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5-Fluoro-[β-^{11}C]-L-tryptophan is a functional analogue of 5-hydroxy-[β-^{11}C]-L-tryptophan in vitro but not in vivo

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ABSTRACT

Introduction: 5-Hydroxy-[β-^{11}C]-L-tryptophan ([^{11}C]HTP) is an established positron emission tomography (PET) imaging agent for neuroendocrine tumors (NETs). It has also been used for other clinical research purposes in neurology and diabetes. However, its widespread use is limited by the short physical half-life of the radionuclide and a difficult radiosynthesis. Therefore, a Fluorine-18 labeled analogue, 5-[^{18}F]Fluoro-L-tryptophan ([^{18}F]FTRP), has been proposed as a functional analogue. There is no published method for the synthesis of L-[^{18}F]FTRP. We have therefore developed a synthesis of 5-fluoro-[β-^{11}C]-L-tryptophan ([^{11}C]FTRP), based on the existing chemo-enzymatic method for [^{11}C]HTP and evaluated the potential usefulness of radiolabeled FTRP as a substitute for [^{11}C]HTP.
Methods: The in vitro and in vivo behavior of [^{11}C]FTRP, including the dependence of key enzymes in the serotonergic metabolic pathway, was investigated in NET cell lines, NET xenograft carrying immunodeficient mice, normal rats and in non-human primate. [^{11}C]HTP was used for direct comparison.
Results: Uptake of [^{11}C]FTRP in NET cell lines in vitro was mediated by enzymes involved in serotonin synthesis and metabolism, similar to [^{11}C]HTP. In vivo biodistribution, either in rodent or non-human primate, was not affected by selectively inhibiting enzymatic steps in the serotonergic metabolic pathway.
Conclusion: [^{11}C]FTRP has in vitro biological function similar to that of [^{11}C]HTP. However, this function is not retained in vivo as shown by biodistribution and PET/CT studies. Radiolabeled FTRP is thus not likely to provide an advantage over [^{11}C]HTP in PET imaging in oncology, neurology or diabetes.

1. Introduction

5-Hydroxy-[β-^{11}C]-L-tryptophan ([^{11}C]HTP) was originally developed for studies of the decarboxylation step, mediated by Dopa Decarboxylase (DDC), of monoamine neurotransmitter biosynthesis in vivo by positron emission tomography (PET) [1,2]. Since then, HTP-PET has found extensive use in the diagnosis and localization of tumors originating from cell types expressing the APUD (Amine Precursor Uptake and Decarboxylation) mechanism. [^{11}C]HTP has been established as a universal imaging agent for neuroendocrine tumors (NETs) [3]. The serotonergic metabolic pathway, expressed in these tumors, contains many different enzymatic steps, from biosynthesis of serotonin through decarboxylation of 5-HTP, to translocation into secretory vesicles or degradation by monoamine oxidases (Fig. 1). More recently, [^{11}C]HTP has been proposed as an imaging biomarker for native pancreatic islets of Langerhans [4–6], as the serotonergic metabolic pathway including DDC is present in the endocrine pancreas [7].

The major obstacle limiting more widespread clinical utilization of [^{11}C]HTP is the difficult radiosynthesis which requires a technically demanding 7-step chemo-enzymatic synthesis pathway [8]. The availability if further complicated by the requirement for individual tracer batches for each HTP-PET examination due to the short half-life of carbon-11 (20.3 min). For these reasons [^{11}C]HTP is currently available at only a few PET sites worldwide while [^{18}F]L-DOPA is commonly used instead [9]. However, [^{18}F]L-DOPA has lower sensitivity for imaging of the monoamine decarboxylation mechanism in vivo and is not a functional analog of [^{11}C]HTP [10,11]. Instead 5-fluoro-[^{18}F]-L-tryptophan and 6-fluoro-[^{18}F]-L-tryptophan have been proposed to alleviate several of the challenges associated with production and use of [^{11}C]HTP for quantification of DDC activity [12,13]. Given the longer half-life of fluorine-18 (109.8 min), each batch would be sufficient for several patients in addition to generating the opportunity of shipping tracer to remote locations from PET facilities with advanced radiochemistry. In a previous study

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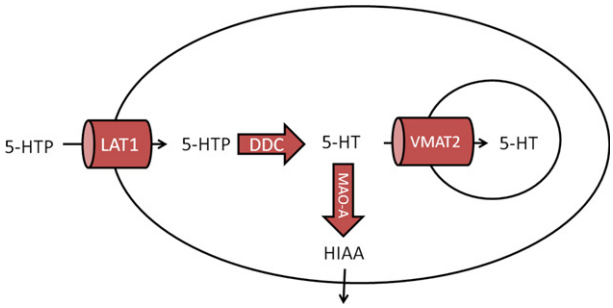


Fig. 1. Schematic depiction of the serotonergic metabolic pathway which is expressed in entirety or in part in tissues such as the central nervous system, in neuroendocrine tumors and in the endocrine pancreas. 5-HTP is transported into the cell by LAT1, and converted in 5-HT (serotonin) by DDC. The fate of 5-HT is either incorporation in secretory vesicles by VMAT2 for extracellular release, or degradation into HIAA by MAO-A.

by Atkins it was found that both 5-fluoro-[^{18}F]-L-tryptophan and 6-[^{18}F]fluoro-DL-tryptophan were promising as potential pancreas scanning agents [12].

There are some potential advantages with 5-fluoro-tryptophan compared to 6-fluoro-tryptophan (which is an irreversible inhibitor of tryptophan hydroxylase, in addition to DDC) in this study. Firstly we would like to avoid irreversible tracer kinetics since the biomathematical handling of data for such a tracer demands on simultaneous measurements of blood flow. A reversible tracer will not be influenced by differences in regional blood flow. Secondly, a crucial step for measurement of DDC activity is to avoid high uptake in tissues other than neuroendocrine. It could be speculated that the liver uptake would be higher with a substrate for tryptophan hydroxylase, such as 6-fluoro-tryptophan, which could cause problems in clinical detection of hepatic metastases of neuroendocrine tumors, in addition to causing specific ligand-receptor interactions other than between tracer and DDC. Hepatic phenylalanine hydroxylase catalyses hydroxylation of tryptophan, and potentially also 5-fluoro-tryptophan.

The synthesis of 5-[^{18}F]fluoro-L-tryptophan is associated with considerable radiochemical difficulties, and we therefore propose to label FTRP with carbon-11 at the β-position for initial preclinical evaluation. Bjurling et al. have earlier shown that the chemoenzymatic synthesis route to [^{11}C]HTP also can be used for the production of [^{11}C]FTRP by simply changing 5-hydroxyindole for 5-fluoroindole [14]. [^{11}C]FTRP is structurally congruent with the proposed [^{18}F]FTRP and thus the in vitro and in vivo evaluation of [^{11}C]FTRP will yield crucial insight in the biological function of FTRP in relation to the serotonergic metabolic pathway.

In this study we evaluated [^{11}C]FTRP in direct comparison with [^{11}C]HTP in cell binding assays and xenograft tumor models using several APUD cell lines, as well as the in vivo biodistribution in rats and non-human primate, targeting both neuroendocrine tumors and pancreatic islets of Langerhans.

2. Method and materials

2.1. Radiosynthesis

[^{11}C]HTP was synthesized as described previously and [^{11}C]FTRP was produced by the same method modified by exchanging 5-hydroxyindole for 5-fluoroindole as shown in Scheme 1 [8]. The tracers were purified by HPLC, filtered through a 0.2 μm sterile filter and delivered in 17 mM acetic acid/1 mM ascorbic acid.

2.2. In vitro cell binding

80,000–200,000 cells of the neuroendocrine cell lines INS-1, QGP1 or H720 were seeded into borosilicate tubes containing 1 ml 50 mM TRIS buffer (pH 7.4). [^{11}C]FTRP or [^{11}C]HTP was added to the tubes (corresponding to 13–114 nM [^{11}C]FTRP or 4–322 nM [^{11}C]HTP). To investigate the uptake dependence of key enzymes in the serotonergic metabolic pathway (see Fig. 1), cells were co-incubated with non-radioactive 5-hydroxy-L-tryptophan (5-HTP; Sigma-Aldrich, St Louis, MO, USA), 5-[^{18}F]Fluoro-L-tryptophan (FTRP; Sigma-Aldrich, St Louis, MO, USA), 2-Amino-2-norbornanecarboxylic acid (BCH; inhibitor of LAT-1; Sigma, St Louis, MO, USA), carbidopa (inhibitor of DDC; Apoteket AB, Stockholm, Sweden), harmine (HAR; inhibitor of MAO-A; Sigma, St Louis, MO, USA) or tetrabenazine (TBZ; inhibitor of VMAT2; BioTrend, Cologne, Germany). The concentrations and intended effect of these compounds on cellular metabolism are outlined in Table 1.

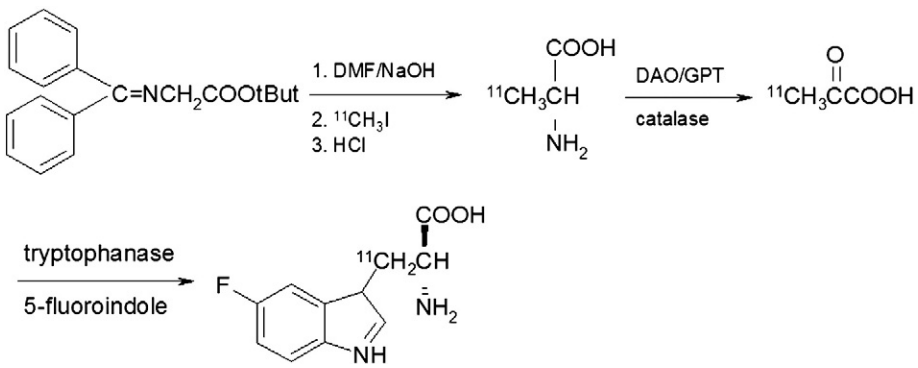
All cell suspensions were incubated at 37 °C for 30 min and then moved onto a 1.2 μm Whatman filter (Brandel, Gaithersburg, MD, USA) by a C-48 cell harvester (Brandel, Gaithersburg, MD, USA). The filter components associated to each incubation tube were measured in a well-counter (GE Healthcare, Uppsala, Sweden). Cell incubation tubes, references and filter binding controls were prepared in triplicates.

2.3. Ex vivo organ distribution

Sprague Dawley rats (n = 33, 373 ± 10 g) were kept under standard laboratory condition with unlimited access to chow and water.The rodent experiments were approved by the local Ethics Committee for Animal Research (C49/10, C242/11) and performed in accordance with local institutional and Swedish national rules and regulations.

2.3.1. Baseline studies

The animals were administered 4.7 ± 0.8 MBq [^{11}C]FTRP or 14.4 ± 4.0 MBq [^{11}C]HTP intravenously through the tail vein under



Scheme 1. Synthesis of [^{11}C]FTRP.