Tissue Iodine Content and Serum-Mediated 125I Uptake-Blocking Activity in Breast Cancer*


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ABSTRACT

In the thyroid, active transport of iodide is under control of the TSH-dependent Na+/I− symporter (NIS), whereas in the breast such control is less well understood. In this study, NIS expression was demonstrated by RT-PCR in 2 of 2 fibroadenomata and 6 of 7 breast carcinoma messenger ribonucleic acid sequences. In addition, mean total tissue iodine levels of 80.9 ± 9.5 ng/mg protein in 23 benign tumors (fibroadenomata) were significantly higher than those in 19 breast cancers taken from either the tumor (18.2 ± 4.6 ng/mg) or morphologically normal tissue taken from within the tumor-bearing breast (31.8 ± 4.9 ng/mg; P < 0.05 in each case). Inhibition of 125I uptake into NIS-transfected CHO cells was observed in serum from 20 of 105 (19.0%) breast carcinoma, 8 of 49 (16.3%) benign breast disease, and 27 of 86 (31.4%) Graves’ patients, but in only 1 of 33 (3.0%) age-matched females controls. IgG purified from serum of patients showing positive 125I uptake inhibition also inhibited iodide uptake, suggesting that such inhibition was antibody mediated. 125I uptake inhibition was significantly associated with thyroid peroxidase antibody positivity (P < 0.05) in sera from breast cancer patients, but not in those with benign breast disease, once again suggesting an association between thyroid autoimmunity and breast carcinoma.


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* This work was supported by the Irish Health Research Board and The Wellcome Trust.

ALTHOUGH the thyroid is the principal organ in the body with the ability to concentrate iodide (1), other tissues such as salivary glands, mammary glands, and gastric mucosa also possess this property (2, 3). Physiological transport of iodide into the thyroid gland has been shown to be under the control of TSH and mediated by the sodium iodide symporter (NIS), an intrinsic membrane-associated protein recently cloned and characterized in the rat (4). The presence of the NIS has been demonstrated using RT-PCR and Northern blot analysis in a variety of tissues other than the thyroid, including breast, colon, ovary, gastric mucosa, and parotid gland (5–7). It has been suggested that the NIS may be a candidate autoantigen in autoimmune thyroid disease (8), and this concept is supported by the finding that sera from 30% of patients with Graves’ disease possessed the ability to block radioactive iodide uptake by CHO cells transfected with NIS (9) and that this blocking activity was IgG related.

A role for iodine in the prevention of breast dysplasia and hyperplasia has previously been described (10). More recently, it has been demonstrated that elemental iodine (I2) rather that iodide (I−) when administered together with the carcinogen dimethylbenzanthracene resulted in a significant reduction in the incidence and size of multiple mammary tumors that developed after carcinogenesis (11, 12). The latter group (12) reported that a higher tumor iodine content together with a significantly reduced tumor size were evident in rats treated with medroxyprogesterone acetate and I2 than in those treated with medroxyprogesterone acetate alone, suggesting that the active uptake of iodine had a suppressive effect on tumor growth. The objective of this study was to investigate NIS expression and the iodine content of human breast tissues of both benign and malignant pathologies. In addition, a modification of a method developed using a CHO-K1 cell line stably transfected with the human NIS (hNIS) (9) was applied to study the ability of serum obtained from patients with both breast cancer and benign breast disease to inhibit iodide uptake.

Subjects and Methods

Ribonucleic acid (RNA) extraction and production of complementary DNA (cDNA)

NIS expression was investigated in two fibroadenoma and seven breast carcinoma tissues. Tissue specimens were also obtained from patients undergoing subtotal thyroidectomy (n = 2) as well as specimens of skin, vein, and prostate, which served as positive and negative controls, respectively. Total RNA was extracted from frozen thyroid and breast cancer tissues using the Ultraspec II total RNA isolation kit (Biotecx Laboratories, Inc., Houston, TX) and was diluted to 1 μg/μL in ribonuclease-free water, following the determination of RNA concentration at 260 nm. RNA was reverse transcribed using oligo(deoxythymidine) (15-mer) and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD). One microgram of total RNA and 1 μg oligo(deoxythymidine) primers (15 mer) in a 5-μL reaction volume were heated to 65°C for 10 min, cooled on ice, and subsequently incubated for 1 h at 37°C with 15 μL of a pool containing 4 μL 5-fold concentrated RT buffer [250 mmol/L Tris-HCl (pH 8.3), 375 mmol/L KCl, and 15 mmol/L MgCl2], 10 mmol/L dithiothreitol, 1 μL RNAsin (40 U/μL), 1 μL deoxy-NTP (10 mmol/L), and 1 μL Moloney murine leukemia virus reverse transcriptase (200 U/μL). The quality of the cDNA was investigated by performing PCR amplification, under the conditions described below, with a pair of primers from the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene sequence (sense, 5′-TCCATGACAACTTTGCGATCGTG-3′; antisense, 5′-GGTGCTGTGTAAGTCACAGGAGAC-3′) predicted to amplify a 380-bp fragment.
mogenates were incinerated in a muffel furnace at 600°C for 180 min. The cation (13) of the alkaline incineration method of Foss was within the tumor-bearing breast. Surgical specimens of thyroid tissue were collected on ice and homogenized in 0.05 mol/L KCl, 0.1% Triton X-100, 1.25 mol/L MgCl2, 1.25 mol/L deoxy-NTP, 0.25 μg/μL primers, and 0.05 U/μL Taq polymerase. The reaction profile consisted of 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 2 min. After amplification, 20 μL of the reaction mix were separated on a 2% agarose gel, and the resulting bands were visualized under UV light after ethidium bromide staining (0.1 μg/μL). Molecular weights were compared to a 1-kb ladder. In all PCR experiments, a negative control, in which water replaced the cDNA template, was included.

Tissue iodine determination

Surgical specimens were collected on ice and homogenized in 0.05 mol/L KH2PO4 containing 10 mM KOH. Homogenates were stored at −20°C until assay. Total tissue iodine concentration was determined in 23 benign tumors (fibroadenoma) and 19 breast carcinomas, including tumor tissue and specimens taken from a site remote from the tumor but within the tumor-bearing breast. Surgical specimens of thyroid tissue (n = 2) served as positive controls. The technique used was a modification (13) of the alkaline incineration method of Foss et al. (14). Homogenates were incinerated in a muffel furnace at 600°C for 180 min. The ash was dissolved in ddH2O and quantified spectrophotometrically at 490 nm using the Sandell-Kolthoff reaction. To account for differences in tissue cellularity, results were expressed as nanograms of I per mg protein.

Iodide uptake inhibition assay

A total of 273 sera were tested for iodide uptake inhibitory activity, of whom 105 had histologically confirmed evidence of breast carcinoma and 49 had benign fibroadenoma. Both breast study groups were asymptomatic for thyroid disease. Sera were also obtained from 33 age-matched healthy female controls and 86 Graves’ disease patients. Sera were tested for iodide uptake inhibitory activity, using a modification of the method of Ajan et al. (9). This assay used a CHO-K1 cell line stably transfected with the hNIS (designated CHO-NIS 9). Cells were first incubated with test serum. After an initial 1-h incubation, an additional 500 μL Ham’s F-12 medium containing 3–4 kilobase pairs of 125I were added, and the cells were incubated for another 30 min. After washing to remove exogenous 125I, the cells were solubilized using 1.0 mL 1 mol/L NaOH, and radioactivity was counted using a γ-counter for 1 min to determine the degree of 125I incorporated. Results were expressed as a percentage of inhibition of iodide uptake. The upper limit of the reference range (30% inhibition of 125I uptake) was determined on the basis of the mean value for 33 control sera ± 3 sd. To investigate whether 125I uptake inhibition was IgG mediated, sera from patients with breast disease (n = 4), Graves disease (n = 6), and normal controls (n = 4) were run through a protein G-Sepharose column with 10 mM phosphate buffer at pH 7.0. These patients were selected on the basis of positive or negative serum 125I uptake inhibitory activity. Bound IgG was eluted with 100 mM/L glycine-HCl at pH 2.8 and extensively dialyzed against PBS before use in the assay.

Serum TPO antibody (TPO-Ab) determination

Sera from 93 patients with breast cancer and 42 patients with benign breast disease in which 125I uptake inhibition had been studied were also assessed for serum TPO-Ab titers. Serum TPO-Ab were measured using a highly sensitive direct RIA (RSR, Cardiff, UK). TPO-Ab titers were recorded as units per mL Medical Research Council Standard 65/93. The upper limit of the reference range was 0.3 U/mL (15).

Statistical analysis

Data were analyzed using Mann-Whitney U and χ² tests.

Results

NIS messenger RNA (mRNA) expression in thyroid, breast, and control tissues

Figure 1 shows the results of NIS RT-PCR analysis after separation on a 2% agarose gel. The upper gel shows GAPDH mRNA amplified from the 14 tissues. The lower gel shows that an amplified fragment of 454 bp sized against a 1-kb ladder corresponding to NIS was observed in 6 of 7 breast cancer specimens (lanes 2–8). Similar bands were observed in 2 fibroadenomata (lanes 9 and 10) as well as in an extract prepared from thyroid tissue (lane 14), which served as a positive control. In contrast, no bands corresponding to the NIS were seen in vein, prostate, and skin tissue samples (lanes 11–13) or from a single breast carcinoma tissue sample (lane 5).

Tissue iodine determination

Results of tissue iodine measurements in breast and thyroid tissue homogenates are shown in scattergraph form in Fig. 2. There was a wide distribution of individual values, but levels in 23 fibroadenomata (median, 69 ng I/mg protein; range, 21–173) were significantly greater than those in 19 breast carcinomas (median, 22 ng I/mg protein; range, 10–78) or remote tissue specimens (median, 15 ng I/mg protein; range, 12–81; P < 0.01 in each case). Tissue iodine concentrations in breast carcinoma were also significantly lower than those in the remote breast group (P < 0.05). All breast tissue iodine concentrations were an order of magnitude lower than those in two thyroid specimens, which had iodine concentrations of 704 and 850 ng I/mg protein, respectively.

Modulation of NIS activity by breast disease sera

The distribution of individual values as well as the percent prevalence of 125I uptake inhibition (>30%) in patient and control sera are shown in scattergraph form (Fig. 3). Thirty-three normal sera were tested for iodide uptake inhibitory activity (median, 17.4%; range, 1.2–38.0%). Of the 105 breast carcinoma sera tested, 20 (19.0%) exhibited significant inhibitory activity (median, 19.0%; range, 4.7–53.5%). In sera from patients with benign breast disease, 8 of 49 (16.3%) showed more than 30% inhibition of iodide uptake (median, 20.0%; range, 5.1–46.3%). These prevalences were not significantly different from each other or from those observed in sera obtained from Graves’ patients of whom 27 of 86 (31.4%) showed positive inhibitory activity (median, 22.7%; range, 1.7–55.1%). Differences in the frequency of iodide uptake inhibition in the breast carcinoma and Graves’ disease patient groups were significantly greater than those in controls, of whom only 1 of 33 (3.0%) was positive (P < 0.05). In contrast, differences in the frequency of 125I uptake inhibition between sera from patients with benign breast disease and healthy controls failed to reach statistical significance.

Purified IgG

Figure 4 shows results of 125I uptake inhibition produced by purified IgG (1 mg/mL) prepared in sera from patients with breast disease, Graves’ disease, and normal controls. IgGs from three patients with breast disease previously
shown to have significant serum $^{125}$I uptake inhibitory activity (36.4–45.7%) uptake also inhibited iodide uptake by 7.3–18.2%. IgGs prepared from six patients with Graves’ disease showed inhibition of $^{125}$I uptake ranging from 8.0–37.5%. In contrast, purified IgG prepared from a single breast disease patient and four controls who were negative for $^{125}$I uptake inhibition did not show significant IgG-mediated $^{125}$I uptake inhibition (range, 2.6% to 2.1%).

Serum TPO.Ab

Table 1 shows a significant association between TPO.Ab positivity and $^{125}$I uptake inhibition in sera from 93 breast cancer patients and 42 with benign breast disease. Of the breast cancer patients, 31 were TPO.Ab positive, and 62 were antibody negative. Significant inhibition of $^{125}$I uptake was observed in 11 of 31 sera positive for TPO.Ab compared to 6 of 62 TPO.Ab-negative sera ($P < 0.01$). No significant association was observed between $^{125}$I uptake inhibition and TPO.Ab positivity in sera from patients with benign breast disease.

**Fig. 1.** Thirty-five cycle GAPDH (a) and NIS (b) RT-PCR amplification of 1 μg/μL total RNA. Lane 1, One-kilobase ladder; lanes 2–8, breast cancer; lanes 9 and 10, fibroadenoma; lanes 11–13, control tissues (vein, prostate, and skin); lane 14, thyroid; lane 15, RT-PCR blank.

**Fig. 2.** Distribution of total iodine (nanograms of I per mg protein) in breast and thyroid tissues.
disease; 2 of 11 sera positive for TPO.Ab showed significant inhibition of $^{125}$I uptake compared to 5 of 31 TPO.Ab-negative sera.

**TABLE 1.** $^{125}$I uptake inhibition positivity in sera from patients with breast disease (TPO.Ab positive or negative)

<table>
<thead>
<tr>
<th>TPO.Ab positive</th>
<th>TPO.Ab negative</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I uptake inhibition positivity (breast cancer)</td>
<td>11/31</td>
<td>6/62</td>
</tr>
<tr>
<td>$^{125}$I uptake inhibition positivity (benign breast disease)</td>
<td>2/11</td>
<td>5/31</td>
</tr>
</tbody>
</table>

**FIG. 3.** Inhibition of $^{125}$I uptake by normal sera (n = 33), breast cancer (n = 105), benign breast disease patients (n = 49), and Graves’ disease (n = 86). Results are the mean of duplicate cultures and are expressed as the percent inhibition of iodide uptake in CHO-NIS 9 cells. Positive sera are classified according to their ability to inhibit iodide uptake by more than the mean $\pm 3$ SD of control sera.

**FIG. 4.** Effect on $^{125}$I uptake by CHO-NIS 9 cells of purified IgG concentrates prepared from patients with breast disease, Graves’ disease, and controls. Results were divided on the basis of positive or negative serum $^{125}$I uptake inhibition assays and are the average of duplicate determinations. BD, Breast disease; GD, Graves’ disease; C, control.

**Discussion**

This study demonstrates that the tissue iodine content of breast carcinoma specimens was significantly lower than that of tissue from a remote site within the tumor-bearing breast, which, in turn, was lower than that of fibroadenomata. Although the requirement for iodide uptake by the breast in providing breast milk iodine content is well established in the lactating breast (16), the iodine requirement of the non-lactating state is less well understood (17). The results of the present study, which demonstrate that the tissue iodine content of breast carcinoma was significantly lower than that in remote tissue from the tumor-bearing breast or in fibroad-
enomata, suggest a disorder in uptake of iodide in breast cancer. The relatively low or absent iodine content of breast carcinoma tissues investigated in the present study is supportive of the previously expressed view that iodine (I\textsubscript{3}) rather than iodide (I\textsuperscript{-}) is required by mammary tissue for normal function (11). These workers demonstrated that molecular I\textsubscript{3} has a therapeutic effect in fibrocytic breast disease (18) and that administration of I\textsubscript{3} alone (19) or in combination with medroxyprogesterone acetate (12) had a suppressive effect on dimethylbenzanthracene-induced rat mammary tumors. The latter group also showed the iodine content of breast tumors in I\textsubscript{3}-treated rats to be significantly greater than that in nonsuppressed tumors.

Studies were focused on the hNIS, the extrathyroidal expression of which has recently been demonstrated (6, 7) using RT-PCR and Southern hybridization in tissues including normal human mammary glands. In the present study expression of the NIS was found to be a feature of both fibroadenomata and breast carcinoma tissues, which suggests that variations in tissue iodine content according to breast pathology may reflect differences in the functional capacity of the hNIS to take up iodide.

The concept of a diminished iodide uptake by breast cancer tissue is supported by the presence of significant iodide uptake inhibitory activity in serum from breast carcinoma patients (19.6%) compared to that in a healthy control population (3.0%). Such inhibitory activity was present in 31.4% of 86 Graves' patients, a prevalence identical to that previously reported using a similar assay methodology (9), but differing from an earlier report demonstrating that approximately 15% and 84% of Hashimoto’s thyroiditis and Graves’ disease sera, respectively, bound to recombinant rat NIS in Western blotting experiments (20). However, the significance of the presence of NIS antibodies in patients with Graves’ disease remains a matter of conjecture. It has been suggested that such antibodies may partially counter the effect of TSAb in Graves’ disease and thus ameliorate the severity of hyperthyroidism (9). Although no such explanation is pertinent in breast cancer, a possible explanation for diminished I\textsuperscript{-} uptake by CHO-NIS 9 cells would be excessive I\textsuperscript{-} content of patient sera. Although iodine excretion was not measured in the individual patients studied, a previous report from this laboratory (21) found no difference in urinary iodine excretion between patients with breast disease and age-matched controls. There is no reason to suspect that patients in the current study cohort had any greater exposure to iodine. Another potential contributor to observed anomalies in 125I uptake is NIS structure. The CHO-NIS 9 cell line expresses a truncated symporter (amino acids 1–612) and therefore may not be the best tool to detect blocking antibodies. However, in preliminary experiments we have performed transient transfection of COS-7 cells with full-length symporter and found complete correspondence between inhibition assays with full-length and truncated symporter in 49 Graves’ disease sera. In particular, no additional sera were positive with the full-length symporter, indicating that CHO-NIS 9 cells are satisfactory for measurement of inhibitory antibodies.

The finding that 125I uptake inhibition, when present in sera from patients with breast disease, resided in the IgG fraction is consistent with the view that such inhibition was immunologically mediated. This is supported by studies using purified IgGs from patients with Graves’ disease, which suggested that NIS inhibitory activity was antibody related. The possibility of an autoimmune-mediated mechanism being responsible for inhibition of iodide uptake in the thyroid has been previously reported (8, 20, 22). This is further supported by the demonstration in the present study of a significant association between TPO.Ab and 125I uptake inhibition positivity in the breast cancer patients tested. Previous studies from our own (23) and other (24, 25) groups have demonstrated an increased prevalence of circulating thyroid antibodies in breast cancer, thus emphasizing a possible link with thyroid autoimmunity. Whether this is a consequence of the disease or part of its pathogenesis is unclear. As tissue iodine content, NIS mRNA, and 125I uptake inhibition analysis were not carried out on the same individual patients, any hypothesis based on such disparate observations must be speculative. However, we postulate that the low iodine content observed in some breast cancers may arise from the presence in patients’ serum of NIS blocking 125I uptake inhibitory activity, perhaps of immunogenic origin. This postulate is more difficult to sustain in the case of benign breast disease, in which higher tissue iodine content compared to either breast cancer or remote tissue was observed despite the finding of iodide uptake blocking activity in 16.5% of the subjects tested. This may reflect the heterogeneity of fibroadenomata (and, hence, NIS expression) or the likelihood that iodide uptake blockade is only one of many mechanisms influencing iodide uptake by the breast. Although, as previously stated, a beneficial role for elemental I\textsubscript{2} in experimental tumorigenesis has been demonstrated in the rat (12, 19), the involvement of iodine in the natural history of breast carcinoma remains to be explored.

Acknowledgments

We greatly acknowledge the expert technical input of D. F. Smith, and the assistance of the staff of the Departments of Surgery and Pathology, St. Vincent’s Hospital, Dublin, in particular Sr. Mary Murray and Ms. Rosaleen Rafferty. We are grateful to Drs. P. A. Smanik and S. M. Jhiang (Ohio State University, Columbus, OH) for the provision of hNIS DNA.

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