Tracer development for detection and characterization of neuroendocrine tumors with PET

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Summary and future perspectives

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Neuroendocrine tumors are slowly growing tumors which originate from neuroendocrine cells. These tumors can secrete several products. In case of overproduction of serotonin, symptoms such as flushing, diarrhea and right-sided heart disease can occur. Next to serotonin, other well known products are e.g. catecholamines.

Amine precursors such as levodopa and 5-hydroxytryptophan are taken up into neuroendocrine tumor cells by large amino acid transporters (LAT) and are decarboxylated by amino acid decarboxylase (AADC) to serotonin and dopamine. These amines are stored in cellular vesicles via the vesicular monoamine transporter VMAT. After release the amines are metabolized by the enzyme monoamine oxidase (MAO) to respectively 5-hydroxyindole acetic acid and homovanillic acid and subsequently excreted.

Staging of neuroendocrine tumors is required for optimal treatment decisions. Because of their slow growth and frequently the induction of non-specific symptoms, these tumors are often metastasized at diagnosis. Different imaging methods for detection of neuroendocrine tumors are currently available. Routine imaging techniques consist of morphological techniques such as computed tomography (CT) or magnetic resonance imaging (MRI) and functional imaging such as somatostatin receptor scintigraphy (SRS). Besides SRS, in neuroendocrine tumors positron emission tomography (PET) is another functional imaging technique which can be useful to improve tumor image quality and sensitivity of tumor detection. Two new interesting PET tracers are available. The PET tracers *L*-6-[^{18}F]fluoro-levodopa ([^{18}F]FDOPA) and *L*-[^{11}C]-5-hydroxytrytophan ([^{11}C]HTP) are of special interest as they take actively part in the biochemical pathways of neuroendocrine tumors.

Therefore, the aim of this thesis was to study the development, biochemical behavior and diagnostic value of new PET tracers for imaging neuroendocrine tumors.

In **chapter 1** a literature review is given of the techniques currently used for staging of neuroendocrine tumors in nuclear medicine. First, an overview of the mechanisms involved in tracer uptake is presented. The different morphological and functional detection methods are described and results obtained in the last decade are shown. The sensitivities of the various tracers in several subtypes of tumors have been compared in Forest plots. This comparison showed, that the metabolic PET tracers [^{18}F]FDOPA and [^{11}C]HTP perform with higher sensitivity than the currently used standard SRS.

These studies with [^{11}C]HTP have been described by the Uppsala group exclusively, as they have been the only PET center worldwide with the capability to produce this tracer. Given their interesting results we decided to investigate the production of [^{11}C]HTP on a Zymark robotic system. The synthesis described in **chapter 2** was started by the production of [^{11}C]methyl iodide and labeling of the precursor *N*-(diphenylmethylene)glycine tert-butyl ester. Subsequent hydrolysis gave racemic [^{11}C]alanine. [^{11}C]HTP was obtained from [^{11}C]alanine in a one-pot synthesis using 4 different enzymes. Average radiochemical yield after HPLC purification was 15 ± 12 % from the time of release of [^{11}C]methyl iodide. Radiation exposure for the radiochemist was reduced to a minimum of 260 µSv for the skin and 40 µSv for the whole body. Currently, [^{11}C]HTP is produced reliably in doses of 400 MBq which is sufficient for patient studies.

Although [^{18}F]FDOPA and [^{11}C]HTP showed interesting results in humans, still little is known about the processes involved in accumulation of these tracers by neuroendocrine tumor cells. In **chapter 3** we therefore evaluated the tracer uptake via LAT transporters, the

influence of the decarboxylase inhibitor carbidopa and of the MAO inhibitors clorgyline and pargyline on tracer accumulation. The effect of carbidopa *in vivo* on metabolism of [^{18}F]FDOPA and [^{11}C]HTP in small animals was studied with a microPET camera. The cellular transport of both PET tracers in the neuroendocrine BON tumor cell line was inhibited by amino-2-norbornanecarboxylic acid and resulted in low IC_{50} values ([^{18}F]FDOPA: 0.01 mM; [^{11}C]HTP: 0.12 mM) after 15 minutes tracer incubation. Inhibition of MAO by clorgyline led to a significant increase in tracer accumulation compared to control ([^{18}F]FDOPA: P=0.02; [^{11}C]HTP: P=0.02) *in vitro* after 60 minutes tracer incubation. We showed that carbidopa did not influence accumulation in tumor cells *in vitro*, but did increase uptake in tumor bearing mice. This increased uptake most likely is the result of inhibition of decarboxylation in peripheral organs which results in better availability of tracer for accumulation in tumor cells. Standardized uptake values (SUVs) of [^{18}F]FDOPA were superior to [^{11}C]HTP 60 minutes after intravenous injection in a neuroendocrine pancreatic tumor animal model (P=0.03). In small animals, neuroendocrine tumors from human origin with weights of less than 20 mg were still clearly visualized with both PET tracers.

In **chapter 4** the diagnostic value of [^{18}F]FDOPA for the detection of patients with carcinoid disease was studied. 53 patients underwent a [^{18}F]FDOPA PET scan which was compared with the standard imaging methods such as SRS and CT. In a patient based analysis [^{18}F]FDOPA had a sensitivity of 100% (95%CI 93 - 100), SRS of 93% (95%CI 82 - 98), CT of 87% (95%CI 75 - 95) and the combination of SRS and CT 96% (95%CI 87 - 100) (P=0.45 for PET versus SRS with CT). However, [^{18}F]FDOPA alone detected many more lesions, more positive regions and more lesions per region than SRS combined with CT. In regional analysis sensitivity of [^{18}F]FDOPA was 95% (95%CI 90- 98) versus 66% (95%CI 57 - 74) for SRS alone, 57% (95%CI 48 - 66) for CT and 79% (95%CI 70 - 86) for SRS with CT (P=0.0001, PET versus SRS with CT). In individual lesion analysis, sensitivities were 96% (95%CI 95 - 98), 46% (95%CI 43 - 50), 54% (95%CI 51 - 58) and 65% (95%CI 62 - 69) for PET, SRS, CT and SRS with CT respectively (P<0.0001 for PET versus SRS with CT). The results of this study showed that PET imaging with [^{18}F]FDOPA is superior for staging carcinoid tumor lesions compared to SRS and CT.

In **chapter 5**, a study is described analyzing the PET tracers [^{18}F]FDOPA and [^{11}C]HTP. As there were no head to head comparisons, it was until now not clear from the literature which PET tracer was superior in which setting. 24 patients with a carcinoid tumor and 23 patients with an islet cell tumor were included in the study. Patients had to have, before performing the PET scans, at least one lesion based on clinical, histological and/ or biochemical findings and detected one abnormal lesion on SRS, CT or MRI. All patients underwent [^{11}C]HTP and [^{18}F]FDOPA PET scans and additional SRS and CT scan.

[^{18}F]FDOPA and [^{11}C]HTP showed more carcinoid tumor positive regions than SRS as [^{18}F]FDOPA sensitivity was 81%, and [^{11}C]HTP sensitivity was 77% (P=0.001 and 0.004 for comparison with SRS respectively). Sensitivity of SRS was 58% and CT 70% (P=ns for the comparison of both PET scans with CT). In islet cell tumors [^{11}C]HTP detected more tumor positive regions (sensitivity 70%) than [^{18}F]FDOPA (sensitivity 44%, P=0.0001 for the comparison with [^{18}F]FDOPA). In carcinoid patients, both [^{11}C]-5-HTP and [^{18}F]FDOPA revealed more tumor positive lesions (sensitivity 78% and 87%) than SRS (sensitivity 49%, $P \leq 0.001$ for the comparison of both [^{11}C]HTP and [^{18}F]FDOPA with

SRS) in carcinoid patients. These results show that [^{18}F]FDOPA performs best in carcinoid tumors and is superior to SRS and CT. Imaging of islet cell tumors gave best results with [^{11}C]HTP. This tracer was superior to SRS and [^{18}F]FDOPA while CT gave similar results as [^{11}C]HTP. For staging both carcinoid and islet cell tumors, these PET scans are, in combination with CT, a promising tool for anatomical localization of the tumor.
[^{11}C]HTP shows interesting results for the detection of neuroendocrine tumors and in particular islet cell tumors. However, only a few centers are equipped with a cyclotron and are therefore capable to produce tracers with short half-lives. If a fluorine-18 labeled tryptophan analogon could be produced with a half-life of 110 minutes this could be transported to other hospitals. It is therefore an attractive alternative for [^{11}C]HTP. We investigated a synthesis route of 5-fluorotryptophan by combining reliable methods used in the synthesis of [^{11}C]HTP and [^{18}F]FDOPA (**chapter 6**). We demonstrated that non-labeled 5-fluorotryptophan accumulates in neuroendocrine tumor cells. Electrophilic fluorodestannylation, a method used in the synthesis of [^{18}F]FDOPA, was chosen to obtain 5-fluoroindole with yields of 15 % from the newly developed precursor 5-trimethylstannylindole. The reversed tryptophanase reaction was used for the synthesis of [^{11}C]HTP. We optimized the synthesis of 5-fluorotryptophan from 5-fluoroindole so far, that 5-fluorotryptophan can now be obtained in reliable yields over 70 % using the reversed tryptophanase reaction. Labeling work is in progress to synthesize [^{18}F]-5-fluorotryptophan from 5-trimethylstannylindole. If successfully developed, [^{18}F]-5-fluorotryptophan might well be a feasible tool for staging neuroendocrine tumors.

Future perspectives

In this thesis it is shown that [^{18}F]FDOPA and [^{11}C]HTP are relevant PET tracers for the visualization of neuroendocrine tumors (chapter 3, 4 and 5). Tracer accumulation in neuroendocrine tumor cells results from the sum of tracer uptake by LAT, decarboxylation AADC and subsequent storage of amines in granules (VMAT). We showed that carbidopa, administered to patients before the imaging to increase tracer uptake, does not influence decarboxylation of tracer in tumor cells. Carbidopa exerts its effect by inhibition of tracer decarboxylation in peripheral organs and therefore more tracer can be taken up by the neuroendocrine tumor. Inhibition of MAO increased tracer accumulation *in vitro* (chapter 3). MAO was overexpressed in the neuroendocrine BON tumor cell line used in our studies. If inhibited, it resulted in high intracellular levels of serotonin or catecholamines. Further preclinical research is required to evaluate whether MAO inhibition should be considered in the clinic to further improve image quality and sensitivity of neuroendocrine tumor detection.
In our studies metabolic tracers [^{18}F]FDOPA and [^{11}C]HTP PET are superior to SRS or morphological techniques such as CT and MRI. However, SRS is used with a gamma camera with lower resolution than PET cameras. ^{68}Ga-octreotide analogues are in development and can be used to image receptor expression in neuroendocrine tumors with PET. To know which technique is superior, a head to head comparison with metabolic tracers such as [^{18}F]FDOPA and [^{11}C]HTP is required. [^{11}C]HTP showed good results for visulalization of neuroendocrine tumors but because of the short tracer half-life the scans have to be started directly after injection and the tracer can be administered to one patient per synthesis only. Tryptophan labeled with ^{18}F would not only allow longer distribution of

tracer but it would also give the possibility to scan for a longer period and would also make the tracer available for two or more patients per synthesis. We showed that 5-fluorotryptophan has favorable characteristics to enter human neuroendocrine tumor cells. A profiling method for metabolites such as 5-fluorotryptamine and 5-fluoroindole acetic acid could show the total accumulation of the tracer in tumor cells and give more insights in the metabolism and biochemical behavior of 5-fluorotryptophan in neuroendocrine tumors. To complete the synthesis towards [^{18}F]-5-fluorotryptophan, labeling of 5-trimethylstannylindole using [^{18}F]F_2 gas should be investigated. Once established, the value of [^{18}F]-5-fluorotryptophan for imaging neuroendocrine tumors can be determined *in vitro* and in a small animal model to get a better understanding of tracer metabolism and accumulation. As [^{18}F]-5-fluorotryptophan contains a fluorine atom further studies with regard to biochemical behavior in organisms are necessary. Other isomers of fluorotryptophan such as 4-,6- or 7-fluorotryptophan may show also affinity for neuroendocrine tumors and therefore, labeled with ^{18}F, they can be attractive tracers in this setting yielding comparable results as [^{18}F]FDOPA and [^{11}C]HTP.