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[^{18}F]FMeNER-D2: A systematic in vitro analysis of radio-metabolism☆

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ABSTRACT

Introduction: The norepinephrine transporter (NET) presents an important target for therapy and diagnosis of ADHD and other neurodegenerative and psychiatric diseases. Thus, PET is the diagnostic method of choice, using radiolabeled NET-ligands derived from reboxetine. So far, [^{18}F]FMeNER-D2 showed best pharmacokinetic and -dynamic properties. However, the disadvantage of reboxetine derived PET tracers is their high metabolic cleavage—resulting in impeding signals in the PET scans, which hamper a proper quantification of the NET in cortical areas.
Methods: Metabolic stability testing was performed in vitro using a plethora of human and murine enzymes.
Results: No metabolism was observed using monoamine oxidase A and B or catechol-*O*-methyl transferase. Incubation of [^{18}F]FMeNER-D2 with CYP450-enzymes, predominantly located in the liver, led to a significant and fast metabolism of the tracer. Moreover, the arising three radiometabolites were found to be more polar than [^{18}F]FMeNER-D2. Surprisingly, definitely no formation of free [^{18}F]fluoride was observed.
Conclusion: According to our in vitro data, the interfering uptake in cortical regions might be attributed to these emerging radiometabolites but does not reflect bonding in bone due to defluorination. Further research on these radiometabolites is necessary to elucidate the in vivo situation. This might include an analysis of human blood samples after injection of [^{18}F]FMeNER-D2, to enable a better correction of the PET-input function.
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1. Introduction

Given the intense influence of the NET in a multitude of psychiatric and neurodegenerative diseases as well as cardiovascular maladies, the importance of non-invasive NET imaging is evident. Therefore, positron emission tomography (PET) as molecular imaging technique presents an excellent method for the in vivo visualization of the underlying pathophysiology on a molecular level. In clinical NET imaging, so far, only radiolabeled reboxetine derivatives have been used [1–8]. Among them, [^{18}F]FMeNER-D2 revealed best properties (i.e. affinity and selectivity, half-life, binding kinetics). However, there are distinct shortcomings regarding its in vivo and in vitro stability. Moreover, present understanding of the metabolic fate of [^{18}F]FMeNER-D2 is limited, but uptake of its metabolite(s) is severely interfering with the interpretation of the NET-PET signal in areas adjacent to the skull (Fig. 1) [9]. This undesired uptake specifically impedes a quantification of NET binding potential (BP) in pre-frontal cortex (PFC), an area highly involved in behavioral and attention control and hypothesized to be altered in attention deficiency hyperactivity disorder (ADHD). Recently, a PET study with an ADHD population was published referring to this problem [9].

Unfortunately, the arising radio-metabolite(s) is(are) hitherto unidentified, but hypothesized to be [^{18}F]fluoride – and thus resulting in bone uptake – hampering quantification of NET BP in cortical regions. Therefore, current research is concomitantly focusing on structural moieties apart from reboxetine as NET PET tracers, including benzoimidazolone and benzothiazole dioxide based NET PET radioligands [10–13]. Among them, [^{11}C]Me@APPI and [^{11}C]Me@HAPTHI are showing promising results [10–13], however they were being used only in in vitro studies so far. Thus, [^{18}F]FMeNER-D2 still presents the radiotracer of choice for clinical use.

To gain insight in the enzymatic mechanisms of [^{18}F]FMeNER-D2 degradation, the aim was to systematically evaluate the metabolic cleavage of the aryloxy morpholine based NET PET tracer. Therefore, incubation of [^{18}F]FMeNER-D2 was performed with various single enzymes and multienzyme complexes and the binding behavior of the parent compound as well as the formed radiometabolites to inorganic bone mineral matrix (hydroxyapatite (HA)) was examined and compared to [^{18}F]FDG and [^{18}F]fluoride. Future understanding of involved

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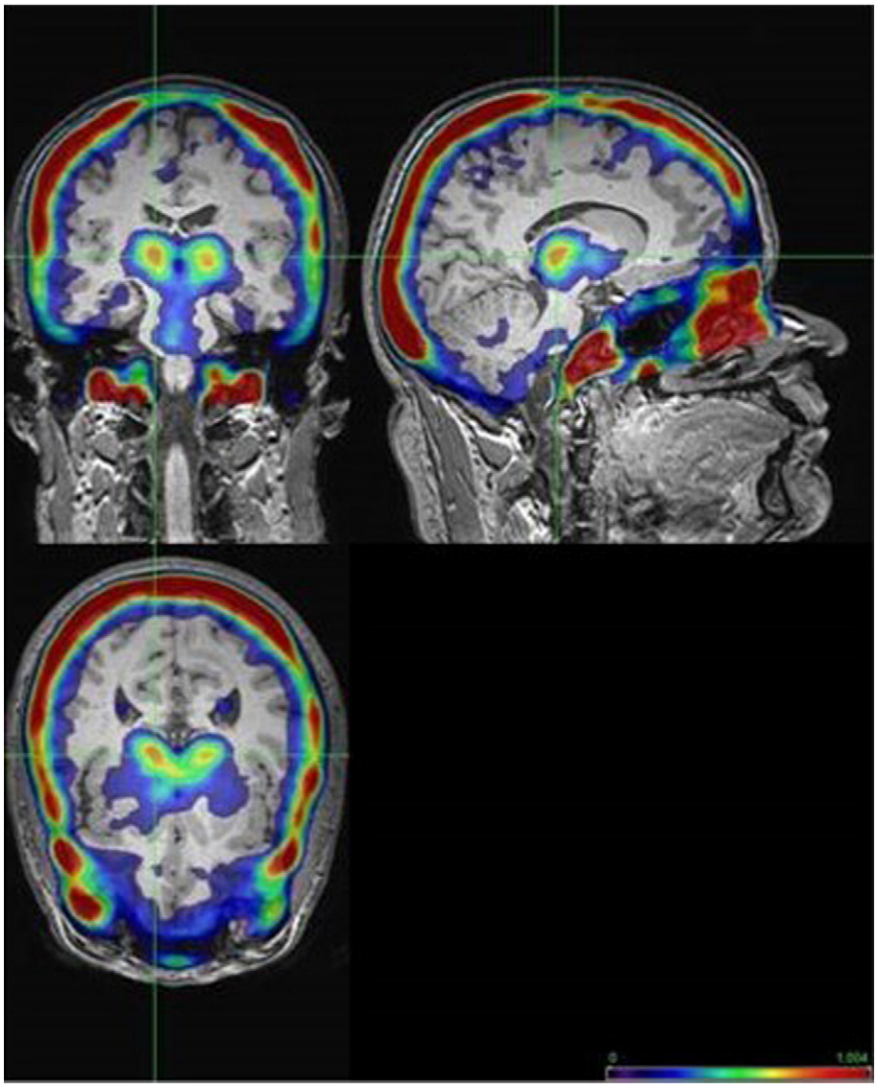

Fig. 1. Human PET-scan of [^{18}F]FMeNER-D2 in a healthy volunteer [9].

enzymatic reactions and arising radiometabolites might enable a correction of the PET input function.

2. Materials and methods

2.1. Materials

For each batch of enzymatic experiments, the radioactive NET-PET tracer [^{18}F]FMeNER-D2 was prepared freshly according to our previously published procedure [3]. Briefly, starting from 20 to 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 performed in Nuclear Interface synthesizer (GE Healthcare, Germany). Radiochemical yields were 1.0–2.5 GBq of formulated [^{18}F]FMeNER-D2 with specific activities between 430 and 1707 GBq/μmol. Radiochemical and chemical purity always exceeded 98%.

Precursor (*S*,*S*)-NER (=(*S*,*S*)-norethyl-reboxetine, =(2*S*, α*S*)-2-[α-(2-hydroxyphenoxy) benzyl]morpholine) and reference standard (*S*,*S*)-FMeNER-D2*TFA ((*S*,*S*)-2-[a-(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)), sodium hydroxide, methanol (MeOH, CHROMASOLV®, for HPLC, ≥99.9%), ammonium formate, hydroxyapatite (reagent grade) and ethanol (absolute) were purchased from Sigma-Aldrich (Vienna, Austria). Sterile water was obtained from a MilliQ® water purification system (Merck Millipore, Germany). Phosphate buffer (125 mM) was prepared by dissolving 0.224 g sodium dihydrogenphosphate monohydrate and 1.935 g disodiumhydrogen phosphate dihydrate (both from Merck, Darmstadt, Germany) in 100 mL sterile water. All other chemicals and solvents were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich (Vienna, Austria) with at least analytical grade and used without further purification.

Microsomal preparations (20 mg/mL in sucrose, human/rat liver microsomes) for stability testing were obtained from BD Bioscience (NJ, USA). Pooled human and rat plasma (Li Heparin) was obtained from Innovative Research (MI, USA). Cytochrom P 450 (CYP) single enzymes, monoamine oxidase A and B as well as porcine catechol-*O*-methyl transferase were purchased from Sigma Aldrich (Vienna, Austria). The NADPH-regenerating system, consisting of solution-A ($NADP^+$, Glucose-6-phosphate and magnesium-chloride in H_2O) and solution-B (Glucose-6-phosphate dehydrogenase in sodium citrate) was obtained from Corning® Gentest™ (NY, USA).

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