Expression of somatostatin receptors in oncocyctic (Hürthle cell) neoplasia of the thyroid

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Summary Ten consecutive patients with Hürthle cell lesions of the thyroid (nodule/adenoma/carcinoma) were studied by 111In-DTPA-D-Phe1-octreotide scintigraphy. Octreotide scintigraphy localized the primary Hürthle cell tumour in eight patients as distinct areas of increased uptake or uptake in draining lymph nodes. A relatively high uptake of 111In was also observed in goiter tissue, which may lead to misinterpretations. The main indication for octreotide scintigraphy in patients with Hürthle cell carcinoma is suspicion of metastatic disease.

Keywords: indium-111-DTPA-D-Phe1-octreotide; scintigraphy; somatostatin receptors; Hürthle (oncocytic) cell tumour; thyroid

Neuroendocrine tumours, including thyroid tumours such as medullary thyroid carcinoma (MTC), have been shown to express somatostatin receptors (sstr) and can thus be diagnosed by scintigraphic techniques utilizing a radiolabelled somatostatin analogue, 111In-DTPA-D-Phe1-octreotide (Baudin et al, 1996; Tisell et al, 1997). Five different subtypes of sstr have been cloned and shown to be G-protein coupled receptors with seven transmembrane regions. Sstr2–3 and sstr5 differ from sstr1 and sstr4 regarding amino acid homology and pharmacological profile. Octreotide binds with highest affinity to sstr2 and with lower affinity to sstr3 and sstr5, while somatostatin binds to all subtypes with high affinity (cf. Bruns et al, 1994). In patients with differentiated thyroid (non-MTC) tumours distant metastases can sometimes be diagnosed and treated with radioiodine. However, one third of these tumours do not accumulate radioiodine (Maxon et al, 1990). Hürthle (oncocytic) cell lesions (nodule/adenoma/carcinoma) belong to this category. The diagnosis of Hürthle cell lesions is today based upon preoperative cytology, or histopathological examination of surgical specimens, demonstrating oncocyctic cell features.

The aims of the present study were to compare the outcome of 111In-DTPA-D-Phe1-octreotide scintigraphy with clinical findings in patients with Hürthle cell lesions of the thyroid and to study the expression of sstr in these lesions by Northern blotting using subtype-specific probes. Furthermore, the 111In concentrations in tumour or normal tissue (T) and blood (B) were determined.

MATERIALS AND METHODS

Patients

During a 3-year period (1995–97) 10 patients (seven women and three men, mean age 68, range 37–86 years) underwent thyroid surgery with subsequent histological demonstration of Hürthle cell lesions. All lesions were reexamined by two pathologists (ON and JM) and classified according to strict criteria (Rosai et al, 1990) as Hürthle cell nodules (n = 2), Hürthle cell adenoma (n = 4) and Hürthle cell carcinoma (n = 4) (Table 1). Eight of these patients had palpable thyroid tumours, where preoperative fine needle aspiration indicated Hürthle cell neoplasia (seven patients) or follicular thyroid neoplasia (one patient). Two patients had previously been treated for Hürthle cell carcinoma and had metastatic disease (patient nos V and IX). Patient no. V had local symptoms and underwent palliative resection, while patient no. IX had no further treatment. All patients underwent scintigraphy with 111In-octreotide, and eight patients were also investigated by conventional thyroid scintigraphy (cf. below) prior to surgery (Table 1). 111In concentrations were determined in both tumour and normal tissues from five patients (Figure 1). Tumour biopsies from four patients (I–IV, two adenomas, one nodule and one carcinoma, cf. Table 1) were investigated by Northern analysis and compared with biopsies of histologically normal thyroid tissue from the contralateral lobe of two patients, who underwent hemithyroidectomy for benign adenomas.

Scintigraphy

Ten patients received i.v. 210–230 MBq 111In-DTPA-D-Phe1 octreotide (10–20 μg) (Mallinckrodt, The Netherlands). The amount of 111In bound to octreotide was higher than 98%. Imaging was done 24–48 h after injection of the radiopharmaceutical. A gamma camera (GE 400 AC/T), equipped with a medium energy parallel hole collimator and connected to a GE STARCAM computer system, was used. Data acquisition was done in a dual window setting (173 and 247 keV). Single photon emission computed tomography (SPECT) of the thoracic cage was performed in all patients (cf. Ahlman et al, 1994). Six patients also underwent thyroid scintigraphy using 99mTc-pertechnetate (75 MBq) and the two patients with distant metastases were scanned with
Measurement of \(^{111}\)In concentration in tissue samples and blood

Surgery was performed 5–7 days after injection of \(^{111}\)In-DTPA-D-Phe\(^1\)-octreotide. The surgical specimens from tumour and normal tissues and blood samples, taken during surgery, were weighed and the \(^{111}\)In concentrations were measured in a calibrated gamma counter equipped with a NaI(T1) well crystal (diameter 7.6 cm, length 7.6 cm, Harshaw, The Netherlands) and a single-channel pulse-height analyser (Elscint, Israel). Corrections were made for background activity and radioactive decay. Tissue-to-blood \(^{111}\)In concentration ratios (Ti/B), were calculated for biopsied tissues, which were subsequently sent for histopathological examination (cf. Forsell-Aronsson et al, 1995). Tumour biopsies were included in the analysis, if the biopsy contained more than 80% tumour tissue. Nonthyroid specimens (lymph nodes, muscle, soft tissue and fat) were included, if they were tumour-free and contained less than 20% of adherent tissue.

Northern analyses of sstr 1–5 mRNA expression

Fresh tumour specimens were rapidly frozen in liquid nitrogen. RNA was prepared by acid guanidinium thiocyanate–phenol–chloroform extraction. Samples of total RNA (20 μg) were heat-denatured and electrophoresed in a 1% agarose gel with 2.2 mol l\(^{-1}\) formaldehyde, 1 mmol l\(^{-1}\) EDTA, 5 mmol l\(^{-1}\) sodium acetate and 20 mmol l\(^{-1}\) morpholine propane sulphonic acid (pH 7.0) as running buffer. RNA was transferred to positively charged nylon membranes (Boehringer, Mannheim, FRG) using a vacuum transfer system (Hybaid, Middlesex, UK) and crosslinked to the membrane by UV-light (Stratalinker, Stratagene, La Jolla, CA). Membranes were hybridized in rotating flasks at 65°C (Hybaid, Middlesex, UK) using \(^{32}\)P-labelled antisense RNA probes. The following probes were used: (1) a 1.126 bp PCR fragment of the human sstr1 gene corresponding to nucleotides 352–1478 (Yamada et al, 1992a); (2) a 1.7 kb Bam HI/Hind III cDNA fragment of the human sstr2 (Yamada et al, 1992a); (3) a 1.9 kb Nco/Hind III cDNA fragment of the human sstr3 (Yamada et al, 1992b); (4) a 2.0 kb Nae I/Xbal cDNA fragment of the human sstr4 (Rohrer et al, 1993); (5) a 1.6 kb Eco RI/Sal III cDNA fragment of the human sstr5 (Yamada et al, 1993), and (6) a 982 bp fragment of human G3PDH.

Specific labelling was detected after 3–6 days exposure on an imaging plate, followed by reading in a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Scintigraphy

The six patients subjected to \(^{99m}\)Tc-scintigraphy had thyroid tumours that were cold. In the two patients with previous thyroidectomy no metastases could be identified by \(^{111}\)In-scintigraphy. \(^{111}\)In-DTPA-D-Phe\(^1\)-octreotide scintigraphy localized the Hürthle cell lesions as distinct areas of increased uptake of radionuclide in all 10 patients (Figure 2A and B; Table 1). In patient no. V with recurrent metastatic disease site localization was obtained by \(^{111}\)In-DTPA-D-Phe\(^1\)-octreotide in the right low neck region prior to surgery. Multiple lung metastases were also visualized in this patient as well as in patient no. IX (Figure 2C).

111In concentration in tissue samples

Ti/B ratios of removed Hürthle cell lesions from five patients (Figure 1) showed large interindividual variations (range 45–94), but were clearly higher than the Ti/B ratios observed for normal soft tissue, muscle and fat (range 0.8–3.6). Lymph nodes (all without tumour involvement) had values ranging from 1.4 to 12. Normal thyroid tissue had similar values as lymph nodes (Figure 1). Higher levels were observed in parts of the glands with colloid goiter (range 26–35).

Patient no. III with Hürthle cell adenoma underwent total thyroidectomy with lymph node dissection on clinical suspicion of a carcinoma. The histopathological examination demonstrated a Hürthle cell adenoma in a thyroid gland with areas of colloid goiter. The Ti/B ratio of the adenoma was higher (45) than the Ti/B ratios found in the contralateral (26, 32) and pyramidal (34) lobes.
with goiter tissue. Fourteen tumour-free lymph nodes were removed with much lower Ti/B values (range 1.4–12).

**Northern analysis**

$sstr$ mRNA was detected in all Hürthle cell lesions investigated as well as in normal thyroid tissue. Patient no. I with Hürthle cell carcinoma and patient no. II with a Hürthle cell nodule expressed mRNA for all five $sstr$. The two adenoma patients (nos III and IV) had similar receptor-profiles except for a lack of $sstr2$. In the controls the normal thyroid tissue selectively lacked $sstr2$ (Figure 3; Table 2).

**Table 2** Expression of somatostatin receptor subtypes in Hürthle cell lesions and in normal thyroid tissue

| Patient | Tumour type   | Expression of somatostatin receptor subtypes |
|---------|---------------|--------------------------------------------|
|         |               | $sstr1$ | $sstr2$ | $sstr3$ | $sstr4$ | $sstr5$ |
| I       | Carcinoma     | +       | +       | +       | +       | +       |
| II      | Nodule        | +       | +       | +       | +       | +       |
| III     | Adenoma       | +       | –       | +       | +       | +       |
| IV      | Adenoma       | +       | –       | +       | +       | +       |
| Control | Normal thyroid| +       | –       | +       | +       | +       |
| Control | Normal thyroid| +       | –       | +       | +       | +       |
DISCUSSION
In a previous study of 21 nonmedullary thyroid carcinomas (papillary, follicular and anaplastic tumour types) $^{111}$In-DTPA-D-Phe$^1$-octreotide scintigraphy visualized specific uptakes in all patients with primary tumours and in 75% of the metastatic lesions. The examination gave additional information to radiiodine scintigraphy, especially with regard to skeletal metastases. However, radiiodine seemed to be superior in detecting pulmonary metastases (Postema et al, 1996). To our knowledge, octreotide scintigraphy has not previously been evaluated for Hürthle cell lesions of the thyroid.

In this study 10 patients with Hürthle cell lesions were subjected to octreotide scintigraphy. The primary lesions, or distant metastases, were in each case localized as distinct areas of increased $^{111}$In uptake. Eight patients were investigated by other scintigraphic techniques ($^{99m}$Te; $n = 6$ and $^{131}$I; $n = 2$). In six patients subjected to $^{99m}$Tc-scintigraphy the thyroid lesion appeared as a cold nodule. In our series two patients with previous thyroidectomy due to Hürthle cell carcinoma had their pulmonary metastases revealed by $^{111}$In-DTPA-D-Phe$^1$-octreotide scintigraphy, but not by $^{99m}$Tc scanning. Using double-phase scintigraphy with $^{99m}$Tc-sestamibi (MIBI) Vattimo et al (1995) reported visualization of primary Hürthle cell tumours. In many centres double-phase MIBI scintigraphy may serve as the first alternative in the evaluation of primary Hürthle cell tumours or carcinomas. Uptake of $^{111}$In in the thyroid in benign disease has been reported previously (Becker et al, 1995). The relative high uptake of $^{111}$In in colloid goiter tissue may lead to misinterpretations. The main indication for octreotide scintigraphy in patients with Hürthle cell tumour is therefore suspicion of metastatic disease.

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