Tracer development for detection and characterization of neuroendocrine tumors with PET

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Introduction and outline of this thesis

Introduction

Neuroendocrine tumors are rare and often slowly growing tumors originating from neuroendocrine cells. These tumors frequently have the ability to secrete peptides and hormones such as serotonin and catecholamines, which can cause symptoms. Best known is the overproduction of serotonin resulting in symptoms such as flushing, diarrhea and right-sided heart disease. These amines are produced in neuroendocrine cells by *u*ptake and subsequent *d*ecarboxylation of *a*mine *p*recursors (APUD) such as 5-hydroxytryptophan (5-HTP) or levodopa (*L*-DOPA) in neuroendocrine cells. Several diagnostic methods for imaging neuroendocrine tumors have been developed in the past years. Therapy can be monitored by morphological techniques such as computed tomography (CT) or magnetic resonance imaging (MRI). However, given specific characteristics of neuroendocrine tumors functional imaging techniques such as somatostatin receptor scintigraphy (SRS) and positron emission tomography (PET) are also of interest as they image respectively the somatostatin receptor expression and intracellular metabolism. In oncology, [^{18}F]fluorodeoxyglucose (FDG) is used as very sensitive but non-specific PET tracer. However, FDG rarely shows sufficient uptake in neuroendocrine tumors. Based on the APUD principle, PET tracers like 6-[^{18}F]fluoro-levodopa ([^{18}F]FDOPA) and [^{11}C]-5-hydroxytrytophan ([^{11}C]HTP) have been developed because of the capacity of neuroendocrine tumor cells to have a high accumulation of these tracers. Only a few clinical studies with these tracers have been performed and little is known about the precise mechanisms involved in the accumulation of these tracers.

Because of their slow growth and non-specific symptoms, neuroendocrine tumors are often already metastasized at diagnosis. Proper staging is important to make the right treatment decisions. PET tracers [^{11}C]HTP and [^{18}F]FDOPA have the potential to improve the yield of diagnostic imaging as they are precursors for respectively the serotonin and catecholamine pathways and can therefore be used to obtain knowledge on the biochemical behavior of neuroendocrine tumors. Following uptake of *L*-DOPA and 5-HTP by large amino transporters (LAT) into tumor cells they are decarboxylated by amino acid decarboxylase (AADC) to their corresponding amines. These amines are then stored into cellular vesicles via the vesicular monoamine transporter (VMAT). After release they are metabolized by the enzyme monoamine oxidase (MAO) to 5-hydroxyindole acetic acid, respectively homovanillic acid and subsequently excreted. Exploring the metabolic pathways with tracers visualizing these pathways could lead to a better understanding of biochemical mechanisms in neuroendocrine tumors. Furthermore the tumor image quality and the sensitivity of tumor detection might be improved by the use of these PET tracers.

PET allows visualization of metabolic pathways by incorporating a positron emitting radionuclide into a molecule taking part in biochemical and physiological processes. These radionuclides can be produced by irradiation of target material with highly accelerated protons or deuterons using a cyclotron. Commonly used radionuclides in PET imaging are carbon-11, nitrogen-13, oxygen-15 and fluorine-18 with relative short half-lives varying from 2 to 110 minutes. Radioactive decay takes place through emission of a positron. A positron has the same mass as an electron but carries the opposite charge. Radiochemical synthesis is used to incorporate the radionuclide into a molecule which is then defined as tracer. Important aspects for radiopharmaceutical preparations are: 1) radiochemical yield, 2) reliability of the production, 3) reaction time and 4) choice of the radionuclide.

Following administration to a patient, the positron interacts after a short distance with an electron from tissue resulting in annihilation. During the annihilation the masses of the positron and electron are converted into energy according to Einstein's formula $E=mc^2$. The energy appears in the form of two photons with an energy of each 511 keV emitted under an angle of 180° from each other. The PET camera consists of a full ring of multiple detectors. Two detectors that are exactly opposite to each other will detect these photons. This allows coincidence detection of two simultaneously emitted photons within 10 nanoseconds. After injection of the radiopharmaceutical, the distribution can be followed in time in a part of the body (dynamic scan) or by moving the bed into several positions (static whole-body scan). PET shows a higher resolution than conventional gamma cameras. While human PET cameras currently have a resolution of 4-5 mm, so-called microPET cameras used for small animal imaging reach a resolution of 1-2 mm.

Aim and outline of this thesis

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

In **chapter 1**, a literature overview is presented on uptake mechanisms of tracers used in nuclear medicine to detect neuroendocrine tumors. Different detection methods and their diagnostic values for several subtypes of neuroendocrine tumors are reviewed.

In order to visualize the serotonin pathway the availability of [^{11}C]HTP was required. In **chapter 2** the enzymatic synthesis of *L*-[^{11}C]HTP on a Zymark robotic system is described. The aim was to optimize the synthesis of enantiomerically pure *L*-[^{11}C]HTP and to obtain radiochemical yields reliable for patient studies. The origin of used enzymes was analyzed for safer human use. For routine production the radiation exposure for the radiochemist had to be minimized. Thereafter [^{11}C]HTP was applied to study its diagnostic value in patients with neuroendocrine tumors and to get more insights in the biochemical behavior of neuroendocrine tumor cells.

In **chapter 3** *in vitro* and small animal studies were performed using the PET tracers [^{11}C]HTP and [^{18}F]FDOPA to analyze tracer metabolism and accumulation. Several inhibitors of LAT, AADC and MAO were administered to a human neuroendocrine tumor cell line to obtain knowledge on the biochemical behavior. The influence of the decarboxylase inhibitor carbidopa on tumor uptake was studied in an animal model using a microPET camera to get a better understanding of the *in vivo* metabolism.

The aim of the study described in **chapter 4** was to determine the diagnostic value of [^{18}F]FDOPA for imaging patients with carcinoid tumors. Fifty-three patients with a metastatic carcinoid underwent a [^{18}F]FDOPA PET scan. The diagnostic sensitivity was compared with combined SRS and CT. Biochemical parameters of the catecholamine and serotonin pathways were compared with the used imaging methods.

As no data are available with a head to head comparison of [^{11}C]HTP and [^{18}F]FDOPA PET imaging we performed the study described in **chapter 5**. The diagnostic value of [^{11}C]HTP and [^{18}F]FDOPA PET in patients with neuroendocrine tumors was evaluated. 24 patients with carcinoid tumors and 23 patients with islet cell tumors were studied with both PET tracers and CT and SRS scan.

Currently several PET tracers for imaging of the catecholamine pathway are available. The serotonin pathway can be visualized with [^{11}C]HTP only. Due to the short half-life of this

tracer, its use is restricted to centers with cyclotron facilities. Therefore we aimed to develop a tracer with a longer half-life and started a synthesis route for ^{18}F labeled tryptophan. First, the affinity of 5-fluorotryptophan to accumulate in a neuroendocrine cell line was investigated. The synthesis of a precursor towards an enzymatic route of [^{18}F]-5-fluorotryptophan and the development of an enzymatic synthesis route towards 5-fluorotryptophan using fluorodestannylation are described in **chapter 6**.
Finally, a summary of the studies performed in this thesis and future perspectives are given in English, Dutch and German.