Nuclear Medicine and Biology 42 (2015) 585–589

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

[^{18}F]FDG is not transported by P-glycoprotein and breast cancer resistance protein at the rodent blood–brain barrier☆

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ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 9 February 2015
Accepted 11 March 2015

Keywords:
Positron emission tomography
2-[^{18}F]fluoro-2-deoxy-D-glucose
Blood-brain barrier
P-glycoprotein
Breast cancer resistance protein
Tariquidar

ABSTRACT

Introduction: Transport of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) by the multidrug efflux transporters P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) at the blood–brain barrier (BBB) may confound the interpretation of [^{18}F]FDG brain PET data. Aim of this study was to assess the influence of ABCB1 and ABCG2 at the BBB on brain distribution of [^{18}F]FDG *in vivo* by performing [^{18}F]FDG PET scans in wild-type and transporter knockout mice and by evaluating changes in [^{18}F]FDG brain distribution after transporter inhibition.
Methods: Dynamic small-animal PET experiments (60 min) were performed with [^{18}F]FDG in groups of wild-type and transporter knockout mice (*Abcb1a/b*$^{(-/-)}$, *Abcg2*$^{(-/-)}$ and *Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$) and in wild-type rats without and with i.v. pretreatment with the known ABCB1 inhibitor tariquidar (15 mg/kg, given at 2 h before PET). Blood was sampled from animals from the orbital sinus vein at the end of the PET scans and measured in a gamma counter. Brain uptake of [^{18}F]FDG was expressed as the brain-to-blood radioactivity concentration ratio in the last PET time frame ($K_{b,brain}$).
Results: $K_{b,brain}$ values of [^{18}F]FDG were not significantly different between different mouse types both without and with tariquidar pretreatment. The blood-to-brain transfer rate constant of [^{18}F]FDG was significantly lower in tariquidar-treated as compared with vehicle-treated rats (0.350 ± 0.025 mL/min/g *versus* 0.416 ± 0.024 mL/min/g, $p = 0.026$, paired t-test) but $K_{b,brain}$ values were not significantly different between both rat groups.
Conclusion: Our results show that [^{18}F]FDG is not transported by Abcb1 at the mouse and rat BBB *in vivo*. In addition we found no evidence for Abcg2 transport of [^{18}F]FDG at the mouse BBB.
Advances in knowledge and implications for patient care: Our findings imply that functional activity of ABCB1 and ABCG2 at the BBB does not need to be taken into account when interpreting brain [^{18}F]FDG PET data.

1. Introduction

Adenosine triphosphate (ATP)-binding cassette (ABC) transporters, such as P-glycoprotein (humans: ABCB1, rodents: Abcb1),[2] breast cancer resistance protein (humans: ABCG2, rodents: Abcg2) and multidrug resistance protein 4 (humans: ABCC4, rodents: Abcc4) are expressed at the luminal membrane of brain capillary endothelial cells forming the blood–brain barrier (BBB) where they control brain distribution of therapeutic drugs by actively effluxing them from brain back into the blood compartment [1]. ABCB1 and ABCG2 possess closely overlapping substrate specificities and have been shown to work together in keeping dual substrates out of the brain [2]. Apart from drugs, several radiotracers for positron emission tomography (PET) or single photon emission computed tomography (SPECT) are recognized by ABCB1 and ABCG2 as substrates and it has now become generally accepted that interaction with efflux transporters should be considered when developing new PET and SPECT tracers targeted to the brain [3]. Efflux transport by ABCB1 and ABCG2 may on one hand limit the brain distribution of radiotracers and thereby provide insufficiently high imaging signals in the brain. On the other hand, efflux transport may confound the interpretation of imaging data when studying diseases, in which cerebral efflux transporter function is altered as part of the pathophysiological process (e.g. epilepsy, Alzheimer's disease) [4]. Moreover, a regional heterogeneity of efflux transporter expression in the brain may invalidate the

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[2] In rodents there are two isoforms of the protein (Abcb1a and Abcb1b). Both isoforms are present in rodent brain, but only Abcb1a is expressed in brain capillaries, whereas Abcb1b is expressed in brain parenchyma.

http://dx.doi.org/10.1016/j.nucmedbio.2015.03.004
0969-8051/

application of reference tissue models for quantification of imaging data [5].

2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG), which is widely used for brain PET imaging, crosses the BBB by active uptake transport mediated by the glucose transporter 1 (GLUT1, SLC2A1). [^{18}F]FDG does not appear to fulfill the structural requirements for a substrate of ABCB1 and ABCG2. Nevertheless, previous studies reported that the accumulation of [^{18}F]FDG was lower in ABCB1-overexpressing than in non-ABCB1-expressing cancer cells and tumors [6–10]. Moreover inhibition of ABCB1 was shown to increase uptake of [^{18}F]FDG in ABCB1-overexpressing tumor cells, which suggested that [^{18}F]FDG may be a substrate of ABCB1 [9,10]. While a recent study found no evidence for ABCB1-mediated transport of [^{18}F]FDG *in vitro* [11], it has so far not been investigated if [^{18}F]FDG is transported by ABCB1 and ABCG2 at the BBB *in vivo*.

Aim of the present study was to investigate *in vivo* the interaction of [^{18}F]FDG with Abcb1 and Abcg2 at the rodent BBB by performing small-animal PET scans in wild-type and transporter knockout mice and in rats without and with pretreatment of animals with the third-generation ABCB1 inhibitor tariquidar [12]. By using this experimental approach we were able to demonstrate that [^{18}F]FDG is not transported by Abcb1 and Abcg2 at the mouse BBB and not by Abcb1 at the rat BBB.

2. Materials and methods

2.1. Chemicals

If not stated otherwise chemicals were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany) or Merck (Darmstadt, Germany) and were of analytical grade and used without further purification. Isoflurane was obtained from Baxter Vertriebs GmbH (Vienna, Austria). Tariquidar dimesylate was obtained from Xenova Ltd. (Slough, UK). For *in vivo* experiments, tariquidar was freshly dissolved prior to each administration in 2.5% (w/v) aqueous D-glucose solution. [^{18}F]FDG was obtained from Seibersdorf Labor GmbH (Seibersdorf, Austria) and diluted for intravenous (i.v.) injection with 0.9% (w/v) aqueous saline solution.

2.2. Animals

Adult female Sprague–Dawley rats (Harlan Nederland, Horst, Netherlands) and adult female *Abcb1a/b*$^{(-/-)}$, *Abcg2*$^{(-/-)}$, *Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ and wild-type mice with an FVB genetic background (Taconic, Germantown, NY, USA) were used for the experiments. The number of animals used as well as body weight, injected [^{18}F]FDG radioactivity, blood glucose and blood radioactivity concentrations at the end of the PET scan is summarized in Table 1. Mice were housed in Makrolon type 2 cages in groups of 5 animals/cage and rats were housed in Makrolon type 4 cages with 2–4 animals/cage. Animals were kept under controlled environmental conditions (22 ± 1 °C; 40–70% humidity; 12-h light/dark cycle) with free access to standard laboratory animal diet (ssniff R/M-H, sniff Spezialdiäten GmbH, Soest, Germany) and water. Before being used in the experiments, the animals were allowed to adapt to the new conditions for ≥1 week. The study was approved by the local animal welfare committee (Amt der Niederösterreichischen Landesregierung) and all study procedures were performed in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EEC). Every effort was made to minimize both the suffering and the number of animals used in this study. The description of the whole study set-up is in accordance with the recommendations given in literature [13].

2.3. Small-animal PET imaging

Mice from all strains were divided into two groups; a vehicle group and a treatment group. In the case of rats, the same animals underwent [^{18}F]FDG PET scans after administration of vehicle and tariquidar on separate days. Two hours before start of the PET scan, animals were anesthetized using isoflurane and pretreated by i.v. bolus injection over 1 min of 15 mg/kg tariquidar or vehicle (2.5% aqueous D-glucose solution) at a volume of 4 mL/kg (mice) or 2 mL/kg (rats). As animal handling has a profound effect on [^{18}F]FDG uptake [14,15], the handling conditions were standardized as follows. Mice were deprived of food for 6 ± 2 h and rats were fasting for 20 ± 2 h prior [^{18}F]FDG injection. Animals had access to drinking water at all times. Prior to PET imaging, animals were placed in an induction box standing on a heating pad which was kept at 38 °C and isoflurane anesthesia (2% isoflurane in oxygen, 0.5 L/min flow) was induced. Anesthesia and warming were maintained during tracer administration and continued throughout the PET measurement. The anesthetic gas mixture was humidified to prevent impairment of the airways. Thereafter, mice were positioned in a custom made dual-mouse imaging chamber and for the rat measurements a dedicated rat holder equipped with earplugs and tooth bar (T8328 animal cradle from Bruker BioSpin MRI, Ettlingen, Germany) was used. Respiratory rate and body temperature of the animals were constantly monitored during the PET scan (SA Instruments Inc, Stony Brook, NY, USA). For tracer administration, the lateral tail vein was cannulated. A 60-min PET scan (microPET Focus220 scanner, Siemens Medical Solutions, Knoxville, TN, USA) was started simultaneously with i.v. injection of [^{18}F]FDG in a volume of approximately 0.1 mL. The administered radioactivity is given in Table 1.

2.4. Postimaging procedures

After completion of the PET scan, blood was withdrawn under isoflurane anesthesia from the orbital sinus vein into pre-weighed micropipettes. Blood samples were weighed and measured for radioactivity in a gamma counter (Perkin-Elmer Instruments, Wellesley, MA,

Table 1
Study parameters for [^{18}F]FDG small-animal PET experiments in mice and rats.

	Study parameter	Mouse				Rat
		Wild-type	*Abcb1a/b*$^{(-/-)}$	*Abcg2*$^{(-/-)}$	*Abcb1a/b*$^{(-/-)}$ *Abcg2*$^{(-/-)}$	
Vehicle	Number of animals	7	5	7	5	4
	Body weight (g)	29.1 ± 2.5	29.9 ± 2.3	30.2 ± 4.3	34.6 ± 2.5	246.4 ± 5.4
	Injected [^{18}F]FDG (MBq)	6.0 ± 0.5	5.6 ± 0.7	5.9 ± 1.0	6.6 ± 1.0	40.8 ± 8.7
	Blood glucose (mg/100 mL)[a]	225 ± 50	200 ± 61	191 ± 71	228 ± 42	105 ± 11
	Blood radioactivity (SUV)[a]	0.53 ± 0.12	0.48 ± 0.08	0.45 ± 0.11	0.56 ± 0.13	0.83 ± 0.06
Tariquidar (15 mg/kg)	Number of animals	7	5	8	5	4
	Body weight (g)	27.9 ± 2.0	27.9 ± 3.9	29.5 ± 3.9	33.1 ± 4.3	256.6 ± 22.6
	Injected [^{18}F]FDG (MBq)	8.4 ± 4.5	8.7 ± 3.7	6.6 ± 1.0	6.2 ± 0.8	45.7 ± 4.4
	Blood glucose (mg/100 mL)[a]	205 ± 52	185 ± 55	202 ± 43	274 ± 64	104 ± 21
	Blood radioactivity (SUV)[a]	0.45 ± 0.05	0.47 ± 0.14	0.53 ± 0.16	0.54 ± 0.23	0.91 ± 0.08

All values are given as mean ± SD.
[a] Measured at end of PET.