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POSITRON EMISSION TOMOGRAPHY (PET) IN NEUROENDOCRINE GASTROINTESTINAL TUMORS

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Positron emission tomography (PET) makes it possible to study effects of medical treatment in vivo. Carcinoid tumors with liver metastases, especially those of midgut origin, produce serotonin via the precursors tryptophan and 5-hydroxytryptophan (5-HTP) and this overproduction contributes to the clinical symptoms of the carcinoid syndrome. Seven patients with histopathologically verified neuroendocrine tumors and liver metastases, five of whom with ileal carcinoids, one a lung carcinoid and one an endocrine pancreatic tumor, were included in the study. All patients had elevation of urinary 5-HIAA with the exception of one patient with a solitary liver metastasis of midgut origin. After an intravenous injection of ^{11}C-5-HTP, PET was performed and the uptake of radioactivity in tumor tissue, normal liver and plasma were compared. All patients with elevated urinary 5-HIAA and also the patient with a solitary liver metastasis and normal urinary 5-HIAA had high accumulation and signs of a high rate of binding of 5-HTP in the liver metastases. The uptake was relatively homogeneous in midgut carcinoid liver metastases but in large necrotic metastases the radioactivity was localized to the periphery. In three patients PET examination was repeated after 3 months of interferon treatment and in agreement with circulating tumor markers and ultrasonography the uptake of 5-HTP was unchanged. Another patient who received the somatostatin analog somatuline progressed on treatment and accordingly the uptake of 5-HTP also increased. The experience with PET in neuroendocrine gastrointestinal tumors is very limited. Our results so far indicate that 5-HTP can be used to visualize serotonin-producing neuroendocrine tumors and furthermore it might prove to be of value to monitor the effects of treatment, possibly also as an early predictive test of the outcome of treatment.

Positron emission tomography (PET) is a radiological technique, which has the unique capacity to provide biochemical and functional information in addition to the morphological data that can be obtained by conventional radiological methods. It has already been shown to be of great clinical value in the diagnosis and management of intracranial tumors, especially pituitary adenomas. In a large number of studies, PET has enabled a better diagnosis in pituitary adenomas in discriminating between active tumor and fibrosis, cysts and bleedings (1). Using a set of selected ligands, PET has also been helpful in the differential diagnosis between different types of tumors (2). It can give information about the dopamine receptor status and thereby guide in the choice of treatment (3). Most importantly, PET has proved to be a valuable tool to monitor treatment, making it possible to get an early indication of the outcome of treatment (4).

Neuroendocrine gastrointestinal tumors like pituitary adenomas belong to the so-called APUD-omas, i.e. they have the property to produce and secrete peptides and

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amines. They are also characterized by a relatively slow tumor growth but nevertheless have a malignant potential and in fact most tumors are diagnosed when liver metastases have developed. Medical treatment, which includes chemotherapy, α-interferons and somatostatin analogs, can control hormonal symptoms by reducing circulating hormone levels for a variable period of time (5). In a small number of patients (10–20%) a significant tumor shrinkage can be seen as an effect of treatment, but in the majority the tumor mass remains unchanged on CT or ultrasonography. Despite the lack of radiological signs of tumor regression, an antitumor effect of α-interferon with a decrease in the number of tumor cells within the lesions has been demonstrated (6). It is quite obvious that conventional imaging techniques are crude and fail to give biochemical information about treatment effects in vivo.

The largest group among our patients are those with classical midgut carcinoids and the carcinoid syndrome, the symptoms of which are partly caused by excessive production of serotonin from the tumors (7). Serotonin is synthesized in the carcinoid tumor cell by two enzymatic steps. First, tryptophan is 5-hydroxylated to form 5-hydroxytryptophan (5-HTP) and then this is decarboxylated to form serotonin by aromatic L-amino acid decarboxylase (Fig. 1). The serotonin circulating in blood is mainly bound to blood platelets. Released serotonin is oxidatively deamidated to 5-HIAA (8), which is excreted in the urine. Elevations of urinary 5-HIAA can also be found in a small number of patients with bronchial carcinoids and endocrine pancreatic tumors (foregut origin) but very rarely in hindgut carcinoids.

To our knowledge the only experience with PET in neuroendocrine gastrointestinal tumors is a single preliminary study that we performed in 1987 (unpublished data). ^{11}C-labelled methionine, tryptophan and 5-HTP were tried as tracer and we found that 5-HTP was taken up in carcinoid liver metastases. Hence, the purpose of the present study was to assess whether 5-HTP can be used more systematically as a tracer substance to visualize metastases in patients with neuroendocrine tumors and signs of overproduction of serotonin. We also wanted to examine whether PET with 5-HTP can be helpful in monitoring treatment effects.

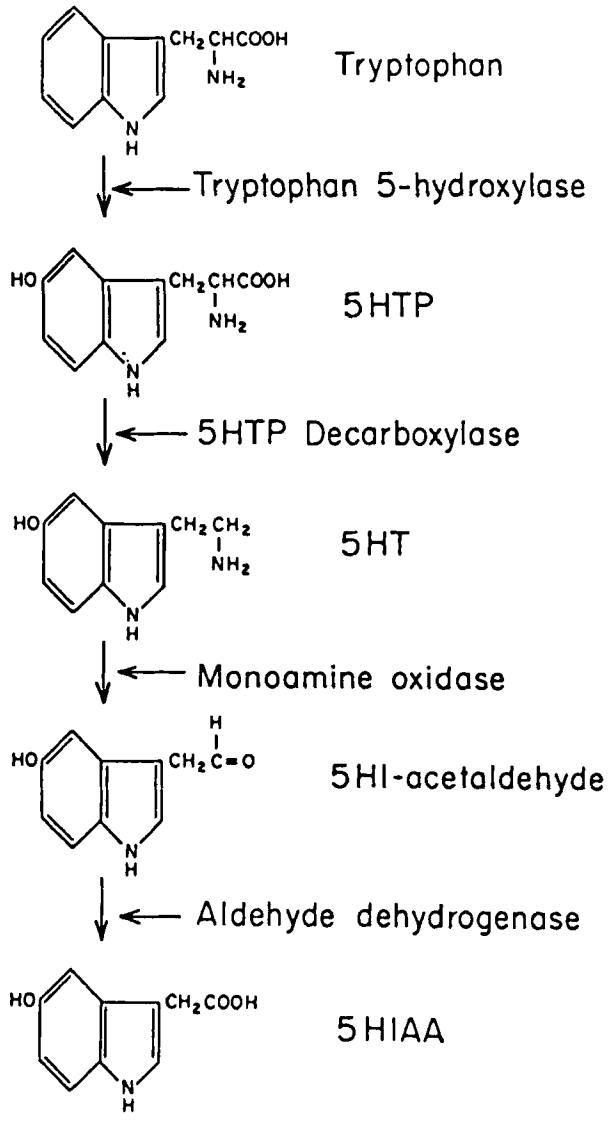


Fig. 1. Metabolic steps in the serotonin synthesis from the first precursor tryptophan to the urinary break-down product urinary 5-HIAA. 5-HTP = 5-hydroxytryptophan; 5-HT = 5-hydroxytryptamine (serotonin); 5-HIAA = 5-hydroxyindole acetic acid.

Material and Methods

Seven patients (4 men and 3 women) with histopathologically verified neuroendocrine tumors and liver metastases, referred to the Medical Department in Uppsala for evaluation and medical treatment, were included in the study. Five patients had classical midgut carcinoids and presented the carcinoid syndrome. The other two patients had no hormonal symptoms; one patient had been operated on because of a lung carcinoid and one had an endocrine pancreatic tumor (EPT) that was unresectable. All patients had elevated urinary 5-HIAA with the exception of one midgut carcinoid with a small tumor burden (one liver metastasis). The histopathological diagnosis was established by ultrasonographically-guided coarse-needle biopsies in each case (9). The Grimelius' (10) and Masson (11) silver staining techniques as well as immunocytochemical stainings against chromogranin and pancreatic hormones were performed (12). For further patient characteristics, see Table.

All patients underwent careful biochemical evaluation by earlier described radioimmunoassays. In addition to urinary 5-HIAA, plasma neuropeptide K (NPK) (13),

chromogranin A + B (14), serum pancreatic polypeptide (PP), and human chorionic gonadotropin (HCG)-a and b, gastrin, and calcitonin were measured in carcinoid patients. In the patient with EPT, serum gastrin, insulin, pro-insulin, calcitonin, plasma vasoactive intestinal polypeptide (VIP), glucagon, and chromogranin were analyzed as well as urinary 5-HIAA. All patients underwent CT and ultrasonography before and at regular intervals during medical treatment.

The PET studies were performed as dynamic sequences with 14 scans obtained during 45 min after a rapid i.v. bolus injection of 5-HTP ^{11}C-labelled in the β-position. Selective utilization for serotonin synthesis in the striatum of rhesus brain has been analyzed using 5-HTP labelled in the β-position (15). The scanning time was successively increased from 60 to 300 s. Twelve plasma samples from a peripheral vein, 'arterialized' by heating, were obtained at similar intervals and analyzed for ^{11}C concentration. The PET unit (Scanditronix GE4096) produced 15 slices with a thickness of about 6 mm and a spatial resolution of 5 mm. The PET tracer substance and the ^{11}C were produced at the PET center, Uppsala University. Regions of interest were selected in the images to represent tumor tissue and normal lever tissue. The quantitative analyses were performed according to the technique of Patlak et al. (16) to examine if an irreversible trapping of ^{11}C 5-HTP occurred in tumor and normal liver tissue using plasma radioactivity as a reference. The PET examinations were performed after informed consent from the patients and with permissions from the Hospital Isotope Committee and the Hospital Ethical Committee.

Recombinant interferon-α2b (Introna, Schering Corp, USA) was administered at doses of 5 MU three times a week subcutaneously in 3 patients. The somatostatin analog somatuline (Henri Beaufour Inst, USA) was given in one patient as a continuous subcutaneous infusion via a peristaltic pump at an inital dose of 750 μg, followed by weekly escalations up to 12 000 μg and then the maximum tolerable dose was given.

Results

All patients with signs of excessive serotonin production, i.e. those who presented elevated urinary 5-HIAA levels and also the patient with only one small lesion in the liver and normal urinary 5-HIAA had a high uptake of ^{11}C-5HTP in the liver metastases (case No. 1, 2 and 4) (Figs. 2, 3, 4). Two of the patients had abdominal lymph node metastases and one had pleural metastases, which also showed increased uptake of 5-HTP in comparison with surrounding tissue (Fig. 5). The radioactivity in the tumor tissue as compared to normal liver tissue studied by the Patlak method indicated a high ratio of binding of 5-HTP in the metastases (Fig. 6). However, with time the slope curved off from linear in several patients (Fig. 6), suggesting release of metabolic products from within the tumor, possibly serotonin.

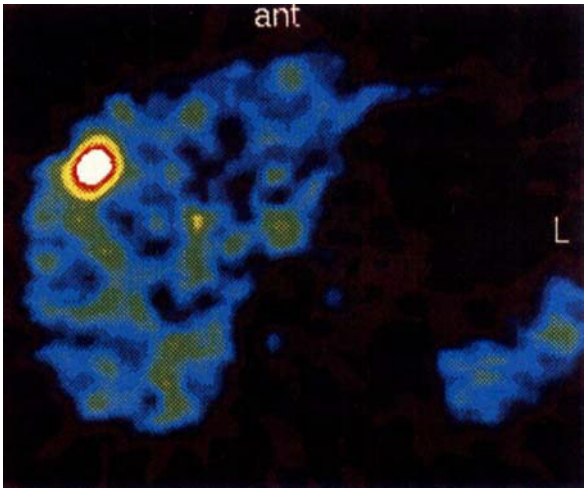


Fig. 2. Patient with an ileal carcinoid (case No. 1) and a solitary liver metastases in the right liver lobe as indicated by ultrasonography. CT failed to detect the tumor. The patient demonstrated normal levels of urinary 5-HIAA but slightly increased chromogranin levels. PET shows an increased and well-defined uptake of ^{11}C-5-HTP in the liver metastasis.

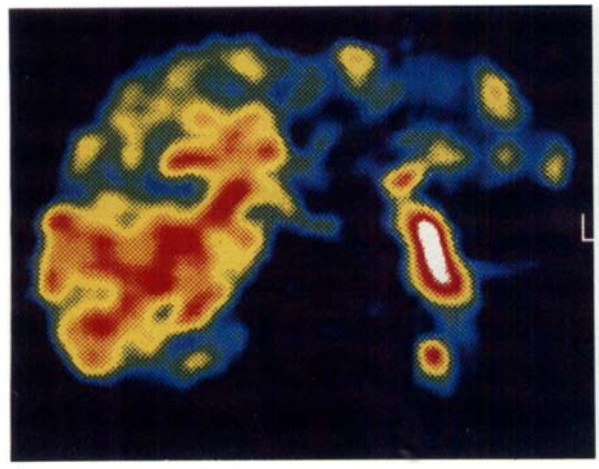
The accumulation of ^{11}C-5-HTP was relatively homogeneous within the lesions in the midgut carcinoids. The patient with a lung carcinoid (case No. 6) had a large metastasis in the right liver lobe with a central necrosis clearly visualized by ultrasonography and by contrast enhanced CT. On the PET images, there was an accumulation of 5-HTP in the periphery of this metastasis (Fig. 7a).

When comparing PET utilizing 5-HTP as a tracer to CT in the carcinoid patients, the morphological information obtained from PET appeared to be superior to that obtained from CT in 4/5 cases and equal in one case (Figs. 2, 3, 4).

The results with PET examination in the patient with a non-functioning EPT (case No. 7) are shown in Fig. 8. Biochemically, she had a slight to moderate increase in urinary 5-HIAA (around 200 μmol/24 h), elevated chromogranin A + B and PP. There was an increased uptake of 5-HTP in one of her liver metastases and a less striking uptake in part of the pancreatic tumor.

Three out of four midgut carcinoid patients were reexamined after 3 months of Introna treatment and in agreement with circulating tumor markers and ultrasonography there was no significant change in the uptake of 5-HTP.

After initial evaluation the lung carcinoid patient (case No. 6) was started on the somatostatin analog somatuline in escalating doses. After 2 months of treatment, urinary 5-HIAA and plasma chromogranin A + B had increased by 65% and 50% respectively and ultrasonography showed an increase in size from 80 mm to 92 mm in diameter of her large liver metastasis. PET performed at that time



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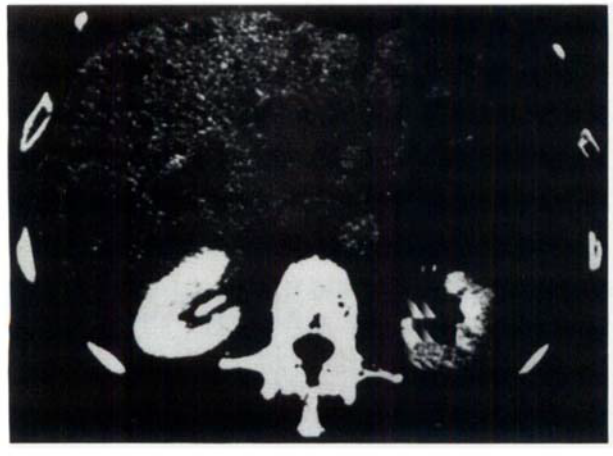
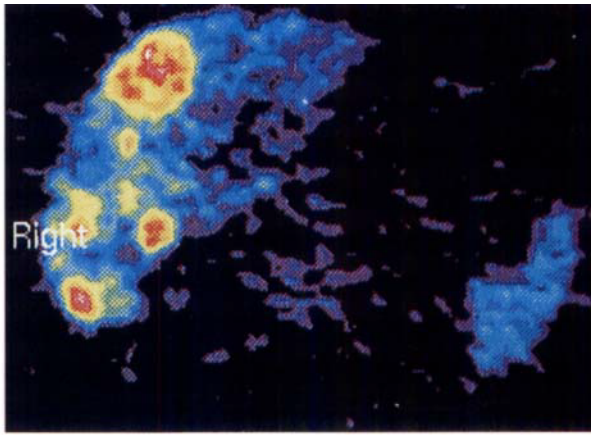


b)

Fig. 3. Patient with an ileal carcinoid (case No. 2), multiple liver and also abdominal lymph node metastases investigated with ^{11}C PET (3a). 5-HTP is taken up in apparently all the liver metastases and also a lymph node metastases adjacent to the pancreas. A CT-scan is shown for comparison (3b).



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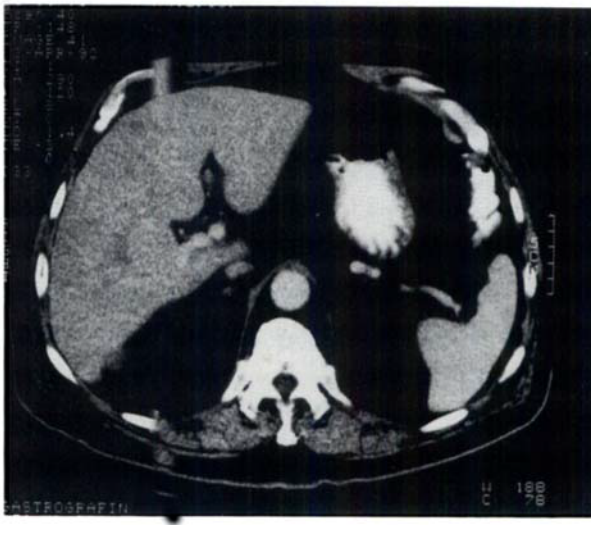


b)

Fig. 4. Patient with an ileal carcinoid (case No. 4) and multiple liver metastases. CT images (4b) show inhomogeneity in the liver, whereas PET with ^{11}C-5-HTP show several well-delineated metastases of different size with a very high uptake of 5-HTP (4a).

demonstrated a clearly higher uptake rate (85% increase) of 5-HTP in the periphery compared to the examination before start of treatment (Fig. 7a and b). However, the increase in tumor size was difficult to discern with PET.

Discussion

PET is an imaging technique that makes it possible to study biochemical events in organs of interest in vivo. For endocrine oncologists, who have had to rely on indirect measurements of serum or plasma hormonal levels and crude conventional radiological methods, this technique will surely have a great impact on tumor detection and therapeutic monitoring in the future.

Due to the minimal experience with PET in neuroendocrine gastrointestinal tumors, the first step in our studies had to be a search for a relevant substance to label in order to visualize the tumors. ^{11}C-labelled methionine, an excellent tracer in the studies of pituitary adenomas, reflecting tumor metabolism (1–4), was attempted in our preliminary study in 1987, but showed low uptake in carcinoid liver metastases or endocrine pancreatic tumors (unpublished data). The uptake in normal pancreas was high, which is not surprising considering the high degree of protein synthesis that normally takes place there (17).

Increased synthesis and metabolism of serotonin remains the most important diagnostic biochemical feature of the carcinoid syndrome. Serotonin or 5-hydroxytryptamine (5-HT) is synthesized via the precursors tryptophan and 5-hydroxytryptophan (5-HTP) and subsequently broken down and excreted as urinary 5-hydroxyindole-acetic acid (5-HIAA). Both precursors, tryptophan and 5-HTP, were labelled with ^{11}C and attempted as tracer in our initial study. Tryptophan showed no accumu-

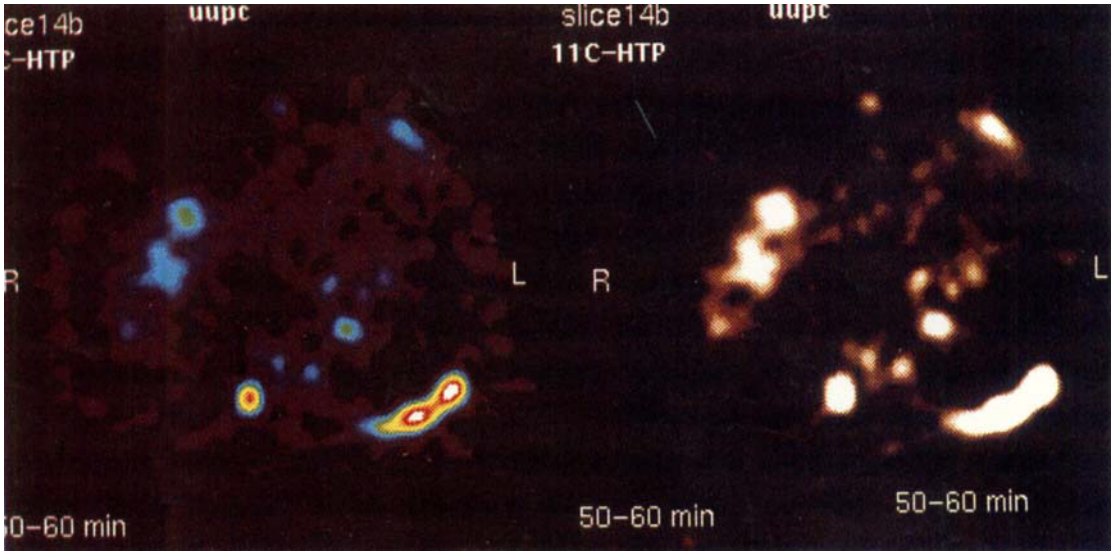


Fig. 5. Patient with an ileal carcinoid (case No. 2), liver lymph node and also pleural metastases. Not only liver and lymph node metastases but also pleural metastases showed an increased uptake of 5-HTP with PET.

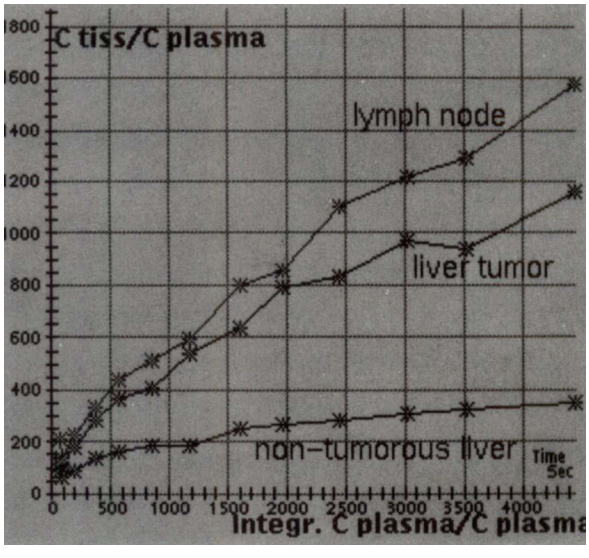
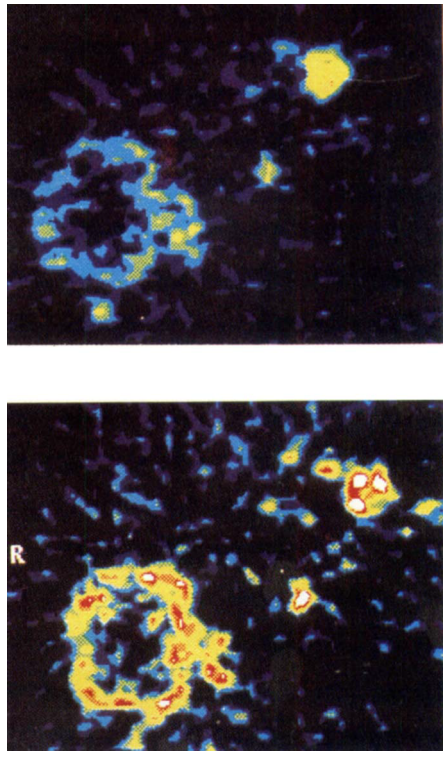


Fig. 6. The rate of trapping of 5-HTP in a liver metastasis and a lymph node metastasis as compared to normal liver is illustrated accordlng to the PATLAK method. The uptake in normal liver is nearly in equilibrium with radioactivity in plasma, as indicated by the horizontal curve. In the metastases, the uptake of 5-HTP indicates a high and virtually irreversible binding of the substance.



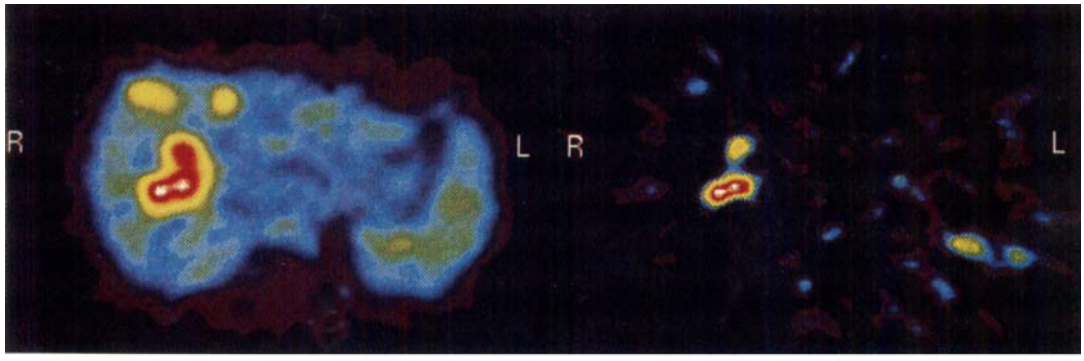
a)

b)

Fig. 7. Patient with a lung carcinoid (excised) (case No. 6) and multiple liver metastases, the largest metastasis with a diameter of 80 mm, located in the right liver lobe. a) PET examination before treatment shows an uptake of 5-HTP only in the active tumor cells in the periphery, whereas the central necrotic area is completely void of activity. b) The patient was reexamined after 2 months of treatment with the somatostatin analog somatuline. Biochemical markers and ultrasonography indicated disease progression PET showed a clearly higher uptake of 5-HTP in the periphery of the metastasis as compared to the examination before treatment.

lation in carcinoid metastases or pancreatic tumors. The uptake in normal pancreas was even higher than with methionine. 5-HTP, on the other hand, was taken up significantly in the liver metastases of a midgut carcinoid patient and this observation was the starting point for our present study.

The correlation between elevated urinary 5-HIAA and uptake of 5-HTP in the metastases is unequivocal. The



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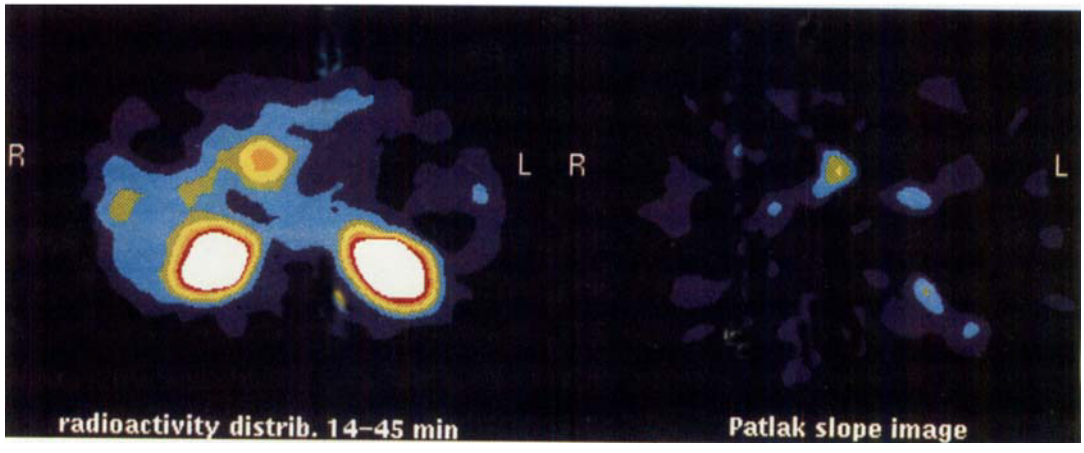

b)

Fig. 8. Patient with a non-functioning endocrine pancreatic tumor and multiple liver metastases (case No. 7). a) PET with ^{11}C-5-HTP shows small distinct areas in the right liver lobe with significantly increased activity. b) The other liver metastases showed scattered uptake and only a part of the pancreatic tumor showed a modest uptake (6b).

midgut carcinoid patients had a homogeneous uptake of the tracer, suggesting an even distribution of serotonin-producing tumor cells. Interestingly, also lymph node and pleural metastases showed the same characteristic. The most puzzling finding in this small group of patients is the very focal uptake of 5-HTP in our case with an EPT. She had multiple liver metastases and also a remaining pancreatic tumor, which showed partial uptake. This observation might suggest that only small localized populations of tumor cells in the pancreas and the liver had the capacity to produce serotonin.

The imaging using PET with 5-HTP in midgut carcinoid patients appears to be excellent and this technique could detect and delineate more metastases in the liver than could CT.

Another important observation is that the uptake of 5-HTP in patients followed during treatment correlated well with treatment results; three patients having stable disease on α-interferon treatment and one patient demonstrating progressive disease on somatuline treatment. This will probably be the most important application in our patients. Since not all patients will respond to treatment it is important to get an early indication of the outcome to spare them from unneccesary side-effects. In the future it will be important to find out how early this prediction can be made.

In conclusion, our present pilot study with PET in patients with neuroendocrine gastrointestinal tumors is promising. We have been able to find a tracer to visualize serotonin-producing tumors with PET. We still have to search for a substance that can function as a tracer in EPT. Furthermore, with the competence available to label growth factors, peptides and macromolecules, such as interferons and other drugs, the PET technique will provide new possibilities to understand the tumor biology, growth regulation and mechanisms of action of various treatments in neuroendocrine gastrointestinal tumors.

Table

Patient characteristics

Patient No.	*Sex*	Location of primary tumor	Tumor burden	Immuno-histochem. liver metastases	Peripheral tumor marker	*PET* uptake of 5-HTP Tumor visibility PET/CT[1]	Type	Treatment result	PET, follow-up
1.	M	Distal ileum	l.m. (solitary)	Grim + Masson + cgA + B +	U-5-HIAA (42) p-cgA + B (460)	PET > CT	Introna	SD	Unchanged
2.	M	Distal ileum	multiple l.m. llgl.m. pleural m.	Grim + Masson + cgA + B +	U-5HIAA (2400) pcgA + B (> 20 000)	PET = CT (PET > CT)[2]	Introna	SD	—
3.	F	Distal ileum	multiple l.m. llgl.m.	Grim + Masson + cgA + B +	U-5HIAA (310) pcgA + B (2300)	PET > CT	Introna	SD	Unchanged
4.	M	Distal ileum	multiple l.m.	Grim + Masson + cgA + B +	U-5HIAA (1110) pcgA + B 23 000	PET > CT	Introna	SD	Unchanged
5.	M	Distal ileum	multiple l.m.	Grim + Masson + cgA + B +	U-5-HIAA (400) pcgA + B (8000)	( )[4]	Introna	SD	—
6.	F	Lung	multiple l.m. multiple s.m. pancreatic m.	Grim + Masson − cgA + B +	U-5HIAA (340) pcgA + B 19 000 HCG-α 9.4	PET = CT	Somatuline	PD	Increased
7.	F	Pancreas	pancreatic tum. multiple l.m.	Grim + Masson − cgA + B +	U-5HIAA (200) pcgA + B (14 700) PP > (6400)	PET = CT (PET < CT)[3]	Somatuline	SD	—

l.m. = liver metastases
llgl.m. = lymph node metastases
Grim = Grimelius' silver staining
pcGA + B = plasma chromogranin A + B
(reference range < 350 μg/L)
U-5-HIAA = urinary 5 hydroxyindole-acetic acid
(reference range 10–80 μmol/d)
PP = pancreatic polypeptide
(reference range < 70 pmol/l)
SD = stable disease
PD = progressive disease
1) Visual comparison PET/CT with respect to number of lesions visible and discernible tumor volume.
2) Liver tumors without brackets, lymph node metastases within brackets.
3) Liver tumors without brackets, result in pancreatic tumors within brackets.
4) No relevant CT available for comparison.

ACKNOWLEDGEMENTS

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