Autoradiographic Evaluation of [¹⁸F]FECUMI-101, a High Affinity 5-HT_{1A}R Ligand in Human Brain

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ABSTRACT: [^{18}F]FECUMI-101 ([^{18}F]1) is a 5-HT $_{1A}$ R ligand demonstrating specific binding in brain regions corresponding to the distribution of 5-HT $_{1A}$ R in baboons. However, we detected moderate uptake of [^{18}F]1 in baboon thalamus, a brain region lacking 5-HT $_{1A}$ R. We sought to investigate the relative binding of [^{18}F]1 to 5-HT $_{1A}$ R, α_1 R, and 5-HT $_7$ R *in vitro*. Using autoradiography in human brain sections, specific binding of [^{18}F]1 to 5-HT $_{1A}$ R was confirmed. However, [^{18}F]1 also showed 26% binding to α_1 R in PFC. The hippocampal for HT $_{1A}$ R, respectively. Thalamus and cerebellum showed very little HT $_7$ R and no effect of temperature on [^{18}F]1 specific binding. [^{18}F]FECUMI-101 is not a completely selective 5-HT $_{1A}$ R ligand. *Key words:* 5-HT $_{1A}$ R, α_1 R, 5-HT $_7$ R, autoradiography, *in vitro*, *in vivo*, measurements of drugs acting at 5-HT $_{1A}$ R *in vivo*.

KEYWORDS: 5-HT_{1A}R, α_1 R, radiotracer, autoradiography, PET, hippocampus

5-HT_{1A}R exists in active and inactive agonist affinity states. The antagonist ligands bind to the active and inactive conformations of 5-HT_{1A}R with equal affinity. Whereas, agonist ligands bind preferentially to the active state of the receptor, thereby providing a more meaningful functional measure of the 5-HT_{1A}R.¹ Although, antagonist 5-HT_{1A}R PET tracers can measure the total receptor binding, they cannot detect changes in the active 5-HT_{1A}R binding in disease states or in the context of drug based treatment. Agonist 5-HT_{1A}R radiotracers are likely to be sensitive to measure the changes in the intrasynaptic concentrations of the endogenous 5-HT to the receptor. Additionally 5-HT_{1A}R agonist radiotracers may be useful to monitor desensitization and sensitization of receptors; to provide a better estimate of 5-HT_{1A}R in diseases; measure receptor occupancy of agonist therapeutic agents; and for the efficacy evaluation of SSRI treatment.¹⁻³ The development of specific 5-HT_{1A}R agonist PET tracers has met with limited success despite three decades of effort.¹ Most of the agonist radiotracers developed to date have the structural core of amino-tetralin, apomorphine, thiochromine, and arylpiperazine based ligands.¹⁻³ We reported 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine tethered arylpiperazines as promising agonist/partial agonist ligands with high affinity for 5-HT_{1A}R based on GTPγS stimulation studies in CHO cells stably expressing human 5-HT_{1A}R.⁴⁻⁶ Based on structure–affinity relationship (SAR) studies of 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine, [¹¹C]CUMI-101

was identified as a partial agonist radiotracer, and it has been tested in both nonhuman primates and human subjects with PET.^{5–13} Another study using membrane preparations from CHO cells also observed 5-HT_{1A}R agonist properties of CUMI-101.¹⁴ However, the same report did not find stimulation of GTPγS binding by CUMI-101 in rat cortex and rat hippocampal membranes. Recently, brain homogenate assays of CUMI-101 in rat, monkey, and human brain homogenates showed increase of GTPγS stimulation compared to basal expression.¹⁵ The dose response curves of the same study were more consistent with that of an antagonist behavior of CUMI-101. In a GTPγS stimulation of rat hippocampal membrane study, buspirone, a partial agonist of 5-HT_{1A}R exhibited very low stimulation to GTPγS binding in comparison to full agonist (R)-8-OH-DPAT.³² The above study indicates that membrane homogenate studies may not provide a definitive answer for 5-HT_{1A}R functional properties of partial agonists.³² Additional findings such as dose–response relationships to behavior and electrophysiological neuron responses in rodents may offer better definitive characterizing of the agonist/antagonist properties of CUMI-101 *in vivo*. While several ¹⁸F-labeled radiotracers based on the CUMI-101 core failed *in vivo* to

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image 5-HT_{1A}R, PET studies of [¹⁸F]FECUMI-101 ([¹⁸F]**1**, Figure 1), a fluoroethyl analogue of CUMI-101, showed

Figure 1. Chemical structure of [¹⁸F]FECUMI-101.

promising imaging characteristics in baboons.¹⁶ FECUMI-101 is a high affinity 5-HT_{1A}R ligand ($K_i = 0.1$ nM) and produced a dose-dependent increase in [³⁵S]GTPγS binding in CHO cells expressing 5-HT_{1A}R with an E_{max} 77% and EC_{50} of 0.65 nM.¹⁶ *In vivo* pharmacological response following subcutaneous administration of FECUMI-101 induce a moderate lower lip retraction in rats, a characteristic response specific to 5-HT_{1A}R activation. These studies further confirm partial 5-HT_{1A}R agonistic properties of FECUMI-101.³³ The highest uptake of [¹⁸F]**1** radioactivity was found in hippocampus, anterior cingulate cortex, insular cortex, prefrontal cortex, and amygdala.¹⁶ Striatum, occipital cortex, and cerebellum showed minimal binding of [¹⁸F]**1**.¹⁶ This distribution is consistent with our previous reports of the parent tracer [¹¹C]CUMI-101 in baboon and human.^{7–11} The thalamus was discrepant showing higher binding of [¹⁸F]FECUMI-101 in comparison to [¹¹C]CUMI-101 and [¹¹C]WAY100635, despite the higher selectivity of FECUMI to α_{1A}R.^{7–13} The binding of [¹⁸F]FECUMI-101 to thalamus cannot be solely attributed to 5-HT_{1A}R binding as it is established that thalamus has low 5-HT_{1A}R concentration.^{13,17} Receptor selectivity assays show that the highest affinity of FECUMI-101, apart from 5-HT_{1A}R ($K_i = 0.1$ nM), is to 5-HT₇R ($K_i = 17.2$ nM), α_{1A}R ($K_i = 21.4$ nM), and α_{1B}R (26.8 nM).¹⁶ Both 5-HT₇R and α₁R are abundant in the thalamus, and α₁R is coexpressed with 5-HT_{1A}R in many brain regions.^{18–20} Since α₁R antagonists are known to produce cardiovascular changes even at 0.01 mg/kg doses in monkeys, it is difficult to perform *in vivo* blocking experiments to test α_{1A}R selectivity of [¹⁸F]**1** in nonhuman primates.^{21,22} The binding affinity measurements of FECUMI-101 were performed at room temperature;¹⁶ however, differences in α₁R binding at room temperature vs 37 °C (physiological temperature) were recently reported for the parent ligand CUMI-101 in membrane homogenate radioligand binding assays.¹⁵

We sought to determine the binding selectivity of [¹⁸F]**1** for 5-HT_{1A}R, α₁R, and 5-HT₇R and test potential temperature effects on 5-HT_{1A}R and α₁R binding by performing experiments at room temperature and at 37 °C using *in vitro* autoradiography in post-mortem human brain sections. Herein, we describe our experiments and results.

The reference standard FECUMI-101 and the corresponding radiolabeling precursor were synthesized using our previously reported procedure.¹⁶ Radiosynthesis of [¹⁸F]FECUMI-101 was achieved with minor modifications of the reported procedure.¹⁶ Briefly, [¹⁸F]fluoroethyl tosylate was synthesized and reacted with the corresponding desethyl precursor in DMSO in the presence of K₂CO₃. The crude product was purified by semipreparative HPLC, followed by elution with C-18 Sep-Pak cartridge, and the radioproduct was reconstructed in ethanol. A portion of the ethanol solution was used for quality control studies and subsequently used for autoradiography studies. [¹⁸F]FECUMI-101 was obtained in 30 ±

5% yield with ≥90% radiochemical purities and 1.5 ± 0.5 Ci/μmol specific activity.

Post-mortem sections (20 μm) from four nonpsychiatric, medication-free normal human brains that included hippocampus, prefrontal cortex (BA9), thalamus, and cerebellum were used for the autoradiography studies.^{23–25} Initially we examined the distribution of [¹⁸F]**1** in slide-mounted post-mortem human brain sections at 37 °C (Figure 2). The binding

Figure 2. Regional distribution of [¹⁸F]FECUMI-101 in post-mortem human brain sections at 37 °C.

data obtained were averaged from two experiments for hippocampus, BA9, and thalamus, four experiments for white matters, and one experiment for cerebellum with measures in triplicate. The highest concentration of [¹⁸F]**1** binding was found in hippocampus, with less binding in other structures (rank order relative to adjacent white matter: hippocampus (28:1) > prefrontal cortex (7.1:1) > thalamus (2.8:1) > cerebellum (2.2:1) > white matter (1:1)). The data for individual regions are corresponding to binding in gray matter relative to adjacent white matter in the same tissue section. The distribution of [¹⁸F]FECUMI-101 was heterogeneous and generally consistent with the known distribution of the 5-HT_{1A}R receptor (Figure 2).

We determined [¹⁸F]**1** binding selectivity in BA9, hippocampus, thalamus, and cerebellum by displacing [¹⁸F]FECUMI-101 uptake with specific blockers; WAY100635 for 5-HT_{1A}R, prazosin for α₁R, and SB269970 for 5-HT₇R at 37 °C (Figure 3). Percentages of binding represented are based on the corresponding specific binding of 5-HT_{1A}R, α₁R, and 5-HT₇R ligands with respect to the total binding of [¹⁸F]FECUMI-101. In BA9, prazosin displaced approximately 26% of [¹⁸F]FECUMI-101 binding, while WAY100635 displaced 98% of

Figure 3. *In vitro* selectivity of [¹⁸F]FECUMI-101 in slide mounted post-mortem human brain sections at 37 °C.

binding suggesting that [^{18}F]FECUMI-101 has high affinity for 5-HT $_{1A}$ R but also modest measurable binding to α_1 R. The hippocampal sections for this experiment were from the most anterior level where only cornu ammonis1 (CA1) is present as dentate gyrus (DG) begins 4 mm posterior to the CA1. WAY100635 displaced all the binding of [^{18}F]FECUMI-101 in the CA1 area, and we did not find any measurable binding for α_1 R as evident from prazosin displacement (Figure 3). In a subsequent experiment, with sections of hippocampus containing both the CA and DG fields, [^{18}F]FECUMI-101 was seen to label both the CA and DG (Figure 4). When the entire

Figure 4. Autoradiograms of [^{18}F]FECUMI-101 in human brain sections at 37 °C. (A) Total binding of [^{18}F]FECUMI-101. (B) Displacement with 1 μM WAY100635. (C) Displacement with 1 μM prazosin.

hippocampus was sampled, prazosin and WAY100635 displaced 51% and 92% of [^{18}F]FECUMI-101 binding, respectively. In the DG, prazosin and WAY100635 provided comparable displacement of [^{18}F]FECUMI-101 binding (84% and 89% respectively); while in CA1, WAY100635 displaced all [^{18}F]FECUMI-101 binding (99%) in CA1 compared to prazosin (13%, Figure 4). In contrast, *in vivo* PET imaging in baboon did not distinguish CA1 and DG subfields and only measured whole hippocampus.¹⁶

In the thalamus and cerebellum, there is comparatively little [^{18}F]FECUMI-101 binding and 69% of that small amount of binding was displaced by prazosin and 87% by WAY100635 in the thalamus and 85% and 87% in cerebellum, respectively (Figure 3). Specificity of cerebellar and thalamus binding of [^{18}F]FECUMI-101 are consistent with *in vivo* baboon PET studies.¹⁶ WAY100635 displaces 42% and 55% binding of [^{18}F]FECUMI-101 *in vivo* in baboon cerebellum and thalamus, respectively.¹⁶ These findings are in contrast to the parent ligand [^3H]CUMI-101 (5-HT $_{1A}$ R K_i = 0.15 nM; α_1 R K_i = 6.5 nM), which shows high amounts of specific binding in 5-HT $_{1A}$ R rich brain regions and no significant binding to α_1 R except for a small level of binding in DG.¹³ The reason for why there is more binding of [^{18}F]FECUMI-101 to α_1 R in prefrontal cortex and hippocampal tissues comparison to [^3H]CUMI-101 is not clear, especially given its lower α_1 R affinity (K_i = 21.4 nM vs 6.5 nM).

The 5-HT $_7$ R ligand, SB269970, did not displace [^{18}F]FECUMI-101 binding in any of the regions measured (Figure 3). This suggests that the [^{18}F]FECUMI-101 has no appreciable binding to the 5-HT $_7$ R or that the density of 5-HT $_7$ R is low in these regions in human brain and hence are not contributing significantly to the total binding.

To determine whether there is a difference in the relative amount of binding to the 5-HT $_{1A}$ R compared to the α_1 R under physiological temperatures, we examined [^{18}F]FECUMI-101 binding displacement by WAY100635 and prazosin at room temperature and 37 °C. At both temperatures, [^{18}F]FECUMI-101 displayed comparable specific binding in hippocampus,

BA9, and thalamus (Figure 5). The binding data obtained are from one experiment in triplicate measurements for hippocampus, BA9, and duplicate measurements for thalamus.

Figure 5. Binding of [^{18}F]FECUMI-101 in post-mortem human brain tissues at 37 °C and room temperature.

In summary, autoradiography studies of [^{18}F]FECUMI-101 in post-mortem human brain sections show 5-HT $_{1A}$ R is the major binding site. The typical cortical lamination of the 5-HT $_{1A}$ R obtained *in vitro* with [^3H]8-OH-DPAT is seen with [^{18}F]FECUMI-101 here.^{26,27} The anatomical distribution of 5-HT $_{1A}$ R binding is consistent with reported *in vivo* and *in vitro* binding of [^{18}F]FECUMI-101 in baboon by PET.¹⁶ While there was measurable specific binding of the radiotracer to α_1 R in hippocampus (mainly DG) and prefrontal cortex, [^{18}F]FECUMI-101 binding was effectively completely displaced by WAY100635 in the prefrontal cortex and CA1 subfield of the hippocampus. The thalamus and cerebellum had little [^{18}F]FECUMI-101 binding, but the binding in these regions was blocked by WAY100635 and prazosin. Because there is displaceable [^{18}F]FECUMI-101 binding in cerebellum it should not be used as a reference region for *in vivo* PET applications. Despite the known high concentration of α_1 R in thalamus and cerebellum, we did not obtain a proportionately high amount of binding of [^{18}F]FECUMI-101 in these regions.^{28–31} Similarly, the *in vitro* binding we observed in human thalamus was much lower than what we measured *in vivo* in baboon.¹⁶ A possible explanation for this discrepancy may be the difference in α_1 R concentration in the thalamus of baboon and human or it could be due to the binding of [^{18}F]FECUMI-101 to an unknown target in thalamus. There is no detectable binding of [^{18}F]FECUMI-101 to 5-HT $_7$ R sites in the tested brain regions. Similarly, we found no significant change of specific binding to 5-HT $_{1A}$ R and α_1 R at room temperature and 37 °C. The sensitivity of *in vitro* autoradiography is much higher than *in vivo* PET; hence, further *in vivo* blocking studies with α_1 R are required to establish *in vivo* α_1 R selectivity of [^{18}F]FECUMI-101. In conclusion, our experiments suggest that [^{18}F]FECUMI-101 is a relatively selective 5-HT $_{1A}$ R tracer that holds promise for occupancy measurement of 5-HT $_{1A}$ R and α_1 R dual or selective drugs *in vivo* with PET.

EXPERIMENTAL PROCEDURES

Author: [^{18}F]FECUMI-101 was synthesized as described before.¹⁶ *In vitro* autoradiography experiments were performed using four nonpsychiatric medication-free normal human brain sections. Brain sections from BA9 and hippocampus came from one subject, whereas cerebellum and thalamus sections were obtained from two other subjects. Three sections (20 μm) each for total and nonspecific binding per brain region were assayed for distribution and specificity

studies. Three brain sections (20 μm) for hippocampus and BA9 brain regions and two brain sections for thalamus were assayed for effect of temperature for total and nonspecific binding. Slide-mounted sections of frontal cortex, hippocampus, thalamus, and cerebellum were brought to room temperature and incubated in 170 mM Tris HCl (pH 7.6) containing 4 mM CaCl_2 and 138 mM sucrose and 5–10 nM of radioligand (1–2.3 mCi in 900–1200 mL of buffer) for 60 min (37 $^\circ\text{C}$ or room temperature). Adjacent sections were incubated in the same buffer with 1 μM WAY100635, 1 μM prazosin, or 1 μM SB269970 to determine nonspecific binding. Sections were washed in same buffer at 4 $^\circ\text{C}$ (2 $\times$ 1 min) and briefly dipped in ice-cold water to remove salts. Slides were quickly dried under a stream of cold air and exposed to ST-phosphor-imaging screen (Packard, wrapped in Mylar film) for 60 min. Screens were scanned with a Packard Cyclone phosphor-imaging system and analyzed with OptiQuant Acquisition and Analysis software (Packard).

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Notes

The authors declare no competing financial interest.

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