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Synthesis and in vivo evaluation of the putative breast cancer resistance protein inhibitor [^{11}C]methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)amino-carbonyl)-2-(quinoline-2-carbonylamino)benzoate

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Abstract

Introduction: The multidrug efflux transporter breast cancer resistance protein (BCRP) is highly expressed in the blood-brain barrier (BBB), where it limits brain entry of a broad range of endogenous and exogenous substrates. Methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl)amino-carbonyl)-2-(quinoline-2-carbonylamino)benzoate (**1**) is a recently discovered BCRP-selective inhibitor, which is structurally derived from the potent P-glycoprotein (P-gp) inhibitor tariquidar. The aim of this study was to develop a new PET tracer based on **1** to map BCRP expression levels in vivo.
Methods: Compound **1** was labelled with ^{11}C in its methyl ester function by reaction of the corresponding carboxylic acid **2** with [^{11}C] methyl triflate. Positron emission tomography (PET) imaging of [^{11}C]-**1** was performed in wild-type, *Mdr1a/b*$^{(-/-)}$, *Bcrp1*$^{(-/-)}$ and *Mdr1a/b*$^{(-/-)}$*Bcrp1*$^{(-/-)}$ mice (n=3 per mouse type) and radiotracer metabolism was assessed in plasma and brain.
Results: Brain-to-plasma ratios of unchanged [^{11}C]-**1** were 4.8- and 10.3-fold higher in *Mdr1a/b*$^{(-/-)}$ and in *Mdr1a/b*$^{(-/-)}$*Bcrp1*$^{(-/-)}$ mice, respectively, as compared to wild-type animals, but only modestly increased in *Bcrp1*$^{(-/-)}$ mice. [^{11}C]-**1** was rapidly metabolized in vivo giving rise to a polar radiometabolite which was taken up into brain tissue.
Conclusion: Our data suggest that [^{11}C]-**1** preferably interacts with P-gp rather than BCRP at the murine BBB which questions its reported in vitro BCRP selectivity. Consequently, [^{11}C]-**1** appears to be unsuitable as a PET tracer to map cerebral BCRP expression.

Keywords: Breast cancer resistance protein; P-glycoprotein; Blood-brain barrier; PET; Inhibitor; Tariquidar

1. Introduction

The adenosine triphosphate-binding cassette (ABC) transporter breast cancer resistance protein (BCRP, ABCG2) can actively efflux a broad range of endogenous and exogenous substrates across biological membranes [1]. BCRP limits oral bioavailability and mediates renal and hepatobiliary excretion of its substrates, and thereby influences the pharmacokinetics of several drugs. In

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addition, BCRP can confer multidrug resistance to tumor cells. Recent work, relying mainly on the use of transporter knockout mice, has revealed important contributions of BCRP to the blood-brain, blood-testis and blood-fetal barriers [1,2].

In the blood-brain barrier (BBB), BCRP colocalizes with P-glycoprotein (P-gp, ABCB1) at the luminal side of endothelial cells of brain capillaries. Interestingly, whereas expression of P-gp is higher than that of BCRP in the murine BBB [3], the opposite seems to be true in humans. Recent data show that mRNA levels of BCRP are about eightfold higher than P-gp mRNA levels in human brain capillaries [4]. However, as BCRP has a substantial overlap in substrate specificity with P-gp [5], the functional role of BCRP at the BBB has remained elusive, despite the availability of BCRP-deficient mice [2].

A powerful strategy to studying function and expression of ABC transporters in vivo is positron emission tomography (PET) together with radiolabelled transporter substrates or inhibitors [6]. We and others have successfully applied this concept to imaging cerebral P-gp by using radiolabelled P-gp substrates, such as (*R*)-[^{11}C]verapamil [7,8] or [^{11}C]-*N*-desmethyl-loperamide [9], and P-gp inhibitors, such as [^{11}C] laniquidar [10], [^{11}C]elacridar [11] or [^{11}C]tariquidar [12]. For translating this promising concept to the visualization of BCRP, the availability of PET probes with high selectivity for BCRP over P-gp is crucial. However, because of the recent discovery of the BCRP transporter, only a few selective BCRP inhibitors have been reported so far. Fumitremorgin C, a diketopiperazine, isolated from *Aspergillus fumigatus*, was reported first, but cannot be used in vivo due to its neurotoxicity [13]. The most potent BCRP inhibitor known to date is the fumitremorgin C analogue Ko143 [14]. The potent third-generation P-gp inhibitor tariquidar (Fig. 1) [15] has been shown to also inhibit BCRP, but at higher concentrations than those at which it inhibits P-gp [16]. It has recently been discovered that structural modifications at the benzamide core of tariquidar result in potent and selective BCRP inhibitors [17]. Out of a series of 10 tariquidar-like compounds, methyl 4-((4-(2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl)phenyl) amino-carbonyl)-2-(quinoline-2-carbonylamino)benzoate (**1**, Fig. 1) was identified as a potent BCRP inhibitor, which inhibits BCRP-mediated transport of mitoxantrone in topotecan-resistant MCF-7 breast cancer cells with a half-maximum inhibitory concentration (IC_{50}) of 60 nM and displays approximately 500-fold selectivity for inhibition of BCRP over P-gp [17].

The aim of this work was the development of a new PET tracer based on **1** to study BCRP expression levels in vivo. Here, we report on the precursor synthesis and ^{11}C-labelling of **1**. Moreover, a first small-animal PET evaluation of [^{11}C]-**1** was performed in wild-type and transporter knockout mice to assess the interaction of [^{11}C]-**1** with BCRP and P-gp at the BBB.

2. Materials and methods

2.1. General

All chemicals were purchased from Sigma-Aldrich Chemie (Schnelldorf, Germany), Merck (Darmstadt, Germany) and Apollo Scientific (Bredbury, UK) at analytical grade and used without further purification. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Advance DPx200 (200 and 50 MHz). Chemical shifts are reported in δ units (ppm) relative to the Me_4Si line as internal standard (s, d, t, m and Cq for singlet, doublet, triplet, multiplet and quaternary carbon, respectively) and *J* values are reported in Hertz. Elemental analysis was performed on a Perkin Elmer 2400 CHN Elemental Analyzer. [^{11}C]CH_4 was produced via the ^{14}N(p,α)^{11}C nuclear reaction by irradiating nitrogen gas containing 10% hydrogen using a PETtrace cyclotron equipped with a CH_4 target system (GE Healthcare, Uppsala, Sweden). [^{11}C]CH_3I was prepared via the gas-phase method [18] in a TracerLab FXC Pro synthesis module (GE Healthcare) and converted into [^{11}C]methyl triflate by passage through a column containing silver-triflate impregnated graphitized carbon [19].

2.2. Animals

Female FVB (wild-type), *Mdr1a/b*$^{(-/-)}$, *Bcrp1*$^{(-/-)}$ and *Mdr1a/b*$^{(-/-)}$*Bcrp1*$^{(-/-)}$ (triple knockout) mice weighing 25–30 g were purchased from Taconic (Germantown, NY, USA). The study was approved by the local Animal Welfare

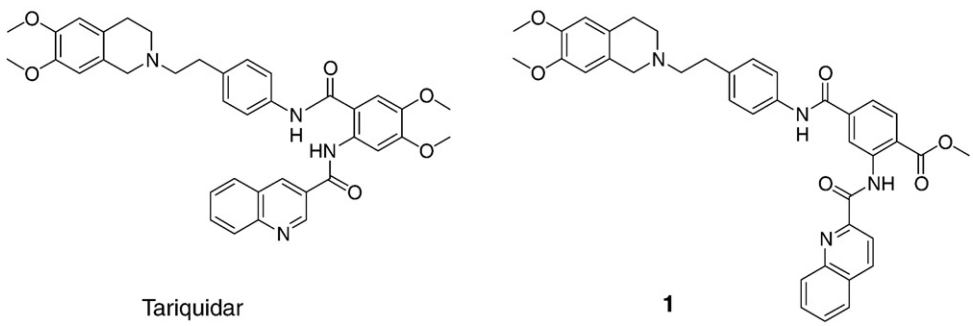

Fig. 1. Chemical structures of tariquidar and **1**.