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Improving metabolic stability of fluorine-18 labeled verapamil analogs

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ABSTRACT

Introduction: Fluorine-18 labeled positron emission tomography (PET) tracers were developed to obtain more insight into the function of P-glycoprotein (P-gp) in relation to various conditions. They allow research in facilities without a cyclotron as they can be transported with a half-life of 110 min. As the metabolic stability of previously reported tracers [^{18}F]**1** and [^{18}F]**2** was poor, the purpose of this study was to improve this stability using deuterium substitution, creating verapamil analogs [^{18}F]**1-*d*$_4$**, [^{18}F]**2-*d*$_4$**, [^{18}F]**3-*d*$_3$** and [^{18}F]**3-*d*$_7$**.

Methods: The following deuterium containing tracers were synthesized and evaluated in mice and rats: [^{18}F]**1-*d*$_4$**, [^{18}F]**2-*d*$_4$**, [^{18}F]**3-*d*$_3$** and [^{18}F]**3-*d*$_7$**.

Results: The deuterated analogs [^{18}F]**2-*d*$_4$**, [^{18}F]**3-*d*$_3$** and [^{18}F]**3-*d*$_7$** showed increased metabolic stability compared with their non-deuterated counterparts. The increased metabolic stability of the methyl containing analogs [^{18}F]**3-*d*$_3$** and [^{18}F]**3-*d*$_7$** might be caused by steric hindrance for enzymes.

Conclusion: The striking similar *in vivo* behavior of [^{18}F]**3-*d*$_7$** to that of (*R*)-[^{11}C]verapamil, and its improved metabolic stability compared with the other fluorine-18 labeled tracers synthesized, supports the potential clinical translation of [^{18}F]**3-*d*$_7$** as a PET radiopharmaceutical for P-gp evaluation.

1. Introduction

P-glycoprotein (P-gp) is an ATP dependent efflux transporter, which is i.a. located on the luminal side of the blood-brain barrier [1]. As such, it mediates the transport of structurally diverse compounds from brain to blood, thereby protecting the brain from xenobiotics. P-gp is the most studied ATP-binding cassette (ABC) transporter and it is linked to various neurodegenerative diseases. It has been shown that P-gp function is diminished in Alzheimer's disease, which may accelerate the disease process, as it is associated with decreased clearance of β-amyloid from the brain [2]. On the other hand, several studies have shown increased P-gp function in epilepsy patients, associated with resistance to anti-epileptic drugs [3]. To obtain more insight into the function of P-gp in relation to these and other conditions, positron emission tomography (PET) can be used to investigate the function of P-gp *in vivo* using substrates labeled with positron emitters [4]. (*R*)-[^{11}C]verapamil is a commonly used PET agent for P-gp research, although limited by its relatively short half-life of 20 min. Originally, verapamil was developed and used as a calcium channel blocker [5], but it is also a substrate of P-gp.

Recently, two fluorine-18 labeled positron emission tomography (PET) tracers were developed [6] ([^{18}F]**1** and [^{18}F]**2**, Fig. 1) to image the function of P-gp in the brain, based on the chemical structure of verapamil. Clearly, these tracers could be useful in clinical studies of Alzheimer's disease or epilepsy, where alterations in P-gp function could be detected in a PET scan by increased or decreased brain uptake. Despite their high specificity for P-gp, a disadvantage of these tracers was their poor metabolic stability, as this may compromise quantification, decrease the signal-to-noise ratio and complicate interpretation.

The metabolic pathway of verapamil has been studied in detail [7,8]. Different metabolites were identified and the most important initial metabolites were D-617, norverapamil and D-703. The metabolites and corresponding enzymes are depicted in Fig. 2 [9, 10]. Previous PET studies have shown the formation of corresponding radiolabeled metabolites of (*R*)-[^{11}C]verapamil *in vivo* [11].

It is known that *N*-demethylation of verapamil by cytochrome P450 enzyme, yielding the metabolite norverapamil occurs via the hydrogen atom transfer (HAT) mechanism [12]. Within this reaction, first a hydrogen atom (H) is abstracted creating a radical carbon atom. Next, an alcohol is formed which is cleaved of to form formaldehyde and a secondary amine. Deuterium substitution of the methyl group could be used to slow down this reaction. Cleavage of the covalent bond of carbon (C) with deuterium (D) requires greater energy than cleavage of the bond with hydrogen, due to the higher mass of deuterium,

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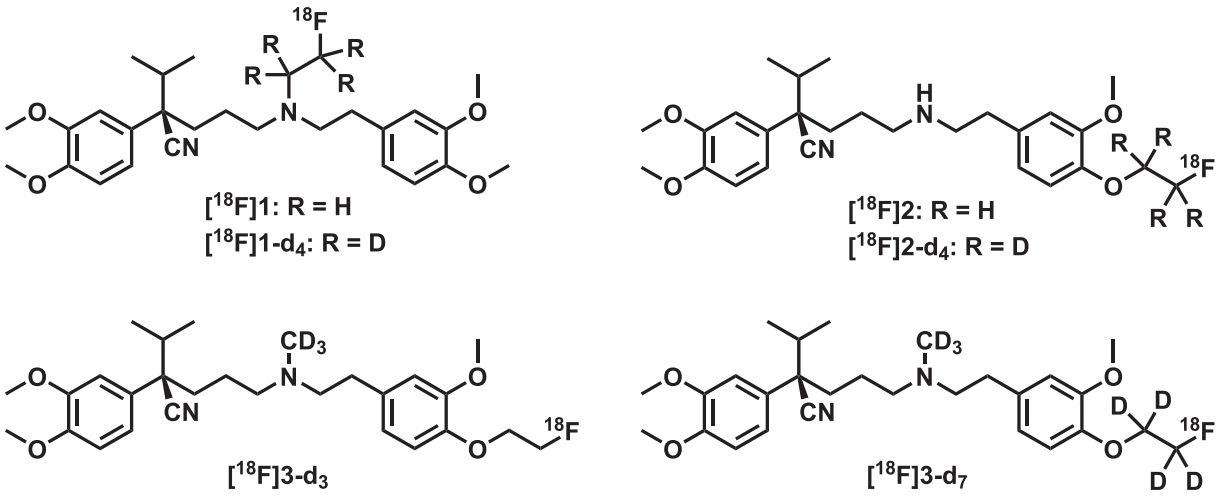

Fig. 1. Chemical structures of deuterated (nor-)verapamil analogs, with measured Log D values.

compared with hydrogen. C—D bonds have a lower vibrational frequency and, thus, lower zero-point energy than an analogous C—H bond. This results in higher activation energy and slower rate for C—D bond cleavage. This rate effect is referred to as the primary deuterium isotope effect [13–15].

The deuterium substitution approach has been used on a number of occasions to fine-tune properties of new pharmaceuticals, primarily related to metabolic stability. The first approval of a deuterium containing drug was provided by the FDA for deutetrabenazine, issued 3rd of April 2017 [16,17]. In addition, in developing new PET tracers, deuterium has occasionally been used to alter properties. The first and most well-known deuterated PET tracer is [^{11}C]L-deprenyl-D_2, which showed slower binding to its target MAO B than the original hydrogen compound resulting in a reduced rate of trapping in (brain) tissue and to improve sensitivity [18]. Multiple deuterated analogs of [^{11}C]- and [^{18}F]-choline showed improved protection against choline oxidation [19,20]. Another example is [^{18}F]deuteroaltanserin, which showed 29% higher ratios of parent tracer to radiometabolites in plasma, compared with [^{18}F]altanserin [21].

In this study, four deuterium substituted analogs were synthesized and evaluated for metabolic stability and *in vivo* behavior. The purpose of this work is to develop a stable fluorine-18 PET tracer for P-gp evaluation, to gain more insight in the metabolic pathways and to investigate which groups are more prone to metabolism.

2. Materials & methods

2.1. General

Chemicals and solvents were purchased from commercial sources Sigma-Aldrich (Zwijndrecht, the Netherlands), Fluorochem (Hadfield Derbyshire, UK), ABCr GmbH (Karlsruhe, Germany) and Biosolve (Valkenswaard, the Netherlands) without further purification unless stated otherwise. Deuterated starting materials ethylene-d_4 glycol, 2-bromoethanol-1,1,2,2-d_4 and Iodomethane-d_3 had an isotopic purity of 98, 98 and ≥99.5 atom % D, respectively. (*R*)-desmethyl-verapamil was kindly donated by Abbott Laboratories (Lake Bluff, IL, USA). Dichloromethane (DCM), 1,2-dichloroethane (DCE), methanol (MeOH) and dimethylformamide (DMF) were dried over 3 Å molecular sieves, for at least 24 h prior to use. Tetrahydrofuran (THF) was first distilled from LiAlH4 and then dried over 3 Å molecular sieves. Thin layer chromatography (TLC) was performed on Merck (Darmstadt, Germany) precoated silica gel 60 F254 plates. Spots were visualized by UV quenching or ninhydrin. Column chromatography was carried out either manually by using silica gel 60 Å (Sigma-Aldrich) or on a Buchi (Flawil, Switzerland) sepacore system (comprising of a C-620 control unit, a C-660 fraction collector, 2 C-601 gradient pumps and a C-640 UV detector) equipped with Buchi sepacore prepacked flash columns. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded

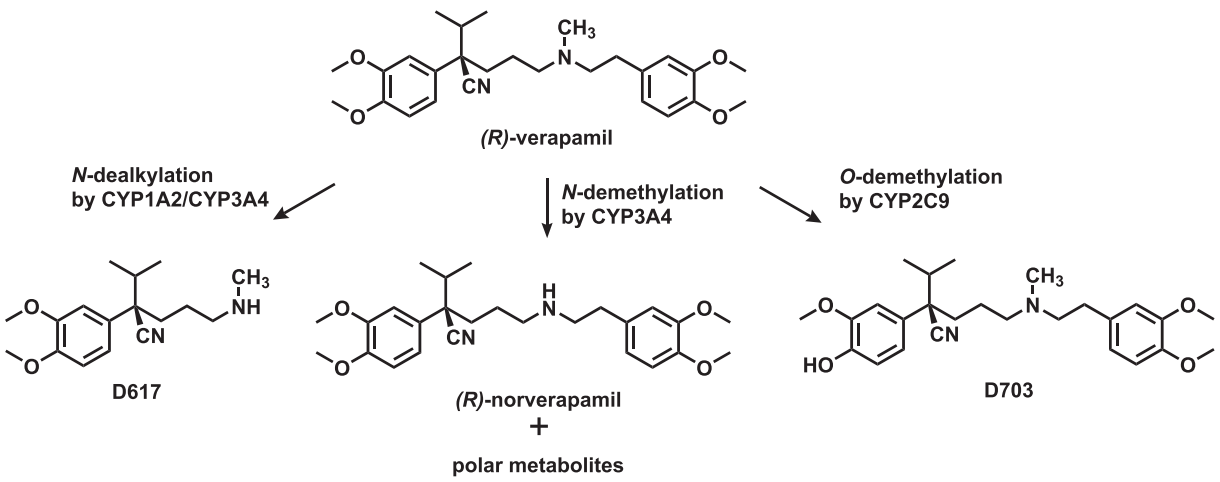

Fig. 2. Metabolic pathway of (*R*) [^{11}C]verapamil as adapted from Luurtsema et al. [11].