[¹⁸F]2-fluoro-2-deoxy-sorbitol PET Imaging for Quantitative Estimation of Blood-brain Barrier Permeability *in vivo*

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Short Report

Keywords: Blood-brain barrier, integrity marker, sorbitol, positron emission tomography

Posted Date: July 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-693599/v1>

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Abstract

Purpose The non-transported and non-metabolized sorbitol derivative [^{18}F]2-fluoro-2-deoxy-sorbitol ([^{18}F]FDS) can be straightforwardly obtained from chemical reduction of commercial [^{18}F]2-deoxy-2-fluoro-D-glucose. [^{18}F]FDS was evaluated as a small-molecule (paracellular) marker of blood-brain barrier (BBB) integrity for PET.

Methods Five mice underwent focused ultrasound (FUS) to generate spatially controlled BBB disruption in the right hemisphere. PET kinetics of [^{18}F]FDS in each brain hemisphere were described by a 1-tissue compartment model using an image-derived input function.

Results BBB disruption resulted in a 2.4 ± 0.8 -fold increase in the brain distribution (V_T , $p < 0.01$) of [^{18}F]FDS. Enhanced brain uptake was associated with an increase in the influx transfer rate K_1 ($+1.4 \pm 0.7$ -fold, $p < 0.05$) and a decrease in the efflux transfer rate k_2 (-1.7 ± 0.4 -fold, $p < 0.01$).

Conclusion Thanks to the quantitative performance of PET compared with other neuroimaging techniques, [^{18}F]FDS PET and kinetic modelling provides a readily available and sensitive method for non-invasive determination of different levels of BBB permeability *in vivo*.

Introduction

The blood-brain barrier (BBB) plays a critical role in protecting the brain from the circulation while controlling brain homeostasis. Integrity of the BBB is mainly carried out by tight junctions between adjacent endothelial cells forming the brain microvasculature [1]. A large body of translational research supports that BBB integrity is compromised in many CNS pathological conditions including multiple sclerosis, hypoxic/ischemic insult, traumatic injury, Parkinson's and Alzheimer's diseases, epilepsy and brain tumors [2]. Different non-clinical models have shown that BBB leakage is a common feature of the neuroinflammatory cascade and contributes to disease-associated brain damage [3, 4].

Quantitative markers are needed to demonstrate loss in BBB integrity in various pathophysiological conditions [5]. In non-clinical *in vitro* and *ex vivo* research, low molecular weight (MW, g/mol) hydrophilic molecules such as fluorescein (MW = 332, fluorescent detection), (radio)labeled analogues of sucrose (MW = 342) or mannitol (MW = 182) are preferred as quantitative "paracellular" (*i.e.* between-cell pathway) markers of membrane integrity (Fig. 1) [2, 6]. Low MW is associated with enhanced sensitivity to subtle change in barrier permeability compared with higher MW compounds such as Evan's blue (EB, MW = 961, highly bound to plasma proteins), radiolabeled proteins (albumin MW = 65,000), dextrans (MW = 1,500 – 70,000) or inulin (MW = 6179) [2, 5].

Imaging techniques enable *in vivo* determination of BBB integrity with translational perspectives. Dynamic contrast-enhanced (DCE) and dynamic susceptibility contrast MRI using gadolinium (Gd)-based contrast agents such as gadoterate (MW = 558) or Gd-DTPA (Gd-diethylene triamine pentaacetic acid,

MW = 546) [7], and brain SPECT using [^{99m}Tc]DTPA (MW = 487) are widely used to detect local BBB disruption associated with some situations such as ischemic stroke or brain tumors [8]. Despite limited quantitative performance, the predominant use of MRI and SPECT to investigate BBB integrity is likely due to the availability of corresponding imaging probes [9]. PET may allow for accurate kinetic modeling and quantitative estimation of the transfer of radiolabeled compounds across the BBB *in vivo* [10]. However, PET using radiolabeled low- or high-MW makers of BBB integrity such as [^{18}F]1-fluoro-1-deoxy-D-mannitol (MW = 183) [11] or [^{11}C]inulin (MW = 6179) [12] did not reach mainstream use, probably due to their limited availability in centers not equipped with a cyclotron.

Sorbitol, a stereoisomer of mannitol, is a non-transported hydrophilic small molecule (MW = 182) that is poorly metabolized in mammals. The fluorinated derivative [^{18}F]2-fluoro-2-deoxy-sorbitol ([^{18}F]FDS, MW = 183) can be virtually obtained in all Nuclear Medicine departments from simple chemical reduction of commercial [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG, Fig. 1) [13]. [^{18}F]FDS shows non-significant brain uptake in healthy rodents and humans [14] and benefits from favorable pharmacokinetic properties for quantitative PET imaging *in vivo* [15].

This study aimed at evaluating [^{18}F]FDS PET and kinetic modelling for quantitative determination of BBB permeability *in vivo*. To this end, a method based on focused ultrasound (FUS) was optimized to induce hemispheric BBB disruption in mice.

Material And Methods

Production of [^{18}F]FDS

Synthesis of [^{18}F]FDS from commercial [^{18}F]FDG and quality control was described by Li et al. [13] and is reported as Supplementary material.

Focused Ultrasound

The method for spatially controlled BBB disruption was optimized from previous work [16] to induce reproducible BBB disruption in the right brain hemisphere only. Seven-week-old female NMRI nu/nu mice were anesthetized with 1.5% isoflurane in O_2 . A catheter was inserted in the tail vein and the animal was transferred to the sonication system. Microbubbles (50 μL) were intravenously administrated in the tail vein before the beginning of the FUS (n=5) or Sham (no FUS, n=3) session. Detailed FUS protocol is reported as Supplementary material.

Evan's blue extravasation test

Evan's blue (EB) (Sigma-Aldrich, Saint-Quentin Fallavier, France) at 4% in NaCl 0.9% was freshly prepared as previously described [17]. Mice received 100 μL EB i.v immediately after FUS. One hour after injection, *i.e.* at the end of PET acquisition, animals were euthanized and brains were removed to visually assess EB extravasation (Fig. 2).

[¹⁸F]FDS PET imaging

Immediately after EB injection, anesthetized mice were transferred to the microPET scanner (Inveon, microPET, Siemens Healthcare, Knoxville, TN, USA). [¹⁸F]FDS was administered intravenously (4.2 ± 0.76 MBq) using a microinjection pump at the rate of $0.2 \text{ mL} \cdot \text{min}^{-1}$ ($n=5$ FUS; $n=3$ Sham). Dynamic PET acquisition (60 min) started with [¹⁸F]FDS injection.

PET images were reconstructed by the 3D OSEM/MAP algorithm and corrected for attenuation, random coincidences and scatter. Volumes of interests (VOI_s) were manually delimited using Pmod software (version 3.8, PMOD Technologies Ltd, Zurich, Switzerland). In the FUS group, extravasation of ¹⁸F-FDS was obvious in the sonicated area on late PET images (Fig. 2A). The region with disrupted BBB was delineated and mirrored to the contralateral hemisphere. In Sham animals, VOI_s were drawn in each brain hemisphere. Another VOI was drawn on the aorta (blood-pool), obvious on early time-frames, to generate an image derived input function (IDIF).

Time Activity Curves (TACs) were corrected for radioactive decay and expressed as standard uptake value (SUV) vs time. Area Under the TAC (AUC) was calculated from 0 to 60 min. A 1-tissue compartment (1-TC) model using IDIF was tested to describe the transport of ¹⁸F-FDS across the BBB and generate parametric mapping of the total volume of distribution (V_T) of [¹⁸F]FDS (Fig. 2B). Data are reported as mean $\pm$ standard deviation (S.D.) and were statistically compared using either a One-way ANOVA or a paired t-test. (GraphPad Prism, La Jolla, CA, USA).

Results

In hemispheres with intact BBB (Sham animals or contralateral hemisphere), brain PET signal increased rapidly with maximal uptake at $T_{\text{max}} \sim 2.0 \pm 0.9$ min. FUS enhanced the PET signal in the sonicated volume consistent with EB extravasation (Fig. 2) and T_{max} was achieved later at 5.5 ± 2.3 min. Brain uptake of [¹⁸F]FDS in the sonicated brain was significantly higher compared with the contralateral area (2.12 ± 1.5 -fold increase, $p < 0.01$) or the Sham group (1.84 ± 1.09 -fold increase, $p < 0.05$) (Fig. 3).

Brain TACs were accurately described by the 1-TC model which demonstrated a significant 2.43 ± 0.8 -fold increase in the V_T of [¹⁸F]FDS in the sonicated area ($p < 0.001$). Enhanced brain distribution was associated with a 1.4 ± 0.7 -fold increase in K_1 ($p < 0.05$) and a 1.7 ± 0.4 -fold decrease in k_2 ($p < 0.01$) (Fig. 4).

Discussion

[¹⁸F]FDS benefits from the characteristics of an “ideal” marker of BBB integrity [5]. This includes clinical safety, metabolic stability, low binding to plasma proteins ($< 0.1\%$) and low baseline brain uptake when

the BBB is intact [14, 18]. Simple production of ^{18}F -FDS from commercial ^{18}F FDG makes it an appealing radiopharmaceutical candidate for determination of BBB integrity using quantitative PET.

Unlike ^{18}F FDG, ^{18}F FDS is poorly taken up by mammalian cells because it does not undergo facilitated transport [13]. ^{18}F FDS PET has been validated in animals and humans to study renal function [15] or detect/estimate bacterial burden in tissues because sorbitol is a specific metabolic substrate of some strains of gram-negative bacteria [19]. Interestingly, ^{18}F FDS was shown to visually accumulate in orthotopic brain tumor xenografts in mice, despite negligible uptake by implanted glioma cells *in vitro* [13]. Our results suggest that enhanced ^{18}F FDS PET signal in the tumor area may be attributed to local BBB leakage.

Pharmacokinetic modelling of brain ^{18}F FDS PET data is relatively simple. The impact of BBB disruption did not restrict to enhanced influx (blood-to-brain) transfer. This suggests that considering the uptake phase of the brain distribution only may underestimate the overall impact of BBB integrity on brain exposure of solutes.

FUS offers unique perspectives to induce safe, reproducible and localized BBB disruption *in vivo* [20]. Interestingly, mapping of ^{18}F FDS brain distribution within the sonicated area displayed a gradient from the center to the periphery (Fig. 2). It may be hypothesized that heterogeneity in intensity of delivered ultrasound may occur within the sonicated area as a consequence of loss of ultrasound transmission relative to the transducer angulation at the skull surface. Interestingly, such phenomenon could not be detected using high-MW markers of BBB integrity such as gadoterate ou EB [17].

There is a critical need for PET imaging markers to offer more quantitative insight into BBB integrity in pathophysiological conditions [11, 21–23]. Outcome parameters and parametric images describing BBB transport of ^{18}F FDS provide absolute quantitative whole-brain mapping of BBB permeability. This may be useful for group comparison or to investigate the dynamics of disease-associated change in BBB permeability in longitudinal studies. In a neuropharmacokinetic perspective, ^{18}F FDS provides a convenient alternative to EB, DCE-MRI or $^{99\text{m}}\text{Tc}$ DTPA SPECT, with better quantitative performance, to interpret the brain penetration of CNS-targeting radioligands with respect to the local permeability of the BBB [24].

Declarations

Funding

Gaëlle Hugon received a PhD grant from the CEA. Louise Breuil received a grant from CEA/AP-HP.

Conflicts of interest/Competing interests

None.

Availability of data and material

All data are available upon reasonable request to the corresponding author.

Code availability

N.A

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Gaëlle Hugon, Sébastien Goutal, Ambre Dauba, Louise Breuil, Alexandra Winkeler, Anthony Novell and Nicolas Tournier. The first draft of the manuscript was written by Gaëlle Hugon and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

All animal experiments were performed in accordance with the recommendations of the European Community (2010/63/UE) and the French National Committees (law 2013-118) for the care and use of laboratory animals. The experimental protocol was approved by a local ethics committee for animal use (CETEA) and by the French ministry of agriculture (APAFIS 16292-2018072609593031 v7).

Acknowledgments

We thank Maud Goislard for helpful technical assistance.

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Figures

Figure 1

Chemical structures of mannitol, sorbitol, [¹⁸F]2-fluoro-2-deoxy-glucose ([¹⁸F]FDG) and [¹⁸F]2-fluoro-2-deoxy-sorbitol ([¹⁸F]FDS)

Figure 2

Brain PET images of [18F]2-fluoro-2-deoxy-sorbitol ([18F]FDS) at the intact and disrupted blood-brain barrier (BBB). Representative SUV-normalized brain PET images (sum 30-60 min) of [18F]FDS uptake after (FUS) or without (Sham) hemispheric ultrasound-induced blood-brain barrier disruptions (A). Corresponding parametric VT maps (1-TC model, B) and Evan's Blue extravasation (C) are reported.

Figure 3

Brain kinetics of [18F]2-fluoro-2-deoxy-sorbitol ([18F]FDS). BBB disruption was obtained in the right brain hemisphere using focused ultrasound (FUS group, A), but not in the Sham group (n=3, in B). In C, corresponding brain exposure (AUC_{brain}) were statistically compared (One-way ANOVA, *p<0.05; **p<0.01). Image-derived input functions are shown in D. Data are mean $\pm$ S.D.

Figure 4

Pharmacokinetic modelling of [18F]FDS brain PET data. Ultrasound-induced BBB disruption (FUS+) was obtained in the right brain hemisphere while BBB in the contralateral brain hemisphere was intact (FUS-). Outcome parameters of the 1-tissue compartment model (V_T , K_1 and k_2) were compared using a paired t-test analysis. Data are reported as mean $\pm$ S.D with individual values (n=5) with *p<0.05, **p<0.01 and ***p<0.001.

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