibi strongly supports the hypothesis that Tc-sestamibi functional imaging may be a method for in vivo imaging of multidrug resistance (31). Comparative functional imaging and pathology studies as well as functional imaging studies after administration of P-glycoprotein inhibitors are required to further clarify a potential prognostic and therapy-guiding role of Tc-sestamibi functional tumor imaging.

REFERENCES


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