Iodine-Labeled Tamoxifen Uptake in Primary Human Breast Carcinoma

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Assessing tumor uptake and retention of 123I-labeled tamoxifen (TX) could increase our understanding of TX’s action and the mechanisms involved in resistance to the drug. Methods: Nine untreated primary breast carcinoma patients underwent whole-body planar and tomographic (SPECT) imaging 30 min and 4–5 h after injection of 185 MBq 123I-TX. Tumor-to-normal tissue uptake ratios (T/N) derived from SPECT images were related to estrogen receptor (ER) and progesterone receptor (PR) status. Results: In 4 of 9 patients, all of whom were ER+/PR+, 123I-TX tumor uptake was clearly depicted. In 2 of them, involved axillary lymph nodes were also visualized. T/N consistently increased over time. All ER+/PR− and ER−/PR− tumors as well as 2 ER+/PR+ tumors were 123I-TX−. Conclusion: These preliminary findings suggest that 123I-TX is preferentially taken up in α-ER+/PR+ breast tumors known to be more likely to respond to endocrine treatment.

Key Words: iodine-labeled tamoxifen; breast carcinoma


The predictive value of breast tumor estrogen receptor (ER) status, assessed either by ligand binding assays or immunohistochemistry, is well documented in the palliative setting. However, response to tamoxifen (TX) therapy is seen in only 50%–60% of ER+ patients with metastasized breast cancer and in up to 10% of ER− patients (1). This means that ER status is a good parameter for predicting outcome to TX treatment in patients with breast carcinoma, though it is certainly not fully reliable.

In vivo imaging of radiolabeled TX tumor uptake, retention, and, eventually, efflux could help to increase our understanding of TX’s action and the mechanisms involved in resistance to the drug. This in turn could provide new targets for the design of therapeutic agents that can both effectively antagonize the ER-dependent growth pathway and circumvent or prevent the emergence of inevitable resistance to the drug in vivo.

In this feasibility study, using planar and SPECT imaging, primary breast tumor lesions were investigated for accumulation of 123I-labeled TX (trans-[123I]iodomethyl-N,N-diethyltamoxifen). 123I-TX uptake was related to tumoral ER and progesterone receptor (PR) status as assessed by immunohistochemistry.

MATERIALS AND METHODS

Nine women (age range, 45–79 y) presenting with an untreated primary breast carcinoma proven by biopsy and with clinically macroscopic disease were included in the study. In all patients, immunohistochemistry for determination of ER and PR was performed. Informed consent according to institutional guidelines was obtained from all patients. The ethical board of the Ghent University Hospital approved of the application of the tracer to humans. 123I-TX was prepared as described previously (2). Briefly, 740 MBq 123I, dissolved in 40 µL 0.01 mol/L NaOH, were added to trans-mesy1-N-diethyltamoxifen (1 mg) in 200 µL acetone. After heating at 80°C for 20 min the reaction mixture was cooled to room temperature and purified by reversed-phase high-performance liquid chromatography (ethanol:acetate buffer, pH 5.0, 70:30 v/v; flow rate, 1 mL/min; detection, ultraviolet 254 nm and Na(Tl)). The fraction containing 123I-TX was isolated, filtered through a 0.22-µm membrane filter into a sterile and pyrogen-free vial, and diluted with up to 10 mL physiologic saline.

Patients were pretreated with potassium iodide orally to block thyroid uptake of free radioactive iodide. The dose was 185 MBq 123I-TX, injected in the arm opposite of the known breast lesion to avoid false-positive uptake in axillary lymph nodes. In all patients, whole-body images (scan speed, 11.4 cm/min; matrix size, 256 × 512), planar spot images (matrix size, 256 × 256), and tomographic images (40 views of 20 s per detector; 60 angles; 3° angle; matrix size, 128 × 128) were obtained using a triple-head gamma camera (Irix; Picker, Cleveland, OH) between 15–30 min and 4–5 h after injection. Raw tomographic data were reconstructed iteratively using ordered-subset expectation maximization (6 subsets and 1 iteration) and postfiltered using a Butterworth filter (cutoff frequency, 0.8; order, 7).

In all patients, routine immunohistochemical assessment of ER and PR was performed on formalin-fixed paraffin-embedded tissue sections of the entire original primary tumor or biopsy sample using, respectively, the mouse monoclonal antibodies clone 6F11 and clone 1A6 (Ventana Medical Systems, Tucson, AZ). ER and PR are expressed in percentage positive staining cells on immu-
and immunohistochemical examination. Tumors were considered ER+ or PR+ when >10% of the cells stained positively.

All studies were reviewed for the presence or absence of focally increased uptake at sites of lesions known from clinical findings or other imaging modalities and for possible additional spots of uptake by 2 observers. One of the observers was blinded to the clinical information and the findings of the other imaging modalities, and both were blinded with respect to the ER and PR status. Disagreements were resolved by consensus.

Quantification of abnormal 123I-TX uptake was performed using regions of interest (ROIs) drawn over areas with increased 123I-TX uptake visualized on SPECT images (i.e., the tumors) and a similar ROI in the contralateral breast or axillary region. For each ROI, the average counts per pixel were calculated. For each lesion, the uptake visualized on SPECT images (i.e., the tumors) and a similar nonspecific uptake (tumor-to-normal tissue uptake ratio [T/N]).

A possible relationship between the maximal tumor diameter assessed by MRI and 123I-TX T/N was assessed using Spearman rank correlation coefficients. Values of \( r \geq 0.05 \) were considered significant.

RESULTS

In 4 of the 9 patients presenting with primary breast carcinoma, 123I-TX uptake was clearly depicted on SPECT images (Table 1; Fig. 1) but not always on planar images among others because of overlap with liver activity. All 4 patients suffered from ER+/PR+ breast tumors. In 2 of these patients, involved axillary lymph nodes were also depicted on 123I-TX images. T/N consistently increased over time. All ER+/PR− and ER−/PR− tumors, as well as 2 other ER+/PR+ tumors, were 123I-TX−.

Correlation coefficients between early and late T/N versus maximal tumor diameter were, respectively, \( r = 0.254 \) (\( P = 0.472 \)) and \( r = 0.430 \) (\( P = 0.279 \)).

DISCUSSION

The majority of TX and its metabolites are bound to serum and only 2%–5% is in the “free” unbound state limiting the amount of bioavailable drug to the tumor. Nevertheless, reported intratumoral levels of TX and its metabolites, after steady state, are 5- to 7-fold greater compared with serum or plasma levels, implying accumulation against a concentration gradient. In patients treated for <2 wk with a dose of 30 mg TX per day, a significant difference in the intratumoral TX concentration between ER+ and αER− tumors was observed (450.1 + 75.3 ng/g and 120.9 + 49.9 ng/g, respectively; \( P = 0.04 \)) (3). However, after ≥2 wk of therapy, approaching steady-state conditions, no significant difference was found, with both ER+ and ER− tumors accumulating high intratumoral concentrations. Thus, both ER+ and ER− breast tumors progressively accumulate TX, but ER+ tumors do this much more rapidly. The use of a bolus injection of 123I-TX and consecutive imaging should allow for this difference in α-ER-related uptake kinetics, with higher expected uptake values for α-ER+ tumors, to emerge visually. However, because the apparent distribution volume for TX is ±50–60 L/kg, which implies extensive tissue binding related to its lipophilic characteristics, a high background activity may be anticipated. In this regard, in a study of patients receiving 40 mg/d for ≥30 d (i.e., steady state), TX and its metabolites were found to accumulate in nonmalignant human tissues 10- to 60-fold above serum levels expressed as ng/g wet weight tissue to ng/mL serum. Nevertheless a depictable 123I-TX uptake compared with background activity was seen in 4 of 6 ER+/PR+ patients but not in the remaining ER+/PR− and ER−/PR− tumors. The progressive and

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>TNM stage</th>
<th>Histology (diff)</th>
<th>ER (%)</th>
<th>PR (%)</th>
<th>Early T/N</th>
<th>Late T/N</th>
<th>Tumor size (cm)</th>
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<tr>
<td>65</td>
<td>T4 N1 M1</td>
<td>Ductal ca (well)</td>
<td>98</td>
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<td>44*</td>
<td>T3 N2 M0</td>
<td>Ductal ca (mod)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6.0</td>
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<td>51*</td>
<td>T2 N1 M0</td>
<td>Lobular ca (mod)</td>
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<td>T</td>
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<td>T4 NX M1</td>
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<tr>
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<td>T1 N0 M0</td>
<td>Ductal ca (well)</td>
<td>60</td>
<td>60</td>
<td>—</td>
<td>—</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Patient is premenopausal.

TNM stage = clinical tumor node metastasis stage; diff = differentiation grade; early T/N = T/N obtained 30 min after injection; late T/N = T/N obtained 4–5 h after injection; ca = carcinoma; well = well differentiated; mod = moderately differentiated; NA = not available.

ER and PR are expressed in percentage positive staining cells on immunohistochemical examination.

TABLE 1
Clinical, Pathologic, and 123I-TX SPECT Quantitative Results
consistent increase in T/N suggests a relative difference in clearance rate of bound and unbound radioligand from tissue and, thus, a specific tumor uptake mechanism in keeping with in vitro and in vivo animal data on 123I-TX. As shown in the Early Breast Cancer Trialists metaanalysis (1), whereas ER positivity confers a 50% response rate to frontline endocrine therapy, associated PR positivity, reflecting ER functionality, increases the likelihood of favorable response to endocrine treatment by 20%–30% (4). In contrast, patients who are ER+/PR− have a <10% response rate to endocrine treatment. Consequently, the lack of discernible 123I-TX uptake compared with background activity in ER+/PR− tumors may prove advantageous for prediction of patient response to TX treatment. Hypothetically, the lack of rapid 123I-TX uptake in ER+/PR− may be explained by a lower affinity of TX to nonfunctional ER.

Currently, there are at least 3 major identified intracellular binding compartments for TX: partition into cellular membranes, binding to high-affinity ERs, and binding to low-affinity antiestrogen binding sites (AEBS) (5). Because partition into cellular membranes is aspecific, a similar degree of accumulation in primary breast tumors compared with surrounding normal breast tissue is to be expected. Given the structural similarity between 123I-TX and other AEBS-binding TX derivatives, some of which are 123I-labeled, retention of 123I-TX in tumor cells may also relate to the presence of AEBS. However, AEBS are low- to moderate-affinity binding sites and depictable 123I-TX retention through AEBS binding would require a steady-state condition and not a bolus injection as performed in the series presented. Thus, increased tumoral 123I-TX uptake compared with the surrounding normal breast tissue in this series most likely reflects binding to functional ER, in keeping with available animal data.

CONCLUSION

These preliminary findings suggest that 123I-TX is preferentially taken up in ER+/PR+ breast tumors known to be more likely to respond to endocrine treatment. However, further confirmation in a larger series is needed.

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REFERENCES

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