Effect of *(Z)*-Isomer Content on [^{11}C]ABP688 Binding Potential in Humans

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ABSTRACT

Purpose: To determine how the low-affinity *(Z)*-isomer of the radiotracer [^{11}C]ABP688 affects binding potential values *in vivo* in humans.

Methods: High resolution [^{11}C]ABP688 PET scans were acquired on 74 healthy volunteers (25 male, 49 female, mean age 20±3.0). Relative content of *(E)*- and *(Z)*-isomer were determined prior to injection using analytical high performance liquid chromatography (r_t*(E)* = 10 minutes, r_t*(Z)* = 8.5 minutes). Mean binding potential ($BP_{ND} = f_{ND} * (B_{avail} / K_D)$) values were calculated in the striatum, limbic regions, and prefrontal cortex using the simplified reference tissue model with cerebellar grey matter as reference.

Results: Mean±SD *(E)*-isomer content in [^{11}C]ABP688 production was 92±3.8% (range 78–97%). Percent *(E)*-isomer was positively correlated with BP_{ND} in the striatum (ρ=0.28, p=0.015) and limbic regions (ρ=0.25, p=0.036). In multiple regression analysis, sex (β=0.39, p=0.001) and *(E)*-isomer content (β=0.23, p=0.040) were significant predictors of BP_{ND}.

Conclusions: Even modest levels of *(Z)*-[^{11}C]ABP688 can reduce estimates of tracer binding *in vivo*. Future studies should use production methods that enrich levels of *(E)*-[^{11}C]ABP688, report tracer isomer ratios, and account for this factor in their analyses.

INTRODUCTION

The radiotracer 3-((6-methylpyridin-2-yl)ethynyl)cyclohex-2-en-1-one-*O*-[^{11}C]methyloxime ([^{11}C]ABP688) is a selective inhibitor at an allosteric site on metabotropic glutamate type 5 (mGlu5) receptors. This receptor is implicated in a number of psychiatric and neurological conditions including mood, anxiety, and substance use disorders [1]. Drugs targeting the mGlu5 allosteric site are currently being investigated pre-clinically and clinically. Accordingly, positron emission tomography (PET) with [^{11}C]ABP688 is a powerful tool to investigate disease pathophysiology and to measure receptor occupancy during drug development.

Due to the presence of an asymmetric C=N double bond, ABP688 exists in two stereoisomeric forms, *(E)*- and *(Z)*-ABP688, with the former binding to mGlu5 receptors with higher affinity than the latter [2]. This differential affinity has a large effect on binding, and, in rats, purified *(Z)*-[^{11}C]ABP688 shows minimal specific binding *in vivo* [3]. In a typical radiochemistry production, the *E*/*Z* isomeric ratio varies depending on the quality of precursor and ^{11}C-labeling conditions. Although [^{11}C]ABP688 can be produced with *(E)*-[^{11}C]ABP688 enriched at a ratio of at least 10:1 [2], these values are not consistently reported and it is not yet known if low levels of *(Z)*-isomer content typical to some production methods have a detectable effect on tracer binding in humans. To investigate this possibility, we examined how [^{11}C]ABP688 binding signal in the healthy human brain is affected by relative isomer content using typical production methods.

MATERIAL AND METHODS

Scans from 74 healthy young adult volunteers were included in this study (25 men and 49 women, mean age 20±3.0 years). Five were current cigarette smokers; there were no other Axis I psychiatric disorders. Participants were recruited from community advertisements (n=25) or from one of two longitudinal cohorts (n=49; Quebec Longitudinal Study of Child Development, n=44; Quebec Study of Newborn Twins, n=5). The study was carried out in accordance with the Declaration of Helsinki and approved by the Research Ethics Board of the Montreal Neurological Institute, McGill University and the ethics committee of the CHU Sainte-Justine Research Center. All participants provided written informed consent.

[^{11}C]ABP688 was synthesized by reacting desmethyl-ABP688 (ABX; Radeberg, Germany) in anhydrous dimethyl sulfoxide (0.5mL) with [^{11}C]methyl iodide in the presence of NaOH (5M, 10μL) at 90^{O}C for 5 minutes. [^{11}C]CH_3I was generated *via* either wet method [4] (n=51) or dry method (Synthra module [Synthra GmbH; Hamburg, Germany]; n=23). The product was purified by semipreparative high performance liquid chromatography (HPLC; Waters, μBondapak C18; mobile phase, acetonitrile: 0.1% phosphoric acid (30:70); flow rate 2mL/min; r_t = 10 min) from the unreacted precursor and [^{11}C]CH_3I, while isomer separation was not achieved. After removal of HPLC eluent by evaporation, the product was formulated in 9.5mL sterile phosphate buffer and 0.5mL EtOH. Radiochemical identity, radiochemical purity, molar activity and diastereomeric ratio were determined by analytical HPLC (MZ Analytical PerfectSil 120 C8 5μm, 100x4.0mm; 45/55 acetonitrile/water at 0.7mL/min; r_{t}*(E)* = 10 min, r_{t}*(Z)* = 8.5 min).

PET scans were acquired using a high-resolution research tomograph (HRRT, CTI/Siemens). A 6-minute transmission scan was first performed with ^{137}Cs to correct for tissue attenuation. Subsequently, a 60-min scan was initiated concurrent with the beginning of a one-minute bolus injection of 372±29 MBq [^{11}C]ABP688. Dynamic data were collected with the scanner in list mode and reconstructed using an ordered subset maximization algorithm including motion correction to the transmission scan.

Mean regional binding potential (BP_{ND}) values were determined relative to nonspecific binding in cerebellar grey matter using the simplified reference tissue model. Grey matter regions of interest (ROIs) were generated using standard masks defined on the MNI152 template and registered to PET images using non-linear transformation. ROIs included the prefrontal cortex, striatum, and limbic areas, with limbic BP_{ND} computed as the mean of values in the insula, hippocampus, and amygdala.

Correlation between regional BP_{ND} and percent *(E)*-isomer was assessed using Spearman's ρ. Multiple regression analysis was used to identify relative effect on regional BP_{ND} of isomer content and other factors including molar activity, sex, and smoking status, which can influence [^{11}C]ABP688 BP_{ND} [5]. Analyses were performed separately for PFC, striatum, and limbic BP_{ND} values.

RESULTS

Radiochemical purity of all [^{11}C]ABP688 batches produced for this study, calculated as the sum of both radioisomers relative to total batch radioactivity, was above 95%. Mean molar activity was 82±31 GBq/μmol (range 29–140 GBq/μmol) for the wet method and 7.9±2.8 GBq/μmol (range 3.7–16 GBq/μmol) for the dry method. Mean *(E)*-isomer content in [^{11}C]ABP688 production was 92±3.8%, with a range of 78–97%.

In the striatum, BP_{ND} was positively correlated with percent of *(E)*-isomer in the product (ρ=0.28, p=0.015, Figure 1). Given the wide range of molar activity, we assessed the effect of injected mass of each isomer (total mass × % *(E)*- or *(Z)*-isomer) on BP_{ND}. Controlling for a chance difference in total tracer mass between men and women (women>men; U=437, p=0.045), BP_{ND} was correlated with mass of (*Z)*-isomer (ρ=-0.30, p=0.011) but not (*E)*-isomer (ρ=-0.19, p=0.11). This suggests a specific effect of *(Z)*-isomer not explained by self-blocking from unlabelled *(E)*-isomer.

Using multiple regression analysis, *(E)*-isomer content (β=0.23, p=0.040) and sex (β=0.39, p=0.001) but neither molar activity nor smoking status were significant predictors of BP_{ND} in the striatum (model R^2=0.25, Table 1). A similar pattern of effects was observed in limbic regions (ρ=0.25, p=0.036; regression *(E)*-isomer β=0.20, p=0.094). BP_{ND} values in the prefrontal cortex were not significantly related to *(E)*-isomer content (ρ=0.12, p=0.29). The effect of sex reflects higher BP_{ND} values in men than in women in this sample, described in more detail elsewhere [6].

DISCUSSION

The imaging results indicate that even modest levels of *(Z)*-[^{11}C]ABP688 can reduce estimates of tracer specific binding *in vivo*. In this sample of 74 [^{11}C]ABP688 scans, average percent *(E)*-[^{11}C]ABP688 in each production batch was over 90%. Despite this enrichment, tracer isomer content was a significant predictor of [^{11}C]ABP688 BP_{ND}.

The two major factors contributing to great variability of the *(E)*- to *(Z)*-[^{11}C]ABP688 ratio are precursor batch quality, i.e. (*E*)-isomer enrichment of the desmethyloxime, and possible *E*/*Z*-

isomerization in the basic condition at high temperatures during ^{11}C-methylation. Because both factors are difficult to control, an effort has to be made to either analyze the isomeric ratio using analytical HPLC during quality control of the tracer or, preferably, separate the two isomers on the HPLC purification step after the radiosynthesis. The preparative HPLC used for these data did not allow for production of the pure (*E*)-isomer.

In rats, *in vitro* estimates of *(Z)*-[^{11}C]ABP688 K_d and, to a lesser extent, B_{max}, were substantially reduced relative to the *(E)*-isomer [3]. Greater *(Z)*-isomer content can therefore artificially reduce binding potential values by reducing the amount of tracer available with high affinity for the target receptor. Indeed, *in vivo* BP_{ND} correlated with isomer ratio using [^{11}C]ABP688 batches in which *(E)*-isomer content ranged from 0 to 100% [3]. Subsequent studies in laboratory animals and humans typically use production methods to enrich *(E)*-[^{11}C]ABP688 content. Despite using such methods, an effect of isomer content was observed on striatal and limbic BP_{ND} values here. Though not statistically significant, a similar pattern emerged in prefrontal cortex. Cigarette smoking was not a predictor of BP_{ND} in this study, likely due to the low number of smokers (n=5).

Regression analyses indicate that for every 1% increase in *(Z)*-isomer content, striatal BP_{ND} will decrease by 0.012. This is 2% of the minimum striatal BP_{ND} value observed (0.71) and 1% of the mean (1.1). Given the range of isomer content of 19 percentage points in this sample, this factor could have a meaningful effect on the ability to detect differences or changes in BP_{ND}. For example, previous studies of clinical populations have identified differences in [^{11}C]ABP688 BP_{ND} of approximately 20% between people with cocaine dependence and healthy controls, and binding reductions of 15-20% have been reported following ketamine administration [7].

While relatively stable in non-human primates [8], variability in [^{11}C]ABP688 BP_{ND} is high in healthy people, and sources of [^{11}C]ABP688 binding variability in humans are not yet well understood [9]. This is evident in the variability observed even at similar levels of isomer content in the present data (Figure 1). Some evidence suggests that binding could be influenced by extracellular glutamate levels [10] and/or by diurnal variation in receptor availability [11]. In order to better understand such potential biological sources of variation, it will be crucial to

identify and minimize technical factors that affect binding. Notably, BP_{ND} values here were computed using a cerebellar reference region. Differences in cerebellar uptake due to differences in nonspecific or off-target binding may have added further variability or bias to BP_{ND} estimates, though cerebellar binding of each isomer was similarly low in rats [3]. Nevertheless, it would be worthwhile to assess isomer effects on other outcome measures, such as V_T.

Future studies should consider using production methods that further enrich levels of *(E)*-[^{11}C]ABP688 or isolate *(E)*-[^{11}C]ABP688 prior to tracer administration. The findings reported here prompted us to refine the preparative HPLC conditions for baseline separation of the two isomers. We can now reliably produce *(E)*-[^{11}C]ABP688 with >99% isomeric enrichment. At a minimum, future studies should report tracer isomer ratios and account for this potential confound in their analyses.

Compliance with Ethical Standards

This work was supported by grants from the Canadian Institutes for Health Research MOP-133537 (ML) and 119509 (CB and ML) and by a grant from FRQS ERA-NET (ML). The authors declare that they have no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Research Ethics Board of the Montreal Neurological Institute, McGill University and the ethics committee of the CHU Sainte-Justine Research Center, and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study. This article does not contain any studies with animals performed by any of the authors.

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Table 1 Multiple regression analysis of BPND values in the striatum

	B	SE	Standardized β	t	p
Constant	-0.225	0.542		-0.415	0.679
Sex	0.165	0.047	0.389	3.534	0.001
% *(E)*-isomer	0.012	0.006	0.233	2.091	0.040
Molar activity	0.000014	0.001	0.071	0.605	0.547
Smoking status	-0.085	0.084	-0.106	-1.004	0.319

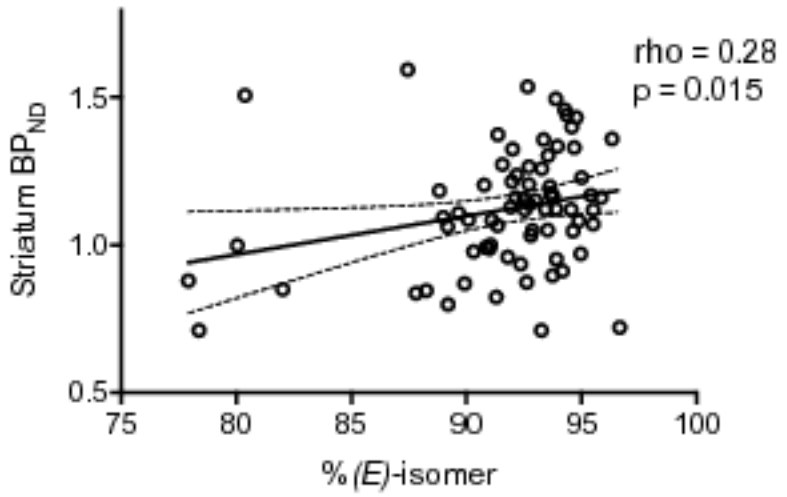

Fig. 1 BP_{ND} in the striatum is correlated with percent of *(E)*-isomer in [^{11}C]ABP688 batch in PET scans from 74 healthy volunteers