**Somatostatin Receptors on Neuroendocrine Tumors — A Way to Intraoperative Diagnosis and Localization**

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Intraoperative radionuclide detection using $^{111}$In-DTPA-D-Phe$^1$-octreotide was evaluated in five patients with midgut carcinoids and in three patients with recurrent medullary thyroid carcinoma. Three different time intervals (24, 48 and 120 hr) from injection of the radiopharmaceutical to surgery were used. At surgery, suspect tumors were measured by probe in situ and ex vivo after excision. All tissue specimens and blood samples withdrawn during surgery were measured for $^{111}$In activity, and tissue/blood activity concentration ratios were calculated. In situ measurements were valuable especially in neck surgery, where the probe was helpful not only in localization of tumors but also in the control of tumor clearance. Ex vivo measurements were helpful in diagnosing tumor tissue. All five patients with midgut carcinoids were somatostatin receptor-positive, while only three out of seven patients with medullary thyroid carcinoma were receptor-positive. The tissue/blood activity concentration ratios and probe measurement ratios were in general higher in patients with midgut carcinoid than in patients with medullary thyroid carcinoma. Of particular interest were the high tissue/blood concentration ratios in all receptor-positive patients at all time intervals studied. This fact suggests a potential role for radiolabelled octreotide in radiotherapy of these tumor types.

**INTRODUCTION**

Somatostatin is a dimer of a 14 amino acid peptide hormone with almost universal distribution in the body and general suppressive effects on gastrointestinal function. Somatostatin receptors are abundant on neuroendocrine cells but may also occur on non-related cells, e.g., lymphocytes [1]. In neoplasia, very high numbers of somatostatin receptors were demonstrated on neuroendocrine tumors using autoradiography of tumor biopsies or binding to tumor cell membranes [2, 3]. Tumor binding of octreotide, a long-acting analogue of somatostatin, has formed the basis for a new clinical imaging technique, octreotide scintigraphy. In the early development of this technique, radioiodine ($^{123}$I and $^{125}$I) was attached to a tyrosine residue, replacing phenylalanine in the receptor-binding site of the octreotide molecule [4, 5]. In the currently used construction, the intact octreotide molecule is labelled with $^{111}$In using diethylene-triamine-pentaacetic acid (DTPA)$^b$ as the chelating agent. This labelling method influences the receptor-binding site minimally and has increased the imaging sensitivity [6, 7].

Today five types of human somatostatin receptors have been cloned [8-11]. Type I mainly occurs in the stomach and jejunum; type II in the brain and kidneys; type III in the brain and pancreatic islets and type IV in the brain. All receptor types belong to the...

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$^b$Abbreviations used: DTPA, diethylene-triamine-pentaacetic acid; CT/US, computed tomography and ultrasound; MC, midgut carcinoid; MTC, medullary thyroid carcinoma; Ti, tissue; B, blood; T, tumor tissue; N, normal tissue.
superfamily of receptors with seven transmembrane domains and are coupled with G-proteins. Octreotide inhibits somatostatin binding to the receptor type II. At this stage, it cannot be excluded that octreotide scintigraphy may also visualize other receptors than type II.

We have previously shown that $^{111}$In-DTPA-D-Phe\textsuperscript{\text{1}}-octreotide scintigraphy has a specificity and sensitivity (86 percent) superior to that of CT/US in the detection of midgut carcinoid tumors (MC). Previously unrecognized tumors were visualized in 19 out of 27 patients [12]. Besides liver metastases, the most common uptake sites were abdominal paraortic, supraclavicular and mediastinal lymph node metastases. The scintigraphic detection of novel tumor sites changed our clinical handling of nine MC patients. These patients were subjected to repeat surgery leading to complete remission in five patients when evaluated biochemically and by postoperative scintigraphy. One major advantage of $^{111}$In-DTPA-D-Phe\textsuperscript{\text{1}}-octreotide scintigraphy is that one single dose of the radiopharmaceutical can be used both for diagnostic purposes preoperatively and for evaluation of the result of surgical treatment postoperatively [12, 13].

In the present study we report on the clinical use of a scintillation detector for intraoperative localization of tumors in patients with MC and medullary thyroid carcinoma (MTC).

**MATERIALS AND METHODS**

**Protocol**

Tumor localization was assessed in 12 patients by scintigraphy and intraoperative scintillation detection after i.v. injection of $^{111}$In-DTPA-D-Phe\textsuperscript{\text{1}}-octreotide. Scintigraphy was performed prior to surgery in all patients and also one to two days after surgery without iterated injection of the radiopharmaceutical. As the optimal interval between injection and surgery has not yet been established, three different time intervals (24, 48 and 120 hr) were used in this study.

**Patients**

Five patients had MC with lymph node and hepatic metastases. Two of these patients (Numbers 1 and 2) had previously undergone intestinal resection and clearance of regional lymph node metastases and had received embolization therapy of the hepatic metastases. The other three patients had recently diagnosed metastatic disease and underwent primary surgery. Seven patients had previously undergone total thyroidectomy with regional lymph node clearance due to MTC. They had subsequently undergone one to four neck dissections due to recurrent tumors.

**Scintigraphy**

All five patients with MC had octreotide treatment, which was discontinued three days prior to injection of the radiopharmaceutical. Each one of the 12 patients received 20 µg of $^{111}$In DTPA-D-Phe\textsuperscript{\text{1}}-octreotide by i.v. injection. The administered activity was 190-350 MBq. A gamma camera (General Electric, 400 AC/T) equipped with a medium energy parallel hole collimator connected to a GE STARCAM computer system was used. Data acquisition was performed in a dual window setting of 173 and 247 keV and evaluated on a GE STAR 3000 system. Static anterior and posterior images from the base of the skull to the pelvis were taken in all patients at 24 hr after injection. Static images were acquired in a 128 x 128 matrix for 10 min, or until 500 kcounts were collected. Single photon emission computed tomography (SPECT) was also performed using a 128 x 128 matrix and 360 degree rotation in 64 steps with 30 sec/step. Pre-filtration was performed
using a Hanning filter with a cut-off frequency of 0.7 cm\(^{-1}\), and transaxial slices were reconstructed with a ramp filter.

**Intraoperative measurements**

The scintillation detector system (TecProbe 2000, Stratec Elektronik, Germany) was equipped with a handheld 17 x 2 cm silver-anodized aluminum tube. The tip of the probe contained a CsI crystal collimated with a lead shield (aperture diameter of 8 mm over a length of 10 mm). The probe was connected to a portable ratemeter. The energy window was 140-200 keV, and measurement time could be chosen between 0.5 and 600 sec. A sterile dressing was drawn over the probe, which was held close to the tissue examined. To avoid contribution of activity from other tissues (e.g., liver, spleen and kidney) the probe was directed away from these tissues if possible. With the scintillation detector, the mean count rate in situ over the suspect lesion was recorded, as was the count rate over adjacent normal tissue. The ratio, \( R_{\text{in situ}} \), between the two measurements was calculated. The count rates of excised tumor and normal tissue were also measured ex vivo with the probe, and the corresponding ratio, \( R_{\text{ex vivo}} \) was calculated (cf. 12).

**Measurements on tissue samples**

Before histopathological examination, the surgical specimens were weighed and the \(^{111}\)In activity was measured in a calibrated gamma counter equipped with Na(Tl) well crystal (diameter 7.6 cm, length 7.6 cm, Harshaw, Holland). The hole in the crystal had a diameter of 3 cm and a depth of 6 cm. A single-channel pulse-height analyzer (Elscint, Israel) was used. Corrections were made for background activity and radioactivity decay. Blood samples withdrawn during surgery were weighed and measured for \(^{111}\)In activity. Tissue (T) to blood (B) activity concentration ratios, Ti/B, were calculated.

**Statistical analyses**

The standard deviation of the difference between the mean number of counts from suspect tumor tissue (T) and normal tissue (N) was estimated:

\[
\sigma = \sqrt{T + N} = \sqrt{T' \cdot t_T \cdot n_T + N' \cdot t_N \cdot n_N}
\]

where \( T' \) and \( N' \) are mean count rates from suspect tumor tissue and normal tissue, \( t_T \) and \( t_N \) are measurement times, and \( n_T \) and \( n_N \) number of measurements for suspect tumor tissue and normal tissue, respectively. If the difference between the mean numbers of counts from suspect tumor tissue and normal tissue exceeded two standard deviations of the difference, there was a 95 percent chance that this difference was not due to random error alone. This difference was regarded as statistically significant \((p < .05)\).

**RESULTS**

All five patients with MC tumors had a receptor-positive preoperative scintigraphy. A positive scintigraphy in the region of planned surgery was found in only three out of seven patients with MTC tumors (Table 1). Four of the MC patients underwent laparotomy and tumor reduction, while one patient had cervical and mediastinal metastases and underwent neck and mediastinal exploration. All MTC patients underwent neck dissection due to recurrent disease. The Table shows the results of probe measurements from patients with positive scintigraphic findings. No new tumor sites besides those identified by scintigraphy were found. The Ti/B ratios relate to normal tissue and surgical specimens with positive histopathological findings.

Our first patient (Number 1) with MC tumor had neck lesions. During dissection, the probe was helpful not only in the localization of lesions but also in the control of adequate
| Time after injection (hr) | Patient number | Disease | Daily dose of (μg) | Preop scintigraphy | Type of surgery | \( R_{in\ situ} \) | \( R_{in\ vivo} \) | Tissue/blood |
|--------------------------|----------------|---------|-------------------|-------------------|----------------|----------------|----------------|--------------|
|                          |                |         |                   |                   |                | Lymph node metastases mean (range) \( n \) | Lymph node metastases mean (range) \( n \) | Tumor tissue mean (range) \( n \) | Normal tissue mean (range) \( n \) |
| 24                       | 1              | MC      | 200               | (+)               | Neck explor.  | 2.3 (1.1-3.3) n=6 | 4.9 (2.2-11) n=6 | 109 (80-170) n=6 | 4.2 (3.8-4.6) n=2 |
| 24                       | 2              | MC      | 200               | (+)               | Laparotomy    | 1.9 (1.1-3.3) n=4 | 8.3 (3.0-16) n=4 | 123 (53-200) n=4 | 11 (10-12) n=2 |
| 48                       | 3              | MC      | 200               | (+)               | Laparotomy    | 1.4 n=1          | 4.3 n=1          | 56 n=1        | 8 (5-11) n=2  |
| 120                      | 4              | MC      | 200               | (+)               | Laparotomy    | 2.8 (2.8-2.8) n=2 | (-)             | 110 n=1      | (-)          |
| 120                      | 5              | MC      | 400               | (+)               | Laparotomy    | 1.6 n=1          | 2.5 n=1          | 46 n=1        | (-)          |

Mean [SEM]: 2.0 [0.2] n=5 5 [1.2] 89 [16] n=5 7.7 [1.9] n=3

| Time after injection (hr) | Patient number | Disease | Daily dose of (μg) | Preop scintigraphy | Type of surgery | \( R_{in\ situ} \) | \( R_{in\ vivo} \) | Tissue/blood |
|--------------------------|----------------|---------|-------------------|-------------------|----------------|----------------|----------------|--------------|
|                          |                |         |                   |                   |                | Lymph node metastases mean (range) \( n \) | Lymph node metastases mean (range) \( n \) | Tumor tissue mean (range) \( n \) | Normal tissue mean (range) \( n \) |
| 24                       | 6              | MTC     | (-)               | (+)               | Neck explor.  | 1.7 (1.3-2.0) n=9 | 6.4 (5.0-9.0) n=5 | 20 (4.1-3.9) n=9 | 4.4 n=1     |
| 24                       | 7              | MTC     | (-)               | (-)               | Neck explor.  | 1.3 (1.2-1.4) n=3 | 2.4 (2.3-4.0) n=3 | 31 (30-32) n=2 | 4.8 (3.8-5.7) n=2 |
| 48                       | 8              | MTC     | (-)               | (+)               | Neck explor.  | 1.0 n=1          | 3.0 n=1          | 18 n=2        | (-)          |
| 120                      | 10             | MTC     | (-)               | (-)               | Neck explor.  |                |                |              |             |
| 120                      | 11             | MTC     | (-)               | (-)               | Neck explor.  |                |                |              |             |
| 120                      | 12             | MTC     | (-)               | (+)               | Neck explor.  |                |                |              |             |

Mean [SEM]: 1.3 [0.2] n=3 3.9 [1.2] n=3 23 [3] n=3 4.6 [0.9] n=2

Table 1. Data on patients with midgut carcinoids (MC) and medullary thyroid carcinoma (MTC) and results from intraoperative scintillation detection and postoperative measurements of tissue samples.
Figure 1. Results from intraoperative radionuclide detection of tumor/normal tissue before (R_in situ) and after excision (R_ex vivo) and determination of tissue/blood activity concentration ratios (T/B) in a patient with medullary thyroid carcinoma undergoing neck dissection. Asterisks indicate statistically significant elevated ratios (p < .05).

clearance of tumor tissue. This was also the case in two (Numbers 6 and 8) of the three patients with somatostatin receptor positive MTC tumors (Figure 1). In abdominal surgery of patients with MC tumors, the probe measurements in situ were difficult to evaluate due to relatively high background activity in parenchymatous organs (liver, kidney and spleen). The discrimination between tumor and normal tissue in situ was better in the pelvic region. In all cases, R_ex vivo measurements of biopsies were reliable in distinguishing between tumor and normal tissue.

There were no evident changes of R_in situ, R_ex vivo and T/B concentration ratios over time, neither for normal nor for tumor tissue. As seen in the Table, the R_in situ values for lymph node metastases of MC tumors were clearly higher than the corresponding values of MTC tumors. R_ex vivo values for both kinds of tumors were higher than the R_in situ values. A marked difference was noted for the Ti/B ratio: MC tumors had a value 12 times higher than for normal tissue, while for MTC tumors, five times higher values were seen.

DISCUSSION

The clinical value of octreotide scintigraphy in our previous diagnostic series was confirmed by the detection of recurrent disease in MC patients assumed to be in complete remission at conventional work-up [12]. The obvious advantage of 111In-DTPA-D-Phe1-octreotide scintigraphy vs. CT/US is the high sensitivity in combination with the whole body screening for tumors [7, 12, 13]. The sensitivity for lymph node metastases is of particular value in MTC patients, since small neck lesions often are difficult to detect by radiological examinations.

The success rate of octreotide scintigraphy relies on the presence of somatostatin receptors in the human tissue. In the vast Rotterdam experience of octreotide scintigraphy, 86 percent of patients with histologically proven MC had known tumor sites visualized. The corresponding figure for MTC patients was 65 percent [14]. In the present series, all five patients with MC tumor had positive scintigraphic findings, while three out of seven patients with MTC had positive findings. Several factors interfering with the visualization of somatostatin receptors have been discussed: endogenous tumor production of somatostatin (MTC), concomitant therapy with octreotide leading to competition or down-regulation, of somatostatin receptors (MC) [14]. In two previous patients with
foregut carcinoid tumors, we have noted the occurrence of both scintigraphically positive and negative metastases, probably reflecting variable differentiation grades [13]. Presence of different subtypes of receptors for specific tumors must also be taken into consideration (MTC) [14, 15].

Intraoperative probe measurements did not give information about other tumor sites than those revealed by preoperative scintigraphy. However, the local extension of tumor growth was clearly outlined by the probe, and successful tumor clearance was thus facilitated. The sensitivity of intraoperative scintillation detection depends on biological factors (e.g., the relations between the concentration of radionuclide in tumor and non-tumor tissues and how these relations are influenced by time) and also on the choice of radionuclide and the performance of the detector system.

Since the concentration in plasma activity decreased six-fold between four and 24 hr after injection, the optimal time for tumor imaging would be at 24 hr or later [16]. Our Ti/B ratios and $R_{\text{in situ}}$ and $R_{\text{ex vivo}}$ ratios seemed to be little influenced by time when studied at 24, 48 and 120 hr. This indicates similar decline in radioactivity of tumor and non-tumor tissue, or blood, after the initial 24 hours. Considering the high Ti/B ratios for tumors, in comparison with the moderate ratios from the probe measurements, technical improvements should be done. For our detector system, we suggest the possibility of choosing a more suitable energy window and a collimator more appropriate for the detection of small tumors. Another way would be to use octreotide labelled with another radionuclide emitting photons with lower energies. Such improvements would reduce the contribution from primary and scattered photons from adjacent tissues [12].

The ex vivo measurements of excised tissue discriminated most reliably between tumor-positive and tumor-negative lymph nodes. This suggests that this technique can be used as a rapid complement to frozen tissue biopsies.

The high Ti/B activity concentration ratios observed indicate a potential role of radio-labelled octreotide for tumor therapy. This possibility can first be explored after detailed pharmacokinetic and dosimetric studies of octreotide labelled with a suitable radionuclide.

Figure 2. Scintigraphic imaging of neck metastases of midgut carcinoid tumor. (A) 48 hr after injection of $^{131}$I-Tyr$^3$-octreotide supraclavicular and mediastinal metastases were indicated. The patient underwent neck dissection but no mediastinal exploration. (B) Control scintigraphy was performed 6 months later using $^{111}$In-DTPA-Phe$^4$-octreotide. The image 48 hr after injection of the radiopharmaceutical clearly demonstrates clearance of neck metastases but persistent mediastinal metastases.
In 1991, we performed our first diagnostic scintigraphies in seven patients with MC tumors using $^{131}$I-labelled Tyr$^3$-octreotide. The image quality was low, as expected, but in three patients, the uptake in tumor tissue corresponded well with the uptake of $^{111}$In-DTPA-D-Phe$^1$-octreotide studied six to 12 months later. One patient underwent surgery for neck metastases, which were first visualized by $^{131}$I-Tyr$^3$-octreotide (Figure 2). For radiotherapeutical purposes, however, $\beta$-emitting radionuclides other than $^{131}$I ought to be considered as first choices.

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