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[^{18}F]FMeNER-D2: Reliable fully-automated synthesis for visualization of the norepinephrine transporter

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ABSTRACT

Purpose: In neurodegenerative diseases and neuropsychiatric disorders dysregulation of the norepinephrine transporter (NET) has been reported. For visualization of NET availability and occupancy in the human brain PET imaging can be used. Therefore, selective NET-PET tracers with high affinity are required. Amongst these, [^{18}F]FMeNER-D2 is showing the best results so far. Furthermore, a reliable fully automated radiosynthesis is a prerequisite for successful application of PET-tracers.
The aim of this work was the automation of [^{18}F]FMeNER-D2 radiolabelling for subsequent clinical use. The presented study comprises 25 automated large-scale syntheses, which were directly applied to healthy volunteers and adult patients suffering from attention deficit hyperactivity disorder (ADHD). Procedures: Synthesis of [^{18}F]FMeNER-D2 was automated within a Nuclear Interface Module. Starting from 20–30 GBq [^{18}F]fluoride, azeotropic drying, reaction with Br_2CD_2, distillation of 1-bromo-2-[^{18}F]fluoromethane-D2 ([^{18}F]BFM) and reaction of the pure [^{18}F]BFM with unprotected precursor NER were optimized and completely automated. HPLC purification and SPE procedure were completed, formulation and sterile filtration were achieved on-line and full quality control was performed.
Results: Purified product was obtained in a fully automated synthesis in clinical scale allowing maximum radiation safety and routine production under GMP-like manner. So far, more than 25 fully automated syntheses were successfully performed, yielding 1.0–2.5 GBq of formulated [^{18}F]FMeNER-D2 with specific activities between 430 and 1707 GBq/μmol within 95 min total preparation time.
Conclusions: A first fully automated [^{18}F]FMeNER-D2 synthesis was established, allowing routine production of this NET-PET tracer under maximum radiation safety and standardization.

1. Introduction

The norepinephrine transporter (NET) is one of the major targets in neuropsychiatric and neurodegenerative diseases like attention deficit hyperactivity disorder (ADHD), depression, Alzheimer's disease (AD), Parkinson's disease (PD) and substance abuse [1]. For treatment of these diseases, selective norepinephrine (NE) reuptake inhibitors (SNRI) are commonly used, which are typically based on reboxetine. The NET itself facilitates the synaptic reuptake of NE from the synaptic cleft at the presynaptic terminals. Blocking of this transporter prolongs the NE action in the synapse, due to an increase in the concentration of NE in the cleft. Perturbation of the noradrenergic system (and the NET-expression) has been reported to trigger many neuropsychiatric disorders and neurodegenerative diseases. Especially, in the locus coeruleus (LC) a reduction of NET levels has been shown in major depression, AD and PD [1–3]. Furthermore, also in ADHD a dysregulation of the NE system was reported [2].

For gaining insight in NET availability and dynamics in both healthy and diseased human brains, a non-invasive molecular imaging protocol has been developed using positron emission tomography (PET). Thus, specific and selective NET-PET radioligands are needed. One of the major prerequisites on these candidate tracers is their affinity towards NET, especially when considering the very low density of NET in cerebellum, striatum and human insular cortex [3–8]. Visualization of NET-rich regions like LC, where NET density is 4–8-fold higher, can be also achieved using ligands with slightly lower affinity [4,7,9]. Besides, affinity of the candidate ligands correlates with their binding kinetics, i.e. longer equilibration times for high-affinity substances (e.g. [^{125}I]iodo-nisoxetine (Ki = 0.7 nM)

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reaches its binding equilibrium 3 h post injection) [10,11]. Moreover, selectivity of the tracers towards NET is required for feasible NET-PET imaging, since NET displays a high similarity to dopamine transporter (DAT) and serotonin transporter (SERT), but shows a significantly lower density in the human brain [5,12,13].

Most described NET-PET tracers used in clinical studies are derived from reboxetine as known NET-selective compound. Both ^{11}C-methylated and ^{18}F-fluoroethylated tracers were proposed [9,14–20]. Thereof, [^{18}F]FMeNER-D2 ((*S,S*)-2-(α-(2-[^{18}F]fluoro[$^{2}H_2$] methoxyphenoxy) benzyl)morpholine) is so far displaying best properties regarding affinity, selectivity and stability. [^{18}F]FMeNER-D2 was developed at the Karolinska Institute (Sweden) in 2004 by Schou and co-workers (Fig. 1) [21–23].

Major drawbacks of this NET-PET tracer were the complexity of the radiosynthesis and lack of an automated preparation sequence. Thus the aim of this work was the set-up of a reliable automated production of [^{18}F]FMeNER-D2 allowing for human NET-PET studies. With this computer-controlled fully-automated synthesis, more than 25 patients were injected with the so-produced [^{18}F]FMeNER-D2 at a dose of 4.7 MBq/kg body weight and, so far, subsequent NET PET scans were successfully acquired.

2. Materials and methods

2.1. Materials

Precursor (*S,S*)-NER (=(*S,S*)-norethyl-reboxetine, =(2*S*, 3*S*)-2-[α-(2-hydroxyphenoxy) benzyl]morpholine) and reference standard (*S,S*)-FMeNER-D2*TFA ((*S,S*)-2-[α-(2-(dideutero fluoromethoxy)phenoxy)benzyl]morpholine trifluoroacetate) were obtained from PharmaSynth AS (Tartu, Estonia).

Acetonitrile (ACN for synthesis of DNA, ≥99.9% (GC) and ACN HPLC grade), dimethylformamide (DMF, p.a, dried over molecular sieves (4 Å)), dibromomethane-d2 (99 atom% D, copper stabilized), sodium hydroxide, methanol (MeOH, CHROMASOLV®, for HPLC, ≥99.9%), ammonium formate, Kryptofix K2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) and ethanol (absolute) were purchased from Sigma-Aldrich (Vienna, Austria). Anion-exchange cartridges (PS-HCO_3) for [^{18}F]fluoride fixation were purchased from Macherey-Nagel (Dueren, Germany). Sterile water was purchased from Meditrade Medicare Medizinprodukte (Kufstein, Austria). Phosphate buffer (125 mM) was prepared by dissolving 0.224 g sodium dihydrogen phosphate-monohydrate and 1.935 g disodium hydrogen phosphate-dihydrate (both from Merck, Darmstadt, Germany) in 100 mL sterile water. For solid phase extraction C18 plus SepPak® cartridges and Silica plus long SepPak® cartridges were purchased from Waters (Waters® Associates Milford, USA). For formulation of the product 0.9% saline solution from B. Braun (Melsungen, Germany), 3% saline solution (Landesapotheke Salzburg, Austria) and 125 mM Phosphate buffer) were used. Low-protein binding Millex® GS 0.22 μm sterile filters were obtained from Millipore (Bedford, USA). All other chemicals and solvents for the syntheses and radiosyntheses were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich with at least analytical grade and used without further purification.

2.2. Instrumentation

[^{18}F]Fluoride was produced within a GE PET trace cyclotron via ^{18}O(p,n)^{18}F reaction (16.5-MeV protons; GE Medical Systems, Uppsala, Sweden). $H_2^{18}O$ (HYOX18; > 98%) was obtained from Rotem Europe (Leipzig, Germany).

Evaluation of reaction conditions was performed manually in a lead-shielded hood with small quantities of initial radioactivity (<1 GBq). After optimization, [^{18}F]FMeNER-D2 synthesis was automated within a Nuclear Interface synthesizer (GE Healthcare, Sweden), remotely controlled by a standard laptop with dedicated processing software (Fig. 3).

Purification of [^{18}F]FMeNER was performed by semi-preparative reversed phase HPLC using the built-in semi-preparative HPLC system equipped with a radioactivity-, a UV-detector (Linear Instruments Model 200 Detector UV/VIS) and a LaPrep HPLC pump (VWR International, Radnor, USA). A Phenomenex® Gemini, C-18 column with TMS end-capping, 10 μm, 250 × 10 mm (Phenomenex®, Aschaffenburg, Germany) and a mobile phase of MeOH/0.1 M ammonium formate (AMF) in water 50/50 v/v% at a flow rate of 12 mL/min was used for purification.

Analytical HPLC was performed on Merck-Hitachi LaChrom HPLC system (L-7100 pump; LaChrom L-7400 UV detector at 254 nm) and a NaI radio-detector (Bertholdt Technologies, Bad Wildbach, Germany) using Raytest software (Raytest, Straubenhardt, Germany). A Phenomenex Prodigy Phenyl-PH3 column; 250 × 4.6 mm, 5 μm (Phenomenex®, Aschaffenburg, Germany) and a mobile phase consisting of ACN/0.1 M AMF in water 50/50 %v/v at a flow rate of 2 mL/min was used. The osmolality was measured with a Wescor osmometer Vapro® 5600 (Sanova Medical Systems, Vienna, Austria) and pH was measured using a WTW inoLab 740 pH meter (WTW, Weilheim, Germany). GC-Analysis was performed with a Bruker Gas Chromatography System 430-GC. Radio-TLC Analysis was performed using silica gel 60 RP-18 $F_{254}S$ plates from Merck (Darmstadt, Germany) with a mobile phase consisting of ACN/water 70%/30% v/v. Analyses of radio-TLC plates were done using a Canberra-Packard Instant Imager (Perkin Elmer, Watford, UK).

PET scans were performed in the Department of Nuclear Medicine, Medical University Vienna, using a GE Advance PET-scanner and kinetic model was done with PMOD 3.0 using a two compartment model.

3. Methods

3.1. Preparation of 1-bromo-2-[^{18}F]fluoromethane-d2 ([^{18}F]BFM)

In Fig. 2 the reaction scheme for the synthesis of [^{18}F]BFM and [^{18}F] FMeNER-D2 is outlined.

Fig. 1. Structures of NER and FMeNER-D2.