Radioiodide uptake and sodium iodide symporter expression in breast carcinoma

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Breast cancer is a common malignancy in women all over the world and novel therapeutic approaches are required for the treatment of patients who become refractory to conventional therapies. Thyroid cancer is being treated successfully with radioiodine since many years. The iodide is transported inside the thyroid epithelial cell via sodium iodide symporter (NIS) which is a trans-membrane protein. The present study was aimed to explore the uptake of radioiodide (RAI) and the expression of NIS in breast tissues of invasive ductal carcinoma patients. Breast tissues from tumor region (Tu-Br) as well as corresponding normal region (N-Br) were collected from patients of invasive ductal carcinoma. In vitro RAI uptake, its efflux and NIS expression were studied. The uptake of RAI (1.98±1.75 × 10⁵ cpm/g) in Tu-Br was significantly higher as compared to that observed in N-Br (0.31±0.27 × 10⁵ cpm/g) and fast efflux was observed in the tissue samples. NIS gene expression was positive in 41.66% (10/24) samples of Tu-Br. None of the N-Br samples expressed NIS gene. In 14 samples of Tu-Br, RAI uptake as well as NIS expression was studied. In 50% of these Tu-Br samples RAI uptake as well as NIS expression was positive. The results indicate that RAI uptake is significantly higher in breast tumor tissues as compared to their normal counterpart and in future radioiodine may be an important agent for treatment of breast cancer.

Keywords: Breast cancer, Radioiodide, RT-PCR, Sodium iodide symporter

Breast cancer is a major public health problem affecting millions of women globally. In India, it is the most common cancer in women in many regions and an increasing trend in the incidence rate has been observed over a period of time. Hence, search for novel diagnostic and therapeutic modalities is an ongoing process more so to treat patients with tumors resistant to conventional therapies such as chemotherapy, radiotherapy and hormonal treatment.

Radioiodine (¹³¹I) has been effectively used for the post-operative management of differentiated thyroid carcinoma for many decades. Iodide is accumulated in the thyroid by means of a highly specialized active iodide transport mechanism catalyzed by the sodium-iodide symporter (NIS), a glycoprotein located in the basolateral plasma membrane of the thyroid follicular cells. NIS-catalyzed iodide accumulation is a sodium dependent active transport driven by the sodium/potassium ATPase. Sodium iodide symporter of rat, human, and mouse has been cloned and gene structures have been deduced. Beside the thyroid, NIS expression has been shown to be present in various normal extrathyroidal tissues like salivary glands, gastric mucosa, mammary glands, ovary, adrenal, heart, lungs, pituitary, pancreas and testis.

The ability of mammary gland tissue to concentrate radioiodide (RAI) has been demonstrated. Various studies have reported the presence of NIS and/or uptake of RAI in human breast cancer tissues. Though this has opened the possibility of exploring the use of RAI in management of breast cancer, there are discrepancies in the percentage of patients expressing the functional NIS protein, cellular location of NIS, amount of radioiodide uptake and retention of iodide in the breast tissue. Therefore, the present study has been aimed to investigate the expression of NIS mRNA in human breast tissue and to determine its ability to concentrate radioiodine.

Materials and Methods

The study subjects comprised of women (n=27) who had breast cancer on excision biopsy or fine needle aspiration biopsy and were admitted to the hospital for total radical mastectomy. The mean age...
was 45 yrs (range: 27-65yrs). No patient was pregnant or lactating at the time of study. Tumor size ranged from 1cm to > 5cm. The pathologic diagnosis which was confirmed by surgical pathology was infiltrating ductal carcinoma of the breast. Breast tumor tissues obtained at surgery were immediately frozen and stored in liquid nitrogen for extraction of RNA to be used in RT-PCR. Part of the fresh tissue was used for radioiodide uptake studies. Normal breast tissue was obtained from peritumoral area of the same breasts from all the patients. The project was approved by our institutional review board.

*In vitro* uptake of $^{125}$I (Na$^{125}$I, Amersham Inc. USA) was studied in tissue samples (n=16) each from normal breast (N-Br) and tumor breast (Tu-Br) tissue. Fresh tissue samples were cleansed and cut into small pieces of 10 mg each. These were then incubated in a shaker water bath at 37°C in 0.02 M phosphate buffered saline (PBS) pH 7.2 containing Na$^{125}$I (10 × 10$^5$ cpm) per vial. After 1 h the medium was aspirated, the tissue was washed 3 times with PBS, blotted on a tissue paper and the iodide uptake was counted in a multi-gamma counter (RIA Star, Packard, UK). The results were expressed as cpm × 10$^5$/g of tissue. The ratio of the iodide uptake in the tumor tissue to the corresponding uptake in the normal tissue was also calculated for the individual patient samples.

The ability of the breast tissue to retain iodide was studied in 3 patient samples. For this, after estimating the iodine uptake at 1 h, fresh PBS was added to the tissue and the vials were incubated in a shaker water bath at 37°C for 10 min. The amount of iodide retained was estimated as explained earlier. The procedure was repeated to estimate the radioactivity at 20 and 50 min intervals respectively.

Total RNA was extracted from breast tissues (n = 48, 24 each of N-Br and Tu-Br) using TRIzol reagent according to the manufacturers protocol (Invitrogen, Life Technologies Inc, USA). The integrity of total RNA was confirmed by 1% agarose gel electrophoresis. cDNA was prepared using Revert Aid cDNA synthesis kit with random hexamer primers as per manufacturers protocol (MBI Fermentas, Lithuania). Complementary DNA for NIS transcription and polymerase chain reaction were carried out at 37°C. cDNA was prepared using M-MuLV reverse transcriptase. Total volume was made to 20 µl with sterile nuclease free water. The reaction was carried out at 37°C for 2 h in a thermal cycler (MJ research, USA). All reagents for reverse transcription and polymerase chain reaction were procured from MBI Fermentas, Lithuania. Primers were synthesized by Operon Biotechnologies, Germany.

**Polymerase chain reaction (PCR)**

The primer pair used for human NIS (sense primer: 5’-CTTCTGAACCTCGGTTCCTAC- 3’, antisense primer: 5’-TCCAGAATGTATAGCGCTC- 3’) and the house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (sense primer: 5’-TGCCTCCTGCACCACCACTG- 3’, antisense primer: 5’-ATGCCAGCCCCAGGTCAAAG- 3’) were those previously described. cDNA (10 µl) was used as template for amplification. The PCR reaction was carried out in 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.08% Nonidet P40, 1.5 mM MgCl$_2$, 1 mM deoxy-NTPs, 2.5 units Taq DNA polymerase and 50 pmoles of each primer. The PCR conditions were: one cycle at 94°C for 3 min followed by 35 cycles at 94°C for 30 sec, 56°C for 30 sec and 72°C for 2 min for NIS and hot start at 95°C for 5 min followed by 35 cycles of 95°C for 30 sec, 61°C for 30 sec and 72°C for 1 min for GAPDH. Final extension was carried out at 72°C for 5 min for both the targets. The amplification product was separated on 2% agarose gel and the resulting bands were visualized under UV light after ethidium bromide staining. Molecular weights were compared to a 100 bp DNA ladder. In all RT-PCR experiments, a negative control in which water replaced the cDNA template was included. The specific PCR products were eluted from the agarose gel using Gene_spin gel extraction kit (Bangalore Genei, India) according to manufacturer’s instructions followed by sequencing of the amplicons on automated ABI 3100 Genetic Analyser using ABI’s AmpliTaq FS dye terminator cycle sequencing chemistry.

Student’s *t*-test was applied to compare the radioiodide uptake in normal and tumor breast tissue. The association between PCR positivity and radioiodide uptake was also elucidated by using the paired *t*-test.
Results

The uptake of Na\(^{125}\)I was studied in normal as well as tumor breast tissue comprising 16 samples each (Fig. 1). The mean uptake in normal breast tissue was \(0.31\pm0.27 \times 10^5\;\text{cpm/g}\) (range \(0.03–1.06 \times 10^5\;\text{cpm/g}\)). In breast tumor tissue the uptake was much higher with mean value of \(1.98\pm1.75 \times 10^5\;\text{cpm/g}\) (range \(0.09–7.5 \times 10^5\;\text{cpm/g}\)). This difference was statistically significant (\(P=0.00038\)). The cut off value calculated for the Tu-Br samples to be scored positive was \(0.84 \times 10^5\;\text{cpm/g}\) (mean uptake in N-Br + two standard deviations) and the positivity was 68.75% (11/16) in samples of Tu-Br. It was also observed that for each individual patient sample, RAI uptake in tumor tissue was higher as compared to the normal counterpart.

The ratio of RAI uptakes in breast tissues of individual patients is depicted in Fig 2. Wide range (1.2 to 22.0) was observed in the ratio of the iodide uptake of Tu-Br to N-Br with median value being 7.17. In three cases, the ratios were greater than 10 with 2 of these showing 20 times more concentration of RAI in tumor tissue as compared to the corresponding normal breast tissue.

The ability of the breast tissues to retain the iodide and its consequent efflux over a period of time was studied in three patients and is shown in Fig. 3. The tissues were incubated with Na\(^{125}\)I for 1 h and then the rate of excretion of iodide was observed at 10, 20 and 50 min respectively. The iodide was rapidly excreted from both N-Br and Tu-Br tissues. In Tu-Br samples the amount of iodide retained was only 10% of that taken up at 1 h and decreased further at 20 and 50 min to less than 5%. As shown earlier in Fig. 1, the iodide uptake at 1 h in N-Br tissue was very less as compared to that in Tu-Br tissue. In the efflux studies the amount of iodide retained at 10 min was negligible which reduced further to base line values at 20 and 50 min respectively.

Total RNA was extracted from normal and tumor breast tissues using TRIzol method and the integrity was checked on agarose gel. Two clear bands of 18S and 28S were visible indicating good quality RNA (Fig. 4). The cDNA prepared was first used to study the expression of GAPDH prior to studying NIS gene expression. A 483 bp GAPDH amplicon of equal intensity was seen in all the samples (Fig. 5.)
NIS gene expression was studied in total of 24 samples each of Tu-Br and corresponding N-Br tissues after extracting total RNA from frozen tissues followed by RT-PCR with NIS specific oligonucleotide primers. Fig. 6 represents the ethidium bromide stained agarose gel showing the NIS specific amplicons. Lane 1 represents the 100 bp DNA ladder. NIS specific transcripts of 454 bp were present in Tu-Br tissues (lane 2-4). Similar band was observed in normal thyroid tissue which was used as positive control (lane 8). No bands were obtained in normal breast tissues indicating the absence of NIS specific gene expression (lane 5-7).

All the breast tissue samples were positive for GAPDH expression whereas NIS gene expression was positive in 41.66% (10/24) of Tu-Br tissues (Table 1). Sensitivity of NIS RT-PCR was 63% in Tu-Br tissue. The specificity was 100% and 60% in N-Br and Tu-Br respectively.

The specificity of NIS and GAPDH amplicons was confirmed by eluting the individual PCR products from the gel and sequencing the same. NCBI nucleotide blast search confirmed 99% homology of both the genes with the genes of homo sapiens.

In 14 Tu-Br samples both the parameters i.e. in vitro radioiodide uptake and expression of NIS gene were studied (Table 2). All these tissues had increased RAI uptake as compared to their normal counterparts as shown earlier (Fig. 1). However, only 7/14 expressed NIS mRNA. The RAI uptake in RT-PCR positive samples was 1.98±1.20 × 10^5 cpm/g and 2.19±2.50 × 10^5 cpm/g in RT-PCR negative tissues. Thus, there was no significant difference between the RAI uptake between the two groups. Also, 50% of the Tu-Br tissues concentrated RAI in spite of lack of NIS gene expression indicating that RAI uptake did not statistically correlate with NIS expression (P ≥ 0.343). NIS RT-PCR positivity was always associated with Tu-Br since no N-Br sample expressed NIS. On the other hand if NIS RT-PCR was negative it could be either N-Br or a Tu-Br tissue.

Discussion

In the present study it has been shown that human breast tissue has the ability to concentrate iodide and the radioiodide uptake is significantly higher in the tumor tissue as compared to the adjacent normal breast tissue in patients of invasive ductal carcinoma. Though iodide uptake is a fundamental feature of the thyroid gland, active iodide accumulation occurs in variety of non-thyroidal tissues including salivary glands, gastric mucosa, choroid plexus, ciliary body of the eye and various types of breast tissues including lactating mammary gland, breast atypia and malignancy. There is also evidence of extrathyroidal thyroxine formation and production of iodinated proteins by resting human breast tissue.

The physiological significance of iodine has not been fully understood in these tissues except in lactating mammary glands where approximately 20% of the trapped iodide is organified as a result of iodide oxidation by peroxidase expressed in the alveolar

<table>
<thead>
<tr>
<th>Tissue samples (n=24)</th>
<th>GAPDH -PCR</th>
<th>NIS-PCR</th>
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<tbody>
<tr>
<td>N-Br</td>
<td>24/24 (100%)</td>
<td>0/24 (0%)</td>
</tr>
<tr>
<td>Tu-Br</td>
<td>24/24 (100%)</td>
<td>10/24 (41.66%)</td>
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N-Br: Normal breast tissue, Tu-Br: Tumor breast tissue. Values in parentheses represent percent PCR positivity.

<table>
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<th>Tissue samples (n=14)</th>
<th>Radioiodide uptake</th>
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<tr>
<td>RT-PCR +ve (7/14)</td>
<td>1.98 ± 1.25 × 10^5 cpm/g</td>
</tr>
<tr>
<td>RT-PCR -ve (7/14)</td>
<td>2.19 ± 2.05 × 10^5 cpm/g</td>
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RAI uptake and NIS-PCR positivity was compared in tissue samples of Tu-Br (n=14). No correlation was observed between the PCR positivity and RAI uptake (a vs b: P ≥ 0.343)
cells of the breast followed by binding to tyrosyl residues of casein and other milk products. Iodine taken up by the lactating breast is transmitted to infants through human maternal milk. Since adequate supply of iodide is required for sufficient thyroid hormone production essential for proper development of the new borns nervous system, skeletal system and lungs. Apart from the lactating breast tissue, increased iodide uptake has been demonstrated in breast malignancies.

In the present study it was also found that breast tumor tissue concentrates significantly higher amount of radioiodide as compared to their normal counterparts. In the present study the uptake of iodide exhibited a wide range and the ratio of tumor to normal tissue uptake varied in each individual patient. It was earlier established that the transport of iodide is catalyzed by sodium iodide symporter (NIS), an intrinsic membrane protein present at the basolateral membrane of the thyrocytes and the cloning and characterization of rat and subsequently human NIS cDNA helped to understand the detailed mechanism of iodide transport in the thyroid epithelial cells. NIS mRNA and protein expression has been demonstrated in various normal extrathyroidal tissues including salivary and lacrimal glands, gastric mucosa, mammary gland etc. suggesting that iodide transport observed in some of these tissues could be through the expression of functional NIS protein. Similarly, NIS also mediates the iodide transport in the lactating mammary gland in rats, transgenic mice with breast tumors, and in collagen cultures of human breast cancer tissue. Presence of NIS in fibroadenoma of breast has been reported though not much work has been carried out in benign breast diseases. More than 80% of patients of ductal carcinoma in situ are positive for NIS expression. High NIS expression at both transcriptional and translational level with ability to transport iodide has been reported in invasive ductal carcinoma of the breast. More than 80% patients of similar histology show positive staining for NIS protein by IHC. The wide range of RAI uptake observed in the present studies could be attributed to the differential expression of NIS in individual breast tumor tissue. NIS is predominantly expressed intracellularly in the cytoplasm with the intensities and positivity varying in different tissue types. Thirty percent of normal appearing peritumoral tissue expressed NIS protein as against 70% of invasive breast cancer tissue with 40% staining strongly positive.

In the present study with 24 samples of normal breast tissue no expression of NIS mRNA was observed using RT-PCR and 41.66% of the breast tumor tissues were positive. Thus presence of NIS mRNA was always associated with Tu-Br tissue. These 41.66% tumor tissues also concentrated RAI indicating the role of NIS in RAI uptake. However, in the remaining tumor tissue samples RAI uptake was observed but NIS expression was absent. This highlights the fact that iodide uptake in breast cancer could be mediated by other transport molecules in addition to NIS. A sulphate iodide transporter was proved to be responsible for the iodide uptake in rat mammary tissue transplant cultures where DIDS (a known sulphate inhibitor) completely blocked the uptake. Also, pendrin is implicated in the iodide transport in MCF-7 breast cancer cells and the apical iodide transporter is responsible for the iodide transport in thyrocytes. Though NIS expression is common in breast tumor tissues only fraction of them exhibit strong positive staining. NIS is located in the membrane of thyroid epithelial cells and lactating breast tissue in rats but it is predominantly present in the cytoplasm in breast tumor tissue. The strong intracellular staining obtained in thyroid and breast tumor tissue in immunohistochemistry with anti-NIS antibodies may be overestimated and was proved to be non-specific.

Retention of iodide in the tumor tissue is mandatory for the effective radiation dose to be delivered leading to tumor cell death. In the present study, though the uptake of iodide was significantly higher in tumor tissue, the same was not retained for long and the efflux of iodide from the tissue was very fast. Similar efflux kinetics with fast excretion of the iodide has been observed in FRTL-5 rat thyroid cell lines with T1/2 being 4 min and also breast cancer cell line MCF-7. Iodide efflux is reported to be fast in MCF-7 cell line. The breast tissue lacks the specific mechanism of organification of the concentrated iodide as is present in the thyroid, though there are reports of the iodide being retained in form of iodinated proteins in lactating thyroid or in some breast tumor tissues. No organification was observed in an in vitro study with MCF-7 breast cancer cell lines. It has been shown that the iodide is retained for longer time without organification in non-thyroidal tumor tissues due to reuptake of the iodide from the blood. Thus, effective treatment may be possible in breast cancer without organification of the
RAI. Various strategies like transfection of the NIS gene, treatment with hormones, retinoids and/or glucocorticoids are being exploited to augment NIS gene expression and the uptake of radioiodide in breast tissue. Preferential uptake of $\text{TeO}_4^-$ in imaging studies and correlation with NIS have been reported in patients of invasive ductal carcinoma. In vivo NIS-mediated iodide accumulation in human breast cancer metastases has also been reported using scintigraphic techniques. In this study, triiodothyronine and methimazole was used to reduce the uptake of iodide to the thyroid gland, thus ensuring significant accumulation in the metastatic breast tissue.

The increased uptake of RAI and the presence of sodium iodide symporter in human breast tumor tissue shown in the present study and other reports emphasize the use of RAI in treatment of breast cancer. The possibility of enhancing the NIS expression and subsequently RAI uptake by various agents while simultaneously blocking the thyroid uptake open newer therapeutic options for management of breast cancer. However, the levels of NIS expression, ability of individual tumor to concentrate iodide, its retention and dosimetry evaluations are certain issues which need to be further addressed for effective use of RAI in treatment of breast carcinoma.

Acknowledgement
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References
24. Shennan D B, Iodide transport in lactating rat mammary tissue via a pathway independent from the Na+/I-


