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Intraoperative Scintigraphic Detection of Abdominal Endocrine Tumors

Development and evaluation of hand held gamma sensitive probes for ^{111}In

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INTRODUCTION

In the surgery of tumor diseases the localization of primary lesions and metastases pre- and peri-operatively is essential, and this is no less true of the endocrine tumors. Because symptoms in patients with endocrine tumors often originate from hormonal excess rather than the tumor mass, the outcome of surgery closely relates to the ability to localize and excise even small tumors and metastases. Conventional radiological methods are often unsatisfactory and may fail to localize such tumors preoperatively. Therefore scintigraphic imaging, with the principal advantage of high specificity, has been explored.

Radiodetection

In 1896 Henri Becquerel discovered gamma rays[1], and the first successful attempt to visualize this radiation was in 1903. The first gamma scintillation camera was built in 1957[2], and utilized a combination of photographic film and Na(Ti) screen to detect radiation emitted by ^{131}I for thyroid imaging. The first commercially available camera using crystals and photomultiplier tubes (PM-tubes) for detection of gamma radiation was constructed in 1964. At the same time ^{99m}Tc labeled radiopharmaceuticals became available and were used for tumor detection and functional diagnosis in organs such as, the liver, lung and kidney. During the

Abbreviations

5-HIAA	5-hydroxyindole acetic acid
%ID/gm	injected dose per gram tissue
CEA	Carciogenic embryonal antigen
CT	computer tomography
GM-tube	Geiger-Müller tube
HAMA	Human anti-mouse antibodies
HPT	hyperparathyroidism
ID	injected dose
MEN 1	multiple endocrine neoplasia type 1
MIBG	metaiodobenzylguanidine
MTC	medullary thyroid carcinoma
PM-tube	photomultiplier tube
PTH	parathyroid hormone
RAID	radioimmuno detection
RAIT	radioimmuno therapy
RIGS	radioimmuno guided surgery
SPECT	single photon emission computed tomography
SSTR	somatostatin receptor type (followed by number 1-5)
US	ultrasonography

1970s, imaging methods were developed for clinical diagnosis of thyroid diseases,utilizing radioactive iodide isotopes. Further refinement of collimators (the lens of the gamma camera) and more sensitive PM-tubes offered higher sensitivity and better resolution. Eventually, digitalization of images and computer technology were introduced with the development of the single photon emission computed tomography (SPECT) camera[3]. This camera provided imaging with higher resolution than earlier and it was generally less sensitive for electrical disturbances, scattering (distortion of gamma rays) and attenuation (absorption of gamma rays)[4].

Reduction of the distance between the detector and area of interest is known to improve the resolution of scintigraphy, generally with preserved specificity for tumor detection. In 1942 a hand-held Geiger-Müller (GM) tube could detect higher uptake and indicate faster biological turn over of ^{32}P in melanoma than in normal skin[5]. Later it was reported that breast masses were correctly classified for malignancy in 24 of 25 cases, by using a probe and ^{32}P preoperatively. However, the method failed to correctly diagnose metastases in axillary and supraclavicular lymph nodes[6]. Hand-held probes, allowing close approximation, have also been exploited during surgery, and were first reported in 1949, when GM tubes (2-5 mm in diameter) were used together with ^{32}P to localize brain tumors in patients undergoing craniotomy[7]. However, the nonspecific ^{32}P tracer had a beta radiation range of 8 mm in soft tissue, was likely to produce false positive results and produced high doses of radiation, that were dangerous to the patient and the environment.

Further development of hand-held radiation detectors has resulted from the improvement in scintillation techniques and by the utilization of other radionuclides. These radionuclides have better physical properties, allowing detection even of deeply located tumors, but sometimes with increased background radiation[8]. In 1956 an application in endocrine surgery was reported, by which a thallium-activated cesium iodide scintillator was used together with ^{131}I to localize thyroid tissue or recurrent thyroid carcinoma in patients undergoing neck exploration[9]. Intraoperative thyroid scintillation has been reported as a valuable determinant of the completeness of thyroidectomy in thyroid carcinoma[10, 11]. Another application of this intraoperative technique is detection of osteid osteoma utilizing ^{99m}Tc, and different kinds of gamma detectors to successfully guide ortopedic surgeons in excision of the lesions[12, 13].

Radio immunodetection (RAID)

In the late 1940s, it was possible to label an antibody with ^{131}I and preserve its immunologic specificity[14]. Some years later, intravenously injected radiolabeled antibodies were found to accumulate to a higher extent in tumors than in normal tissue[15]. Further development of these techniques has resulted from experiments in tumor bearing animals (i. e. immuno-incompetent mice) where preferential uptake of ^{131}I labeled antibodies in tumor samples could be verified by

radioactivity measurements[16, 17]. External radioactivity scanning permitting tumor localization of colorectal cancer with radiolabeled antibodies was described in the 1970s[18, 19].

RAID has subsequently proven useful in the imaging of a number of tumors, such as lung, breast, and ovarian cancers, melanoma, head and neck cancers and soft tissue sarcomas[20-22]. Recently, radiodetection of specific cell receptors by radiolabeled peptides of non-immunological origin has been developed, and RAID has therefore become an inappropriate expression[23]. These tracers tend to exhibit properties similar to antibodies, and will be discussed together with the immunologically generated tracers. With this in mind "radioreceptor detection" may be a more adequate term.

Important considerations for radioreceptor detection

Successful tumor imaging by radioactivity is dependent upon sufficient contrast between tumor and normal tissue, commonly measured as the tumor-to-background ratio or tumor-to-blood ratio of the radioactivity uptake. This is determined by the biodistribution of the particular radiolabeled tracer.

Tracer: The tracer should have high specificity and affinity for the receptor or other specific binding sites. Rapid clearance from the blood and normal tissues is favorable since it provides higher tumor to background ratios. Smaller antibody fragments, peptides obtained by enzymatic cleavage of normal sized antibodies with preserved binding properties are better suited for fast blood clearance[22, 24]. To avoid an anti-mouse antibody response (HAMA) humanized murine or original human monoclonal antibodies have been obtained[25]. Peptides with binding properties to specific receptors, e.g. somatostatin, have been modified, and smaller analogues with preserved or even improved binding capacity have been generated[26, 27].

Radiolabel: The half-life of a radionuclide should suit the biological half-life of the tracer in man, and be safe to administer to patients with respect to dosiometry and toxicity. A long half-life may cause undesirably long exposure to radiation whereas a short half-life may hamper the imaging procedure due to the rapidly vanishing radioactivity[22]. High-emitted energy levels incur a high dose of radioactivity for the patient and necessitate protective measures in the immediate environment. Low-emitted energy levels tend to cause high attenuation in normal tissue, with resulting difficulties in detecting important tracer deposits deep in the body.

The biological properties of the tracer have to be preserved after labeling. Stability *in vivo* and low decomposition of the radiolabeled tracer are important factors to avoid free radionuclides causing disturbing background radiation.

^{125}I is commonly used, although its long half-life of 60 days is rather unsuitable for clinical investigations. However, it has a low-emitted energy of 35 keV with high attenuation in tissues, and tends to reduce undesired background radiation[17]. The physiological accumulation of any I-isotope in the thyroid

interferes with neck imaging, but can be reduced by prophylactic iodide administration.

^{99m}Tc is an other commonly used radionuclide with a half-life of 6 hours and about 165 keV photo-peak. The short half-life of ^{99m}Tc may provide low count-rates if the time between injection of the tracer and imaging is long.

^{111}In requires an emitted energy window that includes two photo peaks at rather high-emitted energy which may cause disturbing background radioactivity[28]. However, the half-life is longer, 66 hours, which makes ^{111}In easy to use in clinical practice (table 1).

Binding sites of tracers: Antibodies and Fab-fragments as well as other tracers consisting of small peptides bind to specific antigens or receptors on cell-surfaces or within cells. The antigens/receptors ought to be present in high concentrations in the target tissue and should be easy for the tracer to reach. The binding sites should be present in low concentration in normal tissue and it is preferable that there be few subtypes with differing distribution and binding capacity[29].

Preoperative tumor imaging: The development of imaging, from simple planar camera images to the three dimensional, computerized reconstructions of the SPECT camera, has resulted in improved resolution of the images. The three dimensional reconstructions make it easier for the clinician to recognize and evaluate any pathological uptake and relate it to normal structures. The theoretical resolution is calculated to be 1 cm^2 at a depth of 5 cm with a tumor to background ratio of 5:1[30]. However, the gamma camera is limited by its physical size and it is not always able to get close to the region of interest. Later it has been confirmed in many clinical studies that a size of 10 mm seems to be the limit for tumor detection by scintigraphy[31-38].

Intraoperative radioimmuno detection

Gamma-sensitive hand-held probes have mainly been evaluated together with radiolabeled monoclonal antibodies as tumor-seeking agents. In animal models using human colorectal adenocarcinoma xenografts on immuno-incompetent mice and ^{131}I-labeled Carciogenic embryonal antigen (CEA) antibodies it has been possible to localize the transplanted tumors with the use of a gamma-detecting

Table 1. Properties of common radionuclides used in the radiolabeling of antibodies and other tracers for radioreceptor detection.

*Radio-metal chelator

Radionucleide	Half-life	Emitted energy (keV)	Attenuation in tissue	Labeling method
^{123}I	13.2 hours	159.0	Medium	Chloramine-T
^{125}I	60 days	35.5	High	Chloramine-T
^{131}I	8 days	364.5	Low	Chloramine-T
^{99m}Tc	6 hours	140.5	Medium	Direct labeling to free thioles
^{111}In	67,4 hours	171.3/245.4	Low	DTPA*

probe[39-41]. However, this method has failed to improve the tumor detection rate in patients with colorectal carcinoma tumors during surgery[11, 28, 35, 42].

In 1985, a technique denoted radioimmuno guided surgery (RIGS) was reported. By utilizing a hand-held detector during surgery of patients injected with an ^{125}I-labeled CEA monoclonal antibody three weeks prior surgery, the surgical field could be searched for tumor uptake[43]. In patients with colorectal cancer RIGS was claimed to provide staging information with impact on the therapeutic strategy[44]. Similar results have been reported by other research groups but the method still needs to be properly evaluated particularly with respect to possible influence on improved patient survival[45-48].

The available gamma-sensitive hand-held probes have so far been designed for radionuclides with low-emitted energy (^{125}I). The studies have revealed that, in concordance with gamma camera imaging, successful detection is critically dependent on the tumor-to-background signal ratio[35].

The hand-held probes have been single channel detectors, with a small collimator made of lead allowing measurements of a circular spot approximately 10 mm in diameter. Examples include the Neoprobe 1000, utilizing a cadmium telluride (CdTe) crystal, and the Tecprobe 2000 and Gammed 2, both utilizing cesium iodide (CsI) crystals. These probes are developed for low energy radionuclides and the thin metal shielding is incapable of preventing disturbance from high-emitted energy radiation. The detectors are connected to a counting system that displays counts per time unit, and they have a sound-signal indicating radioactivity, which can be regulated to ignore registration of background radioactivity[35, 43].

Endocrine tumors

Adrenocortical carcinoma

Adrenocortical carcinoma constitutes a rare tumor with dismal prognosis. If not recognized at an early stage the tumor tends to grow rapidly to conspicuous size, and 5-year survival rates are generally poor, between 16 and 35 %[49, 50]. This neoplasm occasionally responds to chemotherapy, but successful treatment appears to be mainly related to the possibility of radical surgery[51-53].

Preoperative localization of regional and distant metastasis should provide generally desirable information and be beneficial both for surgery and medical strategies[54-56].

Generally, smaller adrenal tumors are often incidentally discovered by computed tomography (CT-scan) of the abdomen (incidentalomas). In such cases there is a need for methods which might discriminate cortical tumors from pheochromocytoma, as well as from other tumors metastasizing to the adrenals[57].

Midgut carcinoid tumors

Carcinoids are rare tumors that develop from neuroendocrine cells in the gastrointestinal tract, and are usually classified according to the part of the gastrointestinal tract where the primary tumor occurs. Tumors located in the proximal parts (stomach, duodenum, pancreas) are called foregut carcinoids, those located in the small intestine and proximal colon midgut carcinoid tumors and those in the distal part of the intestinal tract hindgut carcinoid[58].

The midgut carcinoid tumors are most commonly located in the terminal ileum and secrete mainly serotonin and various tachykinins, but also minor amounts of other peptide hormones. They are commonly associated with the carcinoid syndrome. After a period when only a small (sized 5 - 10 mm) primary manifestation exists in the wall of the small intestine, carcinoid metastases occur to the mesentery. The mesenteric lesion may grow to a conspicuous size of several centimeters, and it usually generates fibrosis in the mesenterico-intestinal surroundings. The advancing disease often induces symptoms of intestinal obstruction and ischemia[59]. The surgical treatment comprises radical removal of the primary tumor and mesenteric metastases, since this may alleviate intestinal symptoms. Removal of accessible liver metastases is also performed of palliating the symptoms of carcinoid syndrome[60]. Treatment with somatostatin analogues and interferon may provide symptomatic relief and biochemical response in patients suffering from the carcinoid syndrome[61].

The small primary carcinoid tumor often escapes radiological detection, whereas CT-scan and percutaneous ultra sound (US) often efficiently demonstrate mesenteric and retroperitoneal lymph node metastases[62, 63]. The development of somatostatin receptor scintigraphy, i. e. [^{111}In]-DTPA-D-[Phe^{1}]-octreotide in combination with SPECT, has contributed to preoperative localization of carcinoid tumors. This method can detect lesions that are occult to other imaging techniques in both the abdomen and in more unusual, distant sites[64, 65].

Midgut carcinoid tumors display argyrophil and argentaffin silver-staining reactions, related to the production of serotinin within the tumor cells[66, 67].

Endocrine pancreatic tumors

Endocrine pancreatic tumors are rare neoplasms. These tumors need to be discriminated from pancreatic adenocarcinoma as their considerably better prognosis justifies aggressive attempts of removal. A minority of endocrine pancreatic tumors appears clinically silent, though they may secrete hormones without clinical effects, and are generally called non-functioning[68]. These tumors tend to cause symptoms due to the expanding tumor mass, which, due to the noninvasive growth of most endocrine pancreatic tumors, often is surgically removable. However, the majority of these tumors are clinically functioning, implying that they are associated with symptoms due to the excessive secretion of specific hormones. These lesions may cause hormonal syndromes even in spite of a minimal tumor mass and then frequently represent a surgical challenge because of

difficulties associated with pre- and intraoperative tumor localization. Some of these lesions (gastrinomas) are to be searched for not only in the pancreas but in the duodenum and regional lymph nodes[69, 70].

Radiological methods may fail at preoperative localization of endocrine pancreatic tumors and somatostatin receptor scintigraphy, i. e. [^{111}In]-DTPA-D-[Phe^{1}]-octreotide in combination with SPECT, has therefore provided the surgeon with valuable complimentary information[64, 71, 72]. This method may also be efficiently used to discriminate the endocrine tumors from the more commonly occurring exocrine pancreatic adenocarcinoma[73].

Men 1 syndrome

Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal, dominantly inherited syndrome, in which carriers of the MEN1 gene develop multiple neoplasms within the parathyroid, the anterior pituitary and the endocrine pancreas. In a few patients the syndrome is associated with adrenal and thyroid adenomas as well[74-76]. Recent identification of the MEN1 gene will in the future simplify family screening for the disease[77, 78]. Apart from relatives to patients with a known MEN1 syndrome the diagnosis should be suspected when hyperparathyroidism (HPT) or endocrine pancreatic tumors occur in young patients[79].

Radical removal of pancreatic and duodenal lesions by surgery has been advocated with the intention that it would favorably influence the long time mortality, although it needs to be emphasized that overly extensive surgery may be associated with unacceptable morbidity due to pancreatic dysfunction[80]. Even in asymptomatic cases the risk for developing malignancy in untreated tumors is considerable[81].

The pancreatic (and duodenal) endocrine pathology in MEN1 typically consists of numerous micro- and macro adenomas measuring up to a few mm and generally only a few larger lesions[82]. Localization of the lesions in the pancreas and the duodenum may sometimes be difficult, because they often display normal radiological findings[83]. Improved surgical results have been obtained by the use of intraoperative ultrasound for tumor detection[84]. Depending on the presence of somatostatin receptors in the tumors preoperative localization with [^{111}In]-DTPA-D-[Phe^{1}]-octreotide and SPECT has sometimes been of benefit for surgical strategy.

Tracers for visualization of endocrine tumors

Neoplasms originating from endocrine cells are often highly differentiated with maintained or characteristically altered secretory regulation. These tumors can be expected to maintain various receptors, which could be used as targets for radioactive tracers. This circumstance has provided the development of

monoclonal antibodies with variable selectivity toward certain endocrine cells for therapeutic and diagnostic purposes.

In 1978 successful treatment of a patient suffering from severe carcinoid syndrome with native somatostatin was reported[85]. However, the short half-life of the two forms of native somatostatin (14 and 28 amino acids) limited its clinical applications, and led to the synthesis of analogues with longer biological half-life. These have subsequently have been utilized for scintigraphic visualization and therapy of a variety of endocrine tumors.

Monoclonal antibodies

Radiolabeled monoclonal antibodies generated against CEA is an example of a successfully utilized tracer for scintigraphic visualization of medullary thyroid carcinoma (MTC)[86-88], and up to 70 % of patients with MTC show positive scintigraphy with ^{131}I-anti CEA-Fab$_2$[89].

Development of monoclonal antibodies against parathyroid chief cells is another example of an endocrine tumor tracer thought to have an application for localization of parathyroid neoplasms[90-92].

Ac5. A monoclonal antibody reactive toward adrenal cortical cells, denoted Ac5, was generated by conventional hybridoma techniques after immunization of mice with cells dispersed from human adrenocortical adenoma[91, 93, 94]. This immunoglobulin (IgG1) antibody recognizes an 80 kd antigen, with unclear function on the surface and within the cytoplasm of normal human adrenocortical cells. Ac5 reacts with cryosectioned parenchyma of adrenocortical adenoma and cortical carcinoma with or without steroid overproduction[95]. Other human tissues (placental syncytiotrophoblasts, ovarian epithelium, Leydig cells, and the intestinal mucosa) have shown variably intense immunoreactivity. Pheochromocytomas, and other normal tissues have failed to react with the antibody[96].

Somatostatin receptor tracers

Somatostatin The peptide somatostatin, consisting of 14 amino acids (fig. 1A), was first described in 1973[97], and its general inhibitory effects were found to include

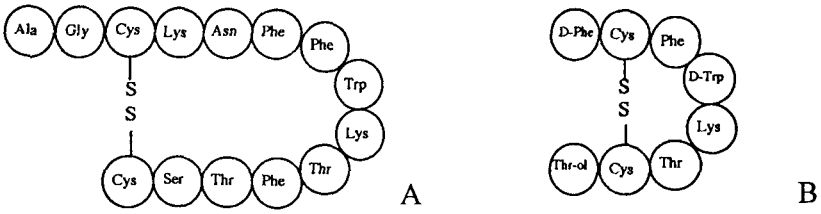

Fig. 1 The aminoacid sequencese for native somatostatin-14 (A) and octreotide (B). (E. Tiensuu Janson, 1995)

suppression of hormone release from various neuroendocrine tumors[98, 99].

Somatostatin exerts its biological effects by binding to specific membrane receptors coupled to GTP-binding proteins, and such receptors have been demonstrated in various tissues the brain, the pancreas, and many endocrine tumors[100-102].

Octreotide Is a somatostatin-analogue consisting of eight amino acids (fig. 1B)[26, 103] with the ability to efficiently block hormone secretion and alleviate symptoms in patients with carcinoid and endocrine pancreatic tumors[104-106]. High dose octreotide treatment has apparently also resulted in a reduction in the size of occasional endocrine tumors[107].

Somatuline (laneotrine, BIM-23014C) and Octastatin (RC-160) are other somatostatin analogues which have recently been introduced[108-111].

Distribution and scintigraphic detection of somatostatin receptors

Subtypes of somatostatin receptors

Further studies of somatostatin receptors have resulted in identification of five subtypes. These subtypes denoted SSTR1 – SSTR5, have variable distributions in normal and neoplastic tissues of different origin. Somatostatin receptor subtype 1 is predominantly found in the stomach and jejunum; subtype 2 is present in brain and kidney and at lower concentration in gastrointestinal tissues; subtype 3 has been found in the brain and the pancreas; subtype 4 in lungs, kidneys, adrenals and the brain[112-117]. Subtype 5 has been detected in the hypophysis, adrenals and brain. The somatostatin analogues seem to have different affinities to the various subtypes, and octreotide binds with high affinity to subtypes 2 and 5 but with intermediate affinity to subtype 3[118]. In tumor tissues subtypes 2 and 5, which both are sensitive to [^{111}In]-DTPA-D-[Phe^{1}]-octreotide, are expressed most frequently, whereas subtypes 3 and 4 are uncommon. However, there is no clear pattern of expression of the various receptor subtypes in different kinds of tumors[119].

Somatostatin receptors in endocrine tumors

Since autoradiographic studies revealed a high content of somatostatin receptors in many endocrine tumors[100, 102, 120], methods were developed for tumor targeting *in vivo*. Initially [^{125}I-Tyr^{3}]- octreotide was utilized for scintigraphy[121-123], but it was later demonstrated that [^{111}In]-DTPA-D-[Phe^{1}]-octreotide in combination with SPECT was more suitable for visualization of endocrine tumors due to reduced bile excretion resulting in less disturbing background radioactivity from intestinal content. As stated above, [^{111}In]-DTPA-D-[Phe^{1}]-octreotide has shown high sensitivity and specificity for visualization of carcinoids and many endocrine pancreatic tumors and their metastases. The method is described to display more than 80% of carcinoid tumors and a high percentage of non-functioning endocrine

pancreatic tumors, glucagonomas and gastrinomas, while insulinomas are reported to be negative in 40 to 50% of the patients[64, 65, 71, 72, 124, 125]. The method is now part of the routine workup for endocrine tumor imaging in many institutions (Fig. 2).

Intraoperative somatostatin receptor detection

In endocrine tumors, encouraging results of intraoperative tumor detection have been reported with the Neoprobe 1000 detector used with intraoperatively injected [^{125}I-Tyr^{3}]-Octreotide[126]. However, the technique demands temporary bile duct obstruction to reduce the intestinal background radiation[126, 127].

[^{111}In]-DTPA-D-[Phe^{1}]-octreotide allows early administration, 24-48 hours before measuring and the background radiation seems unrelated to the bile, which creates more ideal conditions for operative detection. It has been demonstrated that the hand-held Tecprobe 2000 detector may be successfully used together with [^{111}In]-DTPA-D-[Phe^{1}]-octreotide in displaying carcinoid metastases within the thorax and the neck. Contrasting experience was, however, obtained in patients with endocrine tumors located within the abdomen. Thus, the Tecprobe 2000 was generally unable to detect tumors below 10 mm in diameter due to high background radioactivity from the liver, the spleen and the kidneys[128].

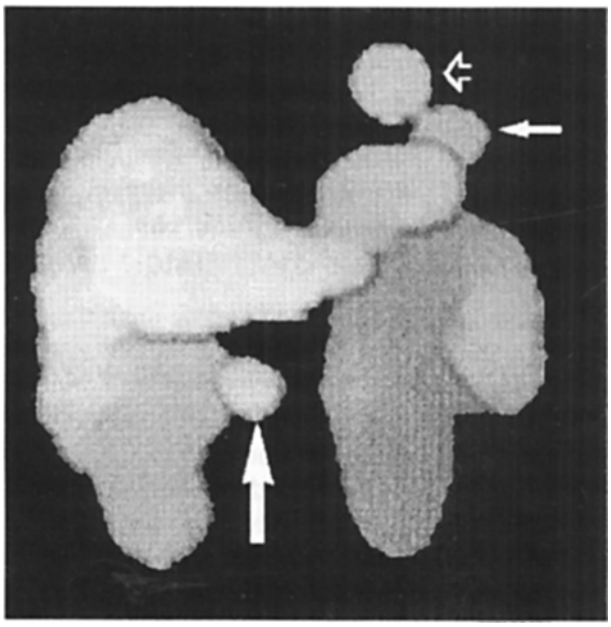

Fig. 2. Scintigraphy with [^{111}In]-DTPA-D-[Phe^{1}]-octreotide. 3-dimensional image from a patient with endocrine pancreatic tumor disease. The volume rendered image shows the thoracoabdominal uptake slightly from the patient's right side. The abdominal tumor (big arrow) is seen under the liver. Uptakes in the pleura (small arrow) and in the skeleton (unfilled arrow) represent distant metastases.
(Westlin 1993)

MATERIAL & METHODS

Tumor cell lines and animals

Two cell lines of human adrenocortical carcinoma SW13 (American Type Culture Collection, Rockville MD. [129]) and T-CAR 1[130] without hormonal excretion *in vitro* were used. Cytospins of SW-13 and T-CAR 1 have been shown to display unequivocal reactivity with Ac5 (15 µg/ml) on examination with a peroxidase-antiperoxidase technique[96]. The cell lines were expanded and maintained at 37° C Viability of the resulting single and small groups of cells routinely exceeded 95 % on Trypan blue exclusion.

Female, 4- to 8-week-old immunoincompetent mice were used for transplantation and tumor propagation. Animals primarily were injected subcutaneously into both proximal thighs with cells. Macroscopic tumors were visible 3 weeks later in approximately 90 % of the thighs. The tumors of single mice bearing SW-13 and T-CAR 1 were removed and cut into cubes whereupon two to three pieces immediately were reinserted subcutaneously into the proximal thigh of other mice. Altogether 25 mice harboring SW-13 and 28 with T-CAR 1 tumors subsequently were analyzed for biodistribution and scintigraphy.

Patient studies

In a biodistribution study twenty-three patients scheduled for routine operation of endocrine tumors were included in the study. Fifteen of them had midgut carcinoid tumors with metastases, and five had endocrine pancreatic tumors (four insulinoma, one gastrinoma), while one single patient had primary aldosteronism due to adrenal cortical adenoma, multiple cutaneous metastases from previously resected paraganglioma, and medullary thyroid carcinoma. Eleven of the patients were intraoperatively examined with the TecProbe 2000 or the Gammed 2 probe.

In a clinical study with *in vivo* radiodetection twenty-one patients scheduled for routine laparotomy were included. Thirteen had metastasing midgutcarcinoid tumors, and eight had endocrine pancreatic tumors, in two of them associated with the MEN-1 syndrome. In these patients intraoperative ultrasound was routinely applied in effort to visualize multiple tumors. Histopathologically verified tumors, measuring 2 mm or more were accounted for, liver metastases were not included. Ten of the patients with disseminated midgut carcinoid tumors were selected for *ex vivo* studies by which the resected intestinal specimen was examined under a gamma camera.

In a following study with the multichannel gammasensitive detector (Matris 16) patients with metastasizing midgut carcinoid tumors (n = 9) or endocrine pancreatic tumors (n = 2), scheduled for routine laparotomy were subjected to investigations with the Matris 16. In ten of the patients the resected surgical specimens were examined *ex vivo*.

Tracer preparation and administration

Monoclonal antibody and Fab´2 fragment Ac5

The Ac5 antibody used in the animal experiment was purified by affinity chromatography

Chloramine $T^{(131)}$ was used to label antibody, which was administered to the mice 4 to 6 weeks after transplantation of tumor tissue, when grafts exhibited palpable diameters of 5 to 15 mm

A Fab´2 fragment of Ac5 was obtained by adding pepsin to the antibody in PBS. The solution was separated into 1 ml fractions on a 1 x 60 cm Sephadex G-100 column (Kabi-Pharmacia, Uppsala, Sweden). Labeling and injection of the fragmented antibody was performed in the same way as described for intact antibody.

[^{111}In]-DTPA-D-[Phe^{1}]-octreotide

Lyophilized DTPA-D-[Phe^{1}]-octreotide and [^{111}In]-chloride were obtained in separate vials (Mallinckrodt Medical, Petten, The Netherlands), and labeling was performed as earlier described$^{(133)}$. Briefly the [^{111}In]-chloride was added to the lyophilized DTPA-D-[Phe^{1}]-octreotide (20 μg) and incubated for 30 minutes at room temperature. In the early study the labeling yield was checked by chromatography$^{(134)}$.

Radiological examination

All patients but the one with medullary thyroid carcinoma were preoperatively investigated by abdominal computed tomography (CT; Somatotom DR2, Siemens, i.v. contrast enhancement 8 mm slice thickness) and percutaneous ultrasound (US, Acuson 128, 3.5 MHz).

Imaging procedure

Scintigraphy of mice

Altogether 13 mice with SW-13 and 10 with T-CAR 1 tumors each received a single subcutaneous 0.2 ml injection of 50 μg radiolabeled Ac5 antibody in PBS, and the same amount of its Fab´2 fragment was administered intraperitoneally into 9 and 15 mice respectively. Thyroid nuclide uptake was reduced by adding 10 mmol/L KI into the drinking water during the 24 hours preceding these injections. Scintigraphy was performed repeatedly up to 8 days after injection of the radiolabeled antibody compounds using a computerized gamma camera (Nuclear Diagnostics, Hägersten, Sweden). Single mice harboring SW-13 and T-CAR 1 tumors received 50 μg of a control antibody. This antibody was radiolabeled as described above, administered subcutaneously, and failed to immunostain human adrenocortical carcinoma including SW-13 and T-CAR 1.

[^{111}In]-DTPA-D-[Phe^1]-octreotide SPECT scanning

The procedure of [^{111}In]- DTPA-D-[Phe^1]-octreotide scanning has previously been described[133, 64]. In order to avoid artifacts caused by intestinal radionuclide accumulation, the patients routinely received laxatives on the day of the [^{111}In]-DTPA-D-[Phe^1]-octreotide administration and throughout the study. The patients obtained 98-244 MBq [^{111}In]-labeled pentatreotide. Planar antero-posterior whole body images, extremities excluded, were collected 4 and 24 hours thereafter. SPECT was performed over the areas of interest. A gamma scintillation SPECT camera (Nuclear Diagnostics, Hägersten, Sweden and London, England) was used for imaging. Scintigraphy was considered positive in patients who demonstrated unequivocal tracer localization to at least one tumor site, being verified in the subsequent operation. The patients were operated on within 12 - 48 h after injecting [^{111}In]- DTPA-D-[Phe^1]-octreotide. The images were presented in three dimensions and presented for and evaluated together with the responsible surgeon[135].

Detectors for intraoperative scintigraphy

Commercial probes

In the first clinical study two probes were tested, the Tec-Probe 2000 (Stratec Electronic, Birkenfeld, Germany) and the Gammed 2 probe (CIS bio international, Gif-sur-Yvette, France), both equipped with CsI-scintillator, a collimator of lead and a standard calibration for ^{111}In. They were connected to a count-rating system where counts per second were shown on the display and documented manually. The Tec-Probe 2000 is 170 mm long and 20 mm in diameter, and the collimator measures 8mm in diameter and 10 mm long[128], the Gammed 2 probe is of similar size.

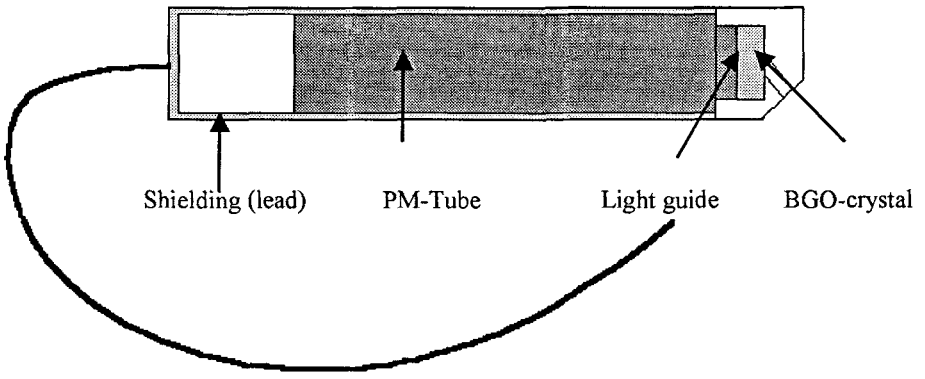

Fig. 3. Schematic transection of the H-probe2 detector. For dimensions see the text.

H-Probe2

In an effort to obtain a probe adapted for ^{111}In the detector H-probe2 was developed in our laboratory (Division of Biomedical Radiation Sciences, Uppsala University, Uppsala, Sweden). The detector is equipped with a cylindrical, Bismuth Germinate (BGO) crystal (Crismatec, Nemours, Cedex, France), connected to a photomultiplier tube (R1635, Hamamatsu Photonics Norden AB, Upplands Väsby, Sweden) by a small light guide. The side and front of the crystal are shielded with 6 mm tantalum, while the rest of the detector has a 5 - 7 mm thick lead shield. The collimator is set at a 42° angle to and has a 3 mm wide opening. The probe has a cylindrical form and is 184 mm long, 24 mm wide, and weighs 730 g (Fig. 3). It is connected to a PC equipped with a preamplifier, single channel analyzer. The probe is supported by high voltage power (1100 - 1200 V). The count rate is presented graphically on the computer screen using specially developed software, and the surgeon could maneuver the computer with his foot via a trackball placed on the floor.

Matris 16

The Matris 16 probe was also constructed in our laboratory, and consists of 16, box shaped, Bismuth Germinate (BGO) crystals 4 x 4 x 7 mm (Crismatec, Nemours, Cedex, France) mounted together in a 4 x 4 matrix, and connected to a 16-cathode photomultiplier tube (H6558, Hamamatsu Photonics Norden AB, Upplands Väsby, Sweden). A parallel hole collimator made of a 10 mm thick tantalum plate with 16 holes (diameter = 3 mm) centered in front of the crystal matrix. The sides and back of the detector are shielded with 7.4 mm thick lead with a capsule in aluminum. It is 142 mm long, 50 x 50 mm thick, and weighs 1650 g. Inside the probe a high voltage supply (750 V) is placed (C6260 Hamamatsu Photonics Norden AB, Upplands Väsby, Sweden), fed with 15 V and pulses are collected in a 16 channel amplifier and discriminator (Phillips Scientific Model 6816) (Fig 4). A PC

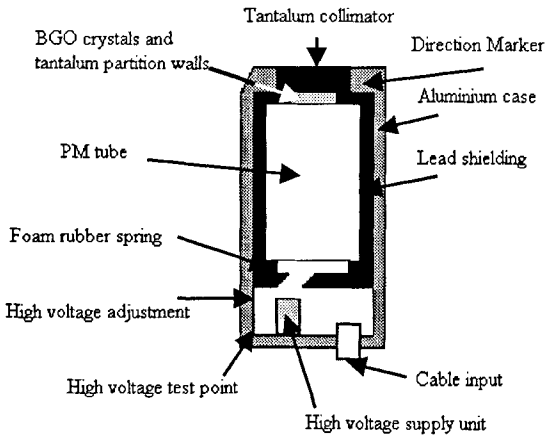

Fig. 4. Transection of the Matris 16. For dimensions see text

equipped with a scaler card registers each pulse from the individual detector channels. A software package (Visual Basic, Microsoft Corporation, USA) allows the user to handle the probe in different modes and provides a graphic presentation of the data.

Characterization of detector efficiency

The Matris 16 was also initially characterized in a test bench. The spatial resolution and the ability of the system to distinguish small hot spots close to high radioactive sources were studied with a phantom. Wells (2 - 10 mm in diameter) were filled with radioactivity solutions (0.96 - 600 kBq) of ^{111}In or ^{99m}Tc (5 - 650 μl). A scanning mode of the detector was tested by moving a single radioactive source (diameter 5 mm) at constant speed in front of the detector.

Histopathology

The purified antibody was biotinylated[94]. Cryosections of 6 μm from excised SW-13 and T-CAR 1 tumors were stained with hematoxylin-eosin for routine analysis, and with the biotinylated antibody (5 μg/ml) by using an avidin-biotin-peroxidase complex (Vectastain, ABC kit; vector Lab, Burlingame, Calif.). Omission of the primary antibody or substitution of a subclass matched antiparathyroid antibody denoted E11 (10 μg/ml) showed no immunostaining of the tumors[91].

In the clinical studies biopsy specimens from apparent and suspect primary and metastatic tumors obtained at operation were histopathologically examined. The specimens were fixed in 10 % buffered-formalin and paraffin-embedded, after which 5 μm thin sections were stained with the van Gieson, haemotoxylin-eosin, Grimelius and Masson stains, as well as with monoclonal chromogranin A antibodies (LK 2H10, Boehringer-Mannheim, Mannheim, Germany) using the avidin-biotin technique. Endocrine pancreatic tumors were also examined immunohistochemically[135]. All neoplasms but the adrenal cortical tumor exhibited the expected argyrophilia and chromogranin immunoreactivity and the intestinal carcinoids were invariably Masson positive. The endocrine pancreatic tumors were insulin or gastrin reactive and the medullary thyroid carcinoma was immunostained for calcitonin. Parenchymal content in carcinoids and pancreatic tumors was analyzed by point sampling utilizing an ocular grid in selected cases.

In vivo measurements with hand-held detectors

The intraoperative measurements were performed with the hand-held gamma-radiation detector probe covered with a sterile plastic tube, after surgical

exploration and overview of the operative field. The detectors were directed as stable as possible towards a suspect tumor and held to minimize influence from adjacent organs, especially the liver, kidneys and spleen. The left thigh was selected as standard for measurement of background radiation.

Commercial probes

At each site of measurement the mean of three, 3 to 5 sec. measurements was obtained. When a tumor gave double or higher count values as compared to the surrounding tissue, it was considered to be detected by the probe.

H-probe2

Measurements were made during 5 or 10 sec towards a suspected tumor. In search of pancreatic tumors the probe was also moved along the pancreatic surface with counts collected during 10 consecutive 5 or 10 sec periods (e g dynamic registrations, referred to as the "sliding" technique). When obtained counts from a tumor exceeded that of the surrounding normal tissue by 2 SD or more the difference was considered significant.

Matris 16

During measurements the detector was directed for 5, 10 or 20 sec towards suspected tumors. In scanning for small tumors the probe was moved along the selected surface with counts collected during consecutive 1, 2 or 3 sec periods. The coefficient of variation for counts obtained at multiple measurements of individual tumors averaged 10 %. Longer registration periods were chosen when the general activity was low due to rapid biological wash out. When the obtained counts from a tumor exceeded that of the surrounding normal tissue by at least 2 SD the tumor was considered detected with the probe. For small tumors this sometimes required only one measurement, due to the activation of only one of the available detector channels.

Statistics

In the measurements with the H-probe2 and Matris 16, SD was calculated as the square root of the number of counts in each measuring.

Surgical specimens

In selected cases of midgut carcinoids (n = 8) parts of mesenterico-intestinal specimens were resected and used for probe examination *ex vivo*. The excised specimens (60 - 160 mm long) were investigated in a test bench, being moved perpendicularly with a speed of 0.3 mm/s as close as possible to the collimator.

The radioactivity was registered during 8 sec intervals, and presented in three-dimensional graphs.

Biodistribution

In the animal experiment up to four mice were killed at predetermined intervals 1 to 8 days after injection of the radiolabeled Ac5 antibody or the Fab'$_2$ fragment. Tumor grafts, both kidneys, spleen, and samples from lung, liver, and small intestine (without content) were blotted dry and weighed wet together with transcardially aspirated blood. Radioactivity of tissue samples was determined by scintillation counting in well-counter (LKB Wallac, 1282 CompuGamma, Turku, Finland) using uninjected mice (n = 4) as controls. The values in blood were expressed as percentages of the injected dose (ID) and the proportion of ID per gram tissue (%ID/gm) was used to determine uptake in murine organs and to calculate tumor-to-blood and tumor to normal tissue ratios.

In the clinical studies peripheral blood samples (0.5 - 1.5 ml) as well as biopsy specimens from tumors (30 - 500 mg) and normal tissues (500 - 1000 mg) were collected at surgery. Cubes of solid tissues were blotted dry and weighed wet. All samples were analyzed immediately after surgery in the well-counter. The counter was calibrated for ^{111}In, with attempts made to standardize geometry of measured samples, and to correct for decay. The tissue/blood radioactivity ratio and also the percentage of injected dose per gram tissue (%ID/gm) were calculated.

RESULTS AND COMMENTS

Biodistribution and scintigraphy with Ac5

Biodistribution

The tumor grafts of both cell-lines (SW-13 and T-CAR 1) preserved the ability to bind both intact antibody and Fab'$_2$ fragment of Ac5 after proliferation in the nude mice.

Initial radioactivity values in blood reached 15 %ID/gm for the intact antibody and 1.5 % for the Fab'$_2$ fragment. Clearance from the circulation was accomplished 4 to 5 days later.

Standardized radioactivity values were low (tumor/blood ratio 1.0) for the two tumor types after injection of the iodinated antibody, whereas Fab'$_2$ fragments were accumulated more efficiently especially with SW-13 grafts (tumor/blood ratio 10.5). This discrepancy between the two types of grafts may relate to different bioavailability of the recognized antigen in vivo.

Dynamics of radioactivity uptake were similar in the two grafted tissues, although the temporal variation was greater for the intact than the fragmented

antibody. Initially there was considerable radioactivity accumulation in lung, liver, spleen and kidney, but this was cleared 3 - 5 days faster than the tumor activity.

Scintigraphy

The tumors became unequivocally detectable by scintigraphy 5 days after injection of the intact antibody. High background radiation was generated from the blood, the liver, the spleen and the kidneys.

The radiolabeled Fab′$_2$ fragments resulted in less scintigraphic background activity, and both tumor types were clearly visualized on the third day after Fab′$_2$ injection (Fig. 5).

Improved detection probably would be achieved with a SPECT camera than with a planar gamma camera. Another radiolabeling technique and radionuclides with radionuclides with more optimal decay characteristics and less decomposition in vivo could improve imaging[137-140].

Today sensitive biochemical markers for primary and recurrent adrenocortical carcinoma exist[141] and a new imaging technique would be convenient to use in early cases of primary or recurrent adrenocortical carcinoma. However, assessment of the clinical value of the present antibody imaging in patients with adrenocortical tumors has to await further studies.

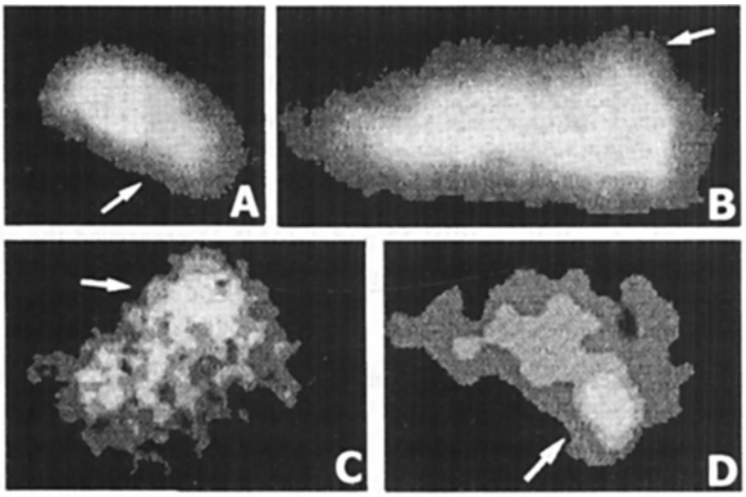

Fig. 5. Scintigraphic view of mice (tails to the right) with SW 13 (A, C) and T-CAR 1 (B, D) tumors in the thigh (arrows) 5 days after injection of radiolabeled Ac5 antibody (A, B) and 3 days after administration of its Fab′2 fragment (C, D)

Preoperative [^{111}In]-DTPA-D-[Phe^{1}]-octreotide scintigraphy

In the first (octreotide-biodistribution study) 23 patients with endocrine tumors 21 were considered positive on preoperative [^{111}In]-DTPA-D-[Phe^{1}]-octreotide and in the following (*in vivo* radiodetection study) 21 patients, 19 were positive.

Carcinoid imaging

All patients with mid gut carcinoids were positive with at least one pathological uptake at the preoperative SPECT. Few primary intestinal carcinoid lesions were preoperatively detected due to their tiny size. Mesenteric metastases were frequently demonstrated due to considerably higher uptake than in the surrounding mesentery and generally conspicuous size. Only larger liver metastases were visualized while smaller ones remained undetected. Occasionally a conglomerate of multiple small liver metastases gave the impression of a single large lesion.

Endocrine pancreatic tumors

Three of four primary tumors in patients with insulinoma were visualized by scintigraphy. The fourth insulinoma failed to be visualized with preoperative scintigraphy as well as with CT and US. In the single patient with gastrinoma the primary lesion and liver metastases were detected with preoperative [^{111}In]-DTPA-D-[Phe^{1}]-octreotide.

Other endocrine tumors

The aldosteronoma was localized to the left adrenal gland by preoperative CT scan but not seen on scintigraphy. The medullary thyroid carcinoma was confined to the thyroid gland, and was distinctly visualized by scintigraphy, as were multiple cutaneous metastases from the paraganglioma.

Detection rate

The number of lesions detected in patients with known somatostatin receptor-positive tumor diseases was analyzed in selected patients. Altogether 56.7 % of 60 extrahepatic abdominal tumors were scintigraphically detected. None of 22 lesions smaller than 9 mm was visualized. In our experience a detailed imaging of the distribution of abdominal endocrine tumors is rarely provided with [^{111}In]-DTPA-D-[Phe^{1}]-octreotide and SPECT analysis, mainly because small tumors generally remain undetected.

Test bench measurements of the developed gamma sensitive detectors.

H-probe2

By choosing tantalum for the collimator, lead for the capsule and utilizing a short light guide, the H-probe2 was efficiently shielded, but still remained handy. The small BGO-crystal with high efficacy for the emitted energy of ^{111}In contributed to keep the detector within small dimensions. The construction resulted in a collimation detection sector of 27° for ^{111}In and 20° for ^{99m}Tc, being estimated as halving of the maximum radioactivity in the test vial. This is considerably smaller and more favorable than the commercially available probes. This kind of collimator is especially adjusted for the detection of small tumors since primarily, these might escape detection. However, further reduction of the collimator detection angle would require increased measurement duration for preserved sensitivity, and this may lead to unnecessary prolongation of the operation time.

Matris 16

In test bench analyses with ^{111}In, the Matris 16 could detect 2 mm wide wells and outline the circumferences of a 6 mm well. Pairs of 3 mm wide wells 4 mm apart, with equal concentration of radioactivity could be separately detected with the probe at a distance of 5 mm. When the probe was moved away to 15 mm from the wells, they had to be 7 mm apart to be discriminated. Wells of 3 and 10 mm with an equal concentration of radionuclide could be discriminated when they were 6 mm apart, while 2 mm apart they could be discriminated only if the radioactivity concentration in the small well ten times exceeded that of the larger. The measurements were simpler and faster than with single channel detector probes. At screening mode the detector mirrored the movement of the wells in front of the collimator. When ^{99m}Tc was used in the phantom the results were comparable to ^{111}In.

Biodistribution

Tumor-to-blood radioactivity ratios varied considerably averaging 183 ±47.4 (0.003 - 0.069% ID/g) in the primary carcinoid tumors, and the ratios were slightly higher, mean 208 ±47.4 (0.012 - 0.059 %ID/g) in mesenteric and liver metastases. The primary lesions had approximately 5 - 100 times higher tumor-to-blood radioactivity ratios than the normal intestine and mesenteric fat. Carcinoid liver metastases had only 1.5 to 6 times higher ratios than the normal liver.

In endocrine pancreatic tumors the mean tumor-to-blood radioactivity ratio was 369 (0.009 - 0.254 %ID/g), and the uptake was twenty times that of the normal pancreas.

Well-counter analysis of normal tissue specimens revealed the highest uptake in spleen (0.054 %ID/g), lower in the liver (0.0067 %ID/g) and the pancreas (0.0042 %ID/g), and least in small intestine, fat and muscle.

Variations in uptake and size of the different lesions can partly explain the variable pre and intraoperative detection rate of tumors. Other factors influencing detection rate could be disturbing radioactivity from normal organs, presence of larger neighboring lesions, on variable distribution of different somatostatin receptor subtypes with different affinity to octreotide[115].

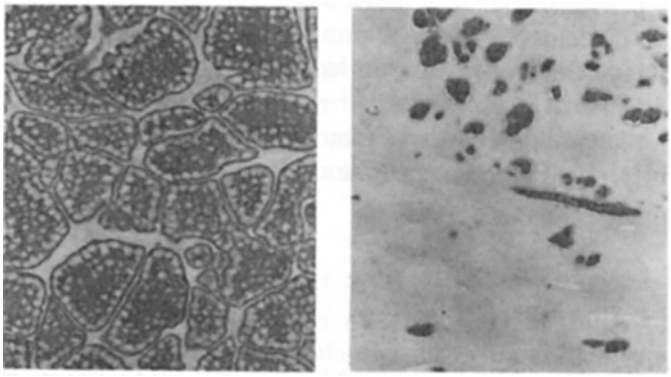

Fig. 6. Sections of liver (left) and ovarian (right) metastaes of midgut carcinoid tumors stained with Grimelius silver stain.

Histopathological examination

At histopathological examination especially the carcinoid exhibited variable degrees of fibrosis with parenchyma cells occupying 13.2 - 61.2 % (mean 39 %) of the sectioned area. However, there was no significant correlation between the histological proportion of tumor parenchyma and the registered tracer accumulation in the well-counter measurements (Fig. 6).

Intraoperative gamma detection

Commercially available probes

Intraoperative radioactivity measurements with the hand-held probes demonstrated altogether 15 of 21 tumors. The carcinoid mesenteric metastases (n = 9, 20 - 50 mm in diameter) showed higher count-rates than the surrounding mesentery. The count-rates of liver metastases were variable, some being lower than the normal liver, others exceeded this activity by up to three times. One 3 cminsulinoma displayed a count rate (85 c/s) being less than twice the value of the surrounding pancreas (54 c/s). Count-rates of primary carcinoid tumors measuring <10 mm in diameter, and the cutaneous metastases from the paraganglioma measuring 8 - 15 mm, were no higher than the normal surrounding tissue. In normal organs count-rates varied considerably with the highest values for the kidneys, spleen and liver, whereas background values in the thigh were low (1 - 10 c/s, mean 3 c/s). The

Gammed 2 probe was similar to the Tecprobe 2000 with respect to size, weight and counting system, but seemed to provide generally lower count-rates.

The presently investigated probes essentially adapted for radiation energies of ^{99m}Tc or ^{125}I were found to be insufficiently shielded for use with the ^{111}In labeled octreotide. Small tumors located within or close to the liver, the spleen, or the kidneys were not identified with these probes. Our experience is that lesions possible to detect by these probes could be displayed already on a preoperative SPECT, and therefore these detectors would not provide new information with impact on the surgical strategy. Our conclusion is that these commercial probes seem to have better applications in neck surgery[10] and colorectal surgery (i e RIGS)[43] than in surgery for abdominal endocrine tumors[128]. Together with ^{111}In, hand-held probes apparently need more accurate collimation and efficient shielding.

H-probe2

The intraoperative investigations with the H-probe2 revealed 32/35 (91,4 %) histopathologically verified lesions (Table 2). The visualized tumors measured 5 mm or more, and undetected pancreatic tumors in a patient with MEN-1, were all smaller (n = 3). Sliding measurements over a pancreatic tumor resulted in a graph with prominent peak radioactivity. Several palpable lesions, suspected to represent tumors, displayed low count-rates, and were found to be normal tissue at histopathology. However, one case of false positive localization within the pancreas was apparently due to accumulation of radioactive bile in the pancreatic duct. Also with this probe, count-rates of liver metastases equaled or were lower than the count-rates of normal liver (in all cases except one).

Probe measurements of normal organs such as the liver, the spleen and the kidneys displayed high count-rates, whereas low count-rates were registered from the aorta, the stomach, the intestine and the intestinal mesentery.

The H-probe 2 seems to have appropriate shielding against non-specific radiation from beside and behind the detector and appears suitable for ^{111}In diagnosis within the abdominal cavity. However, it is still necessary to avoid organs with high unspecific radioactivity in front of the collimator, e.g. the liver, the spleen and the kidneys.

H-probe 2 was clearly more efficient in detecting tracer accumulation in small

Table 2. Number and size of intraoperatively detected tumors with the H-probe2 (number of undetected tumors within parentheses)

Size	2-4mm	5-10mm	>10mm
Carcinoid tumors	0	5	16
Pancreatic tumors	0 (3)	5	6
Total	0 (3)	10	22

endocrine tumors than the preoperative SPECT/[^{111}In]-DTPA-D-[Phe^{1}]-octreotide examination, and the commercial probes. The principal value of intraoperative probe measurements would be to identify palpable lesions as likely to represent endocrine tumors. Screening for unpalpable lesions in the abdomen would be difficult and time consuming, since each individual registration spot required a 5-10 second measurement. Higher injected doses (e.g. > 175 MBq) of octreotide would result in more intense tumor signals, and better discrimination between tumors and normal tissue could possibly also be achieved by increased duration of individual radioactivity registrations, but this would also prolong the procedure.

Nevertheless, the H-probe2, with its efficient collimation and shielding and its computerized counting rate system represented an improvement of the intraoperative gamma detection.

Matris 16

The Matris 16 (Fig. 7) is described as capable of to detecting intestinal, mesenteric and pancreatic tumors in altogether 18 of 34 (53 %) of the tumors *in vivo*. However, as with the other probes, tumor deposits in the liver could not be identified due to high background radioactivity. Moreover, the form and size of the detector limited the accessibility to lesions in the liver. Small tumors (measuring 2 - 10 mm) required 10 times higher radioactivity uptake than the surrounding normal tissue to be detected. Larger tumors were visualized if the radioactivity was 4 times that of the normal tissue. Mesenteric lymph nodes without tumor did not cause false positive registrations. The detector also made it possible to register radioactivity in larger areas of interest within a reasonable period of time.

Ex vivo gammadetection of surgical specimens

Gamma camera

The *ex vivo* images with the gamma camera revealed more tumors than SPECT, though 24 of 35 carcinoid tumors (68.6 %) were detected in the 10 resected intestines. However, tumors smaller than 5 mm in diameter (n = 4) and tumors in close proximity to larger ones (n = 7) remained undetected. Two false positive registrations were obtained, obviously due to intestinal content with high concentration of nonspecific radioactivity. Because of the close approach to the object this technique provided a more detailed image than the preoperative SPECT. Evidently a small gamma camera or a multichannel detector optimized for intraoperative use would be a contribution for detection of small abdominal endocrine tumors. Such a hand-held planar multichannel detector (diameter 2 cm), adapted for ^{125}I, primarily used for imaging of the thyroid has previously been reported[11] but not yet used for intraoperative applications.

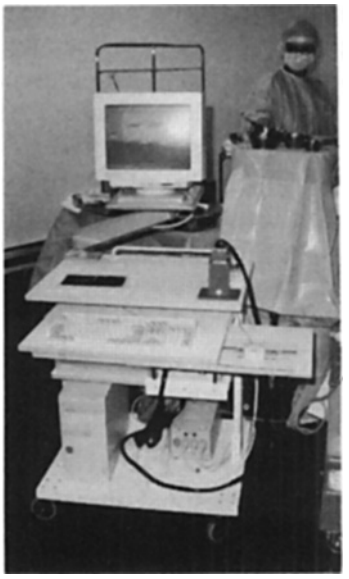

Fig. 7. The detector Matris 16 connected to the computer unit placed in the operating theatre.

H-probe2

The probe analyses of operative specimens *ex vivo* occupied 1 - 1.5 hours. All 18 palpable tumors were easily recognized by radioactivity peaks on three-dimensional diagrams. The count-rates of the radioactivity peaks were 31 - 146 counts per 8 sec, and they exceeded the background value from the normal intestine by at least 3 standard deviations. Examination of the graphs substantiated the possibility to detail the location of tumors and to grossly appreciate differences in size. Tumors situated at least 2 mm apart could be discriminated with the probe. The sensitivity was found to exceed that of careful intraoperative palpation since the *ex vivo* investigations revealed 4 non-palpable lesions 2 - 3 mm in diameter. Dissection of the specimens confirmed that recognition of two intraoperatively occult tumors apparently was prevented from detection by their close apposition to a 15 mm sized lesion.

Thus under ideal circumstances *ex vivo* the H-probe2 substantiated a sensitivity allowing recognition of neoplasms measuring only a few mm in diameter in specimens from patients who had received a standard [^{111}In]-DTPA-D-[Phe^{1}]-octreotide dose before surgery.

Matris 16

In the *ex vivo* study with the Matris 16 29 of 34 (87 %) tumors were detected. The minimal distance between two detected tumors was about 2 - 3 mm in the *ex vivo* experiments. The difference in detection rate *ex vivo* and *in vivo*

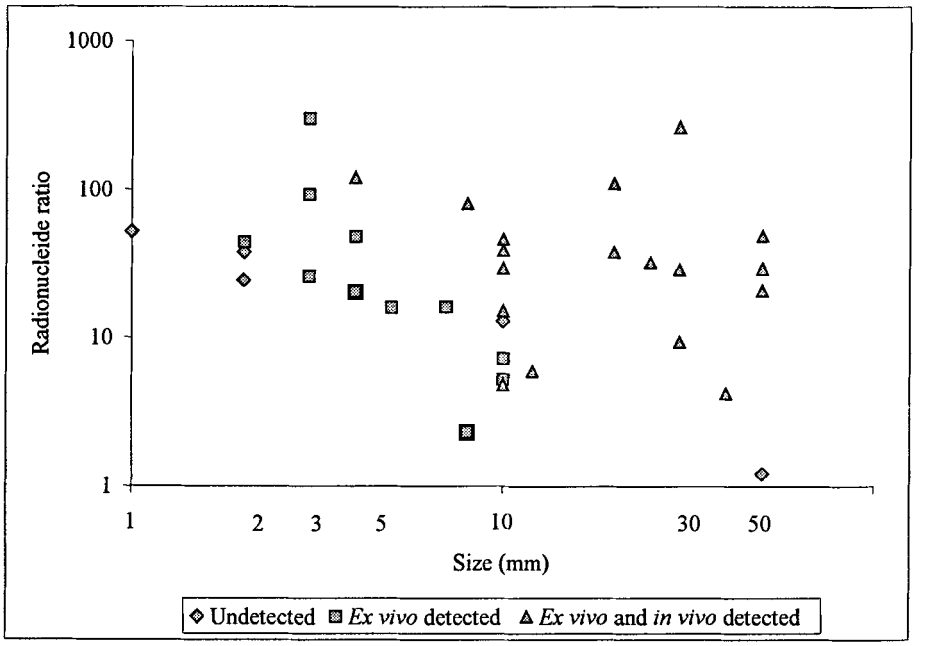

Fig. 8. Diagram of tumor detection *in vivo* and *ex vivo* in relation to tumor size and radioactivity ratio between the tumor and the surrounding tissue (logarithmic scales).

measurements is described in figure 8. *Ex vivo* measurements were generally more sensitive, locating 11 more tumors than in vivo registrations (n = 18). Tumors 2 - 3 mm in diameter were detected (fig. 9) if they contained at least 10 times higher radionuclide uptake than the surrounding tissue. One false negative tumor (10 mm) occurred probably due to prominent background radiation and irregular uptake of the tracer.

GENERAL COMMENTS

Preoperative radiological and scintigraphic methods both have limitations for the visualization of small abdominal endocrine tumors. Moreover, even lesions correctly localized preoperatively may sometimes be difficult to identify during laparotomy. Intraoperative visualization of somatostatin receptor positive tumors might therefore improve the efficacy of surgery. The H-probe2 and Matris 16 were able to detect small tumors, some even inaccessible to surgical palpation, more efficiently than preoperative SPECT. These devices comprise new developments to improve the detection of abdominal endocrine tumors by adoption of the specific emitted energy levels of ^{111}In, taken in consideration aspects like: high gamma energy of ^{111}In, data analysis and clinical utility. Matris 16 resembles a hand held gamma camera by its ability to display radioactivity distribution as an image. It allows registration, with preserved resolution, within a considerably larger area (16 times larger) than the other probes. The images of Matris 16 improve the clinical

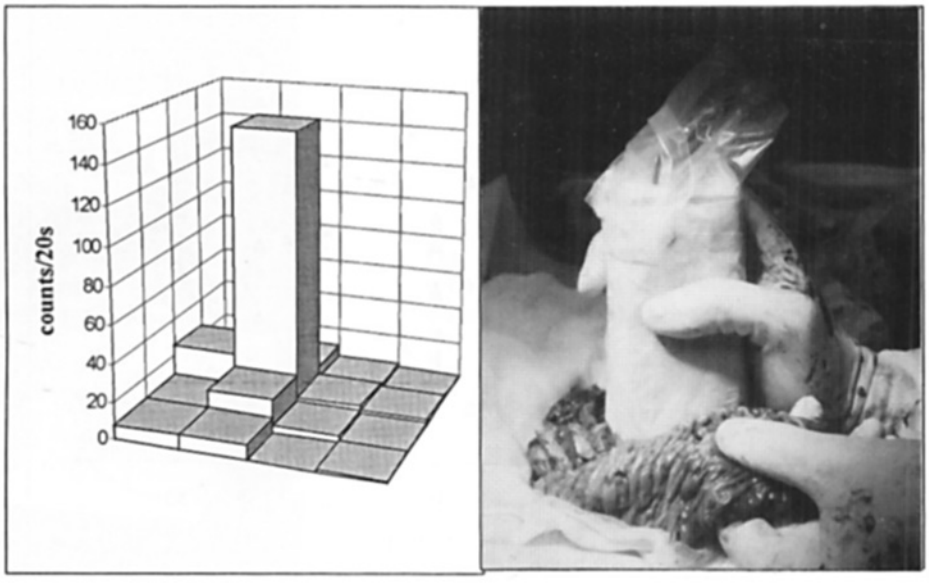

Fig. 9. *Ex vivo* measuring of a 3 mm gastrinoma in the duodenal mucosa measured on the operative specimen. A three-dimensional diagram of the activity (left). The Matris 16 detector probe during measuring (right).

applicability of the probe in two ways. First, The signal from a small tumor is usually found in 1 - 5 channels while the other channels mainly reflect background radiation. This makes evaluation of signal/background radioactivity possible to do in one single measurement. Secondly, repeated registrations and small probe movements help to focus the probe on the tumor and to adequately register the position of the uptake.

Impact on therapy

Intraoperative gamma detection has the potential to become an important contribution to achieving radical resection of endocrine tumors. Inefficient localization of such tumors may result in unnecessarily extensive exploration in search of small, easily overlooked lesions. Mesenterico-intestinal resections in patients with carcinoids may be more efficient, and it would be less likely to overlook small tumors or metastases if these were securely identified intraoperatively. Similarly, duodenal interventions, pancreatic exploration and the search for lymph node metastases in patients with gastrinoma could be simplified if the lesions were easily found at operation. This should be particularly true in patients with MEN-1 syndrome who are likely to harbor multiple lesions, and where removal may prevent or postpone tumor progression.

Future considerations

Tracers

Further development of scintigraphic techniques awaits the production of new tracers with improved uptake in tumors and faster biological clearance. Experience indicates that decreasing the molecular size of tracers while preserving or even improving receptor-binding properties would provide a lower disturbing background activity. There are reports of a new radiolabeled somatostatin analogue (Lanreotide, BIM-23014C) with improved receptor affinity for all described subtypes of somatostatin receptors, resulting in higher tumor to background activity ratios. With SPECT this tracer has been reported to provide improved imaging of endocrine tumors[141].

Radionuclides

Newly applied radionuclides and *in vivo* stable labeling will improve the measuring situation providing higher signals and lower background. More radiolabeling possibilities will facilitate the choice of a suitable radionuclide with half-life well adapted to the scheduling of the operation in order to provide optimal activity at the time of the intraoperative detection.

Intraoperative gamma detectors

New crystals and new solid state detector technology will in the future provide us with a neater hand held gamma detector device. Applied together with radionuclides with lower emitting energy this will considerably decrease the size and weight of the detector and provide improved usefulness. A small detector would also be easier to handle in all parts of the abdomen and would improve applicability in areas like the neck and axilla.

Future applications of the intraoperative gamma detectors include sestamibi scintigraphy for parathyroid neoplasia, CEA scintigraphy for localization of medullary thyroid carcinoma metastases and axillary intervention for lymph node metastases in breast cancer. Recently, a different tracer technique has been introduced. Administration of ^{99m}Tc colloidal solution in or close to, a primary tumor is used to identify the first station of lymphatic spread, so called sentinal node, in melanoma and breastcancer. This technique might have a role in surgery for gastrointestinal cancer.

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