PET Imaging Evaluation of Four Novel Sigma-1 Radiotracers in Nonhuman Primates

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Short running title: Evaluation of Four Sigma-1 PET Tracers
ABSTRACT
Sigma-1 receptors (S1R) are implicated in a variety of diseases including Alzheimer’s disease and cancer. Previous positron emission tomography (PET) S1R radiotracers are characterized by slow kinetics or off-target binding that impedes their use for human brain imaging. Here, we report the first PET imaging evaluation in rhesus monkeys of four $^{18}$F-labeled spirocyclic piperidine-based PET radiotracers ($^{18}$F-1 to $^{18}$F-4) that have previously shown good binding characteristics in rodents and pigs. **Methods:** Baseline scans for the four radiotracers were obtained on an adult male rhesus monkey. Blocking scans were performed with administration of the S1R selective agonist SA4503 before injection of $^{18}$F-2 and $^{18}$F-4. Arterial input functions were measured and binding parameters were determined with kinetic modeling analysis. **Results:** In the rhesus brain, all four radiotracers showed high and fast uptake. Tissue activity washout was rapid for $^{18}$F-2 and $^{18}$F-4, and much slower for $^{18}$F-1 and $^{18}$F-3, in line with their respective in vitro S1R binding affinities. Both the 1-tissue compartment (1TC) and multilinear analysis-1 (MA1) kinetic models provided good fits of time-activity curves (TACs) and reliable estimates of distribution volume ($V_T$). Regional $V_T$ values were highest in the cingulate cortex and lowest in the thalamus for all radiotracers. $^{18}$F-4 showed greater differential uptake across brain regions and three-fold higher binding potential ($BP_{ND}$) than $^{18}$F-2. SA4503 at the dose of 0.5 mg/kg blocked ~85% ($^{18}$F-2) and ~95% ($^{18}$F-4) of radiotracer binding. **Conclusion:** Tracers $^{18}$F-2 and $^{18}$F-4 displayed high brain uptake and fast tissue kinetics, with $^{18}$F-4 having higher specific binding signals than $^{18}$F-2 in the same monkey. Taken together, these data indicate that both radiotracers $^{18}$F-2 and $^{18}$F-4 possess the requisite kinetic and imaging properties as viable PET tracers for imaging S1R in the primate brain. **Key Words:** sigma-1 receptor; PET; radioligand; fluorine-18; rhesus monkey
INTRODUCTION

S1R are chaperone proteins localized at mitochondrial associated endoplasmic reticulum membranes that have been shown to play a role in a wide range of diseases, including addiction, amnesia, Alzheimer’s disease, amyotrophic lateral sclerosis, and cancer (1,2). Several studies have detailed the roles of S1R in regulating potassium channels, neuritogenesis, calcium signaling, memory, and drug addiction (3-5). A variety of psychoactive chemicals and neurosteroids have been shown to interact with S1R, including haloperidol, (+)-N-allylnormetazocine (SKF-10,047), cocaine, and progesterone (6-8). Maurice et al. (9) demonstrated that the S1R agonists (+)-pentazocine, PRE-084, and SA4503 exhibit anti-amnesia effects in a dose dependent manner in mice with β25-35-amyloid induced amnesia. These results demonstrate a link between S1R and the pathologic states affecting the cholinergic and glutamatergic systems, which may be of therapeutic importance in the process of aging (9).

Given the diverse interactions of S1R in pathophysiology, a PET imaging agent for use in humans would allow for the noninvasive investigation of S1R in vivo and lead to new understandings of its function and dysfunction in disease states. It will also make it possible to correlate and translate preclinical findings in animal models to humans and help in the development of novel therapeutic agents.

Several PET radioligands for S1R have been developed, including 18F-FPS, 18F-FBP, 18F-FTC-146, and 11C-SA4503 (Fig. 1) (10-13). An ideal tracer would possess appropriate affinity, high selectivity for S1R versus S2R and the vesicular acetylcholine transporter (VACHT), alongside with fast, reversible tissue kinetics. Furthermore, it should have the requisite lipophilicity (Log D = 1-3) to cross the blood brain barrier (14). (R)-(+) and (S)-(−)-18F-fluspidine (18F-1 and 18F-2, respectively) demonstrated good S1R binding affinity (18F-1 Ki =
0.57 nM; \(^{18}\text{F}-2 K_i = 2.30 \text{ nM}\) and favorable kinetics when tested in rodents and pigs (15-17). Kranz et al. (18) also evaluated \(^{18}\text{F}-2\) in four healthy human subjects for dosimetry calculations. Li et al. (14) described a series of spirocyclic piperidine derivatives with subnanomolar affinity for S1R and >100 fold selectivity over S2R and VACHT. In a subsequent paper, Chen et al. (19) described the synthesis and evaluation of \(^{18}\text{F}-1'-(4-(2\text{-fluoroethoxy})benzyl)-3H-spiro[2-benzofuran-1,4'-piperidine] (^{18}\text{F}-3)\) and \(^{18}\text{F}-1'-(6-(2\text{-fluoroethoxy})pyridin-3-yl)methyl)-3H-spiro[2-benzofuran-1,4'-piperidine] (^{18}\text{F}-4)\) in mice. In baseline scans and blocking studies with SA4503, faster clearance and greater specific binding was observed for the N-pyridinyl analog \(^{18}\text{F}-4\) than the N-benzyl analog \(^{18}\text{F}-3\). The spirocyclic piperidine series thus demonstrated promising properties to image S1R with PET based on their high selectivity towards S1R and good binding characteristics in rodents, pigs, and a preliminary human study (14,16-19). Here, we report the first PET imaging evaluation in nonhuman primate of four radioligands (\(^{18}\text{F}-1-4, \text{Fig. 1}\)) from this series to assess their pharmacokinetic and \textit{in vivo} binding properties, and to select the most suitable tracer for advancing to evaluation in humans.
MATERIALS AND METHODS

Chemistry

Precursors and reference standards for $^{18}$F-1 and $^{18}$F-2 were prepared at Westfälische Wilhelms-Universität Münster, as previously reported (15). Precursors and standards for $^{18}$F-3 and $^{18}$F-4 were synthesized at Beijing Normal University, as described before (14).

Radiochemistry

Instrumentation for radiochemistry procedures and the production of $^{18}$F-fluoride have been described previously (20,21). Radiosynthesis of $^{18}$F-1 to $^{18}$F-3 was achieved via nucleophilic displacement of the corresponding tosylate precursors (5-7, Fig. 2) with $^{18}$F$^{-}$ in the presence of Kryptofix 2.2.2 and potassium carbonate (14,15). $^{18}$F-4 was synthesized in a two-pot, two-step synthesis, first isolating $^{18}$F-fluoroethyl tosylate followed by its reaction with the 2-pyridinol precursor (8, Fig. 2) (19,22). Chemical purity, radiochemical purity, and specific activity were determined by high performance liquid chromatography (HPLC) analysis of the final product solutions. Identities of the labeled compounds were confirmed by co-injection of the products with their respective unlabeled reference standards.

PET Imaging Experiments in Rhesus Monkeys

PET Procedures. Experiments were performed in rhesus monkeys (Macaca mulatta) according to procedures approved by the Yale University Institutional Animal Care and Use Committee and described previously (20).

Three animals were used in this study. The animals were immobilized with ketamine (10 mg/kg intramuscularly) and anesthetized with 1.5-2.5% isoflurane. An arterial line was placed in the radial or femoral artery for blood sampling. Scans were performed on a FOCUS 220 camera. Before radioligand injection, a 9-min transmission scan was obtained for attenuation correction. Baseline scans were obtained over 4 h on a 7-year-old male rhesus monkey (13.8 kg). Each tracer was injected intravenously over 3 min as a slow bolus (~185 MBq in 10 mL). Two-hour blocking scans of $^{18}$F-2 and $^{18}$F-4 were performed with a dose of SA4503 (6) (0.5 mg/kg) given
intravenously 10 min prior to radioligand administration. Two additional baseline scans were obtained for $^{18}$F-2 and $^{18}$F-4 on a 12-year-old female (6.1 kg) and a 9-year-old female (9.7 kg) monkey, respectively, for comparison with baseline scans obtained in the 7-year-old male. Eight PET scans were obtained in total.

**Metabolite Analysis and Arterial Input Function Measurement.** Procedures for measurement of the arterial input function, including sample preparation, metabolite analysis, and data processing have been described previously (20). Arterial samples were collected at preselected time points and the radioactivity concentrations in the whole blood and plasma were measured. During the 4 h baseline scans, samples at 3, 8, 15, 30, 60, 90, 120, 180, and 240 min after injection were processed and analyzed by analytical HPLC using a modified column-switching system (23) to determine the fraction of unmetabolized tracer over the course of the scan. A biexponential function was fitted to the measured parent fractions to produce a continuous function describing the parent fraction over time. The input function was calculated as the product of the total plasma activity and interpolated parent fraction at each time point. The measured input function values were fitted to a sum of three exponentials, and the fitted values were used as inputs for kinetic analyses.

**Plasma Free Fraction and Log D Measurement.** The free fraction in plasma ($f_P$) was measured via ultrafiltration of 0.3 mL aliquots of plasma spiked with a small amount (~740 kBq) of radioligand, repeated in triplicate. The amount of radioactivity in the filter and filtrate was counted, and $f_P$ calculated as the ratio of the concentration (radioactivity/mL) of the filtrate to the total activity. The Log $D$ of each tracer was determined by the shake-flask method as described previously (24).

**Image Analysis and Kinetic Modeling.** Procedures for PET image reconstruction, definition of regions of interest (ROIs), and kinetic analysis have been detailed previously (20). Emission data were attenuation corrected using the transmission scan, and dynamic images (33 frames over 120 min or 57 frames over 240 min) were reconstructed using a filtered back-
projected algorithm with a Shepp-Logan filter. ROIs were defined from a single representative anatomic rhesus MR image registered to a template image. Registration parameters were derived to apply ROIs to each PET scan, and time activity curves (TACs) were generated for the following 16 cortical and subcortical brain regions: amygdala, brainstem, caudate, cerebellum, cingulate cortex, frontal cortex, globus pallidus, hippocampus, insula, nucleus accumbens, occipital cortex, pons, putamen, substantia nigra, temporal cortex, and thalamus.

Regional volumes of distribution \( (V_T, \text{mL} \cdot \text{cm}^{-3}) \) were determined by kinetic analysis of the TACs, using the metabolite-corrected arterial plasma input function according to 1- and 2-tissue compartment (1TC, 2TC) models, and the multilinear analysis-1 (MA1) method as described previously \((25,26)\). Standard errors were compared to determine the optimal model for regional \( V_T \) estimates.

Comparison of \( V_T \) between tracers was used to determine relative regional \( BP_{ND} \) by graphical methods \((27)\). In this analysis, \( V_T^A \) of one tracer is plotted on the x-axis and \( V_T^B \) of another is plotted on the y-axis. A linear regression yields the following equation:

\[
V_T^B = \frac{f_P^B}{f_P^A} \frac{k_D^B}{k_D^A} V_T^A + V_{ND}^B \left(1 - \frac{BP_{ND}^B}{BP_{ND}^A}\right)
\]  

(1)

More negative values for the y-intercept indicate \( BP_{ND}^B > BP_{ND}^A \), and vice versa. Since \( f_P \) was measured for all tracers, the ratio of equilibrium dissociation constants \( (K_D) \) can be determined from the slope of the regression \((27)\).

Sigma-1 receptor occupancies with SA4503 were calculated using \( V_T \) values from all 16 ROIs to create occupancy plots according to the method of Cunningham et al. \((28)\). For \(^{18}\text{F}-2\) and \(^{18}\text{F}-4\), regional \( BP_{ND} \) values were calculated using the nondisplaceable volume of distribution \( (V_{ND}) \) obtained from the occupancy studies, where \( BP_{ND} = (V_T/V_{ND}) - 1 \) \((26)\).

RESULTS

Radiochemistry
All tracers were synthesized in >96% radiochemical purity and high specific activity (296.9 GBq/µmol average at end of synthesis, n = 7). Total synthesis time was 110 ± 30 min. Radiochemical yields (±SD where applicable) are 2.0%, 6.2 ± 1.7% (n = 3), 7.8%, and 6.5 ± 9.2% (n = 3), respectively, for $^{18}$F-1, $^{18}$F-2, $^{18}$F-3, and $^{18}$F-4.

**In Vivo Evaluation in Rhesus Monkeys**

After a bolus injection of the tracers (181.5 ± 10.6 MBq; specific activity of 242.4 GBq/µmol average at time of injection; injected mass of 0.45 ± 0.29 µg, n = 7) into an adult male rhesus monkey, total plasma activity and parent activity exhibited a rapid rise and clearance, followed by a stabilization or slow decrease over time for $^{18}$F-1 to $^{18}$F-3, and a slight increase for $^{18}$F-4 (Fig. 3A). Metabolism rates were moderate, with 37%, 35%, 18%, and 19% of parent fraction, respectively, for $^{18}$F-1 to $^{18}$F-4 at 60 min post injection (Fig. 3B). Blocking with SA4503 increased plasma activity particularly for $^{18}$F-4 (Fig. 3A) and increased metabolism for $^{18}$F-2 and $^{18}$F-4 (Fig. 3B). Typical metabolite profiles over time under baseline conditions are presented in Supplemental Fig. 1-4. Polar metabolites have been previously suggested for these tracers that should not enter the brain and interfere with PET quantitation (14,15,17).

Plasma $f_P$ values were measured at 2%, 2%, 8%, and 17%, respectively, for $^{18}$F-1-4, consistent with their respective measured $\log D$ values of 2.80, 2.80, 2.55, and 2.50. A summary of in vitro $K_i, f_P$, and $\log D$ values are shown in Table 1.

Regional TACs (Fig. 4A-D) were generated and analyzed with 1TC and 2TC models (26), as well as the MA1 method (25) using the metabolite corrected arterial plasma input function. The 1TC model was found to provide better fits than the 2TC model, with the 2TC model producing high standard error (SE) across many regions (e.g. >20% SE in 28% of regions under all conditions, and >150% SE in 75% of regions for $^{18}$F-4 under blocking condition). Therefore, the 1TC would be considered an appropriate model for analysis of imaging data.

Regional $V_T$ values estimated by MA1 showed good correlation with 1TC values (e.g. for $^{18}$F-4, $V_T(\text{MA1}) = 0.975 V_T(\text{1TC}) + 1.239, r^2 = 0.996$). Listed in Table 2 are the 1TC-derived $V_T$ values for
the tracers across brain regions, including those under blocking conditions. Values under baseline conditions are generally consistent with regional distribution trends seen in the TACs. Blocking with 0.5 mg/kg SA4503 for $^{18}$F-2 and $^{18}$F-4 reduced regional disparities, with $V_T$ in high uptake regions trending down to levels seen in low binding regions (Table 2). The time stability of $V_T$ values was also determined for the four tracers, revealing bias and regional errors associated with shorter scan times (Fig. 5).

Administration of the S1R agonist SA4503 blocked 85% and 95% of specific binding of $^{18}$F-2 and $^{18}$F-4, respectively (Supplemental Fig. 5). Values of $V_{ND}$ were calculated for $^{18}$F-2 and $^{18}$F-4 from the occupancy plots using 1TC and MA1 derived $V_T$ values (28). This analysis yielded $V_{ND\,(1TC)} = 6.87$ and $V_{ND\,(MA1)} = 7.30$ for $^{18}$F-2, and $V_{ND\,(1TC)} = 9.29$ and $V_{ND\,(MA1)} = 10.44$ for $^{18}$F-4. These values were then used to calculate the binding potential ($BP_{ND}$), as a measure of specific binding signal, across the brain regions (Table 3). Relative $BP_{ND}$ and $K_D$ for all tracers was also assessed by the graphical methods of Guo et al. (27) by comparing baseline $V_T$ values, which showed for rank order of $BP_{ND}$: $^{18}$F-3 > $^{18}$F-1 > $^{18}$F-4 > $^{18}$F-2 and for $K_D$: $^{18}$F-4 > $^{18}$F-2 > $^{18}$F-1 > $^{18}$F-3.

**DISCUSSION**

In this paper, we describe the evaluation of four $^{18}$F-labeled sigma-1 receptor tracers in nonhuman primates. (R)-(+)- and (S)-(−)-$^{18}$F-Fluspidine ($^{18}$F-1 and $^{18}$F-2, respectively) were previously found to have high affinity for S1R, metabolic stability, and appropriate binding kinetics in mice (16) and pigs (17). $^{18}$F-2 was also evaluated in healthy human subjects and found to have an effective dose (21.0 $\mu$Sv/MBq) within acceptable imaging limits (18). Spirocyclic piperidines of related structure ($^{18}$F-3 and $^{18}$F-4) also showed good affinity and promising binding kinetics for S1R in mice and rats (14,19). Hence, we evaluated these four PET tracers in nonhuman primates to compare their pharmacokinetic and binding characteristics and assess feasibility for use in human subjects.
In the rhesus monkey brain, all four tracers demonstrated high uptake as shown in TACs presented in Fig. 4A-D. Activity peaked between 10-20 min post injection, indicating fast uptake kinetics. Fast tracer washout was observed for $^{18}$F-2 and $^{18}$F-4, with slow washout for $^{18}$F-1 and $^{18}$F-3. TACs depict $^{18}$F-4 as having the most heterogeneous uptake across brain regions, further displayed in summed images of the adult male monkey (Fig. 6A-D). For all tracers, regional TACs show the highest uptake in the cingulate cortex, insula, and frontal cortex; lower in the hippocampus, temporal and occipital cortices; and lowest in the caudate and thalamus (Fig. 4A-D). While this distribution pattern is in agreement with results from in vivo imaging study with $^{11}$C-SA4503 and in vitro autoradiography study with (+)-$^{3}$H-3-PPP in rhesus monkey brain (29,30), it differs somewhat from ex vivo autoradiography studies with $^{18}$F-3 and $^{18}$F-4 in rats (14,19), which showed high accumulation in the temporal cortex, frontal cortex, and vermian lobule of the cerebellum; moderate uptake in the hippocampus, hypothalamus, and thalamus; and low accumulation in the nucleus accumbens. Studies with $^{18}$F-fluspidine in mice (16) showed highest uptake in the facial nucleus, moderate uptake in the cerebellum, and low binding in the thalamus and caudate/putamen. PET imaging studies using $^{18}$F-1 and $^{18}$F-2 in pigs (17) demonstrated narrow regional differences, with highest uptake in the midbrain, pons, and thalamus; moderate uptake in the hippocampus, temporal and occipital cortices; and lowest in the frontal cortex. The distribution patterns of these four new tracers in monkeys also align well with that of $^{11}$C-SA4503 in humans (31), with high uptake in cortical and limbic areas and lower uptake in the caudate, putamen, and thalamus. Species differences in tracer uptake between rodents, pigs and primates highlight the importance of tracer evaluation in non-human primates before translation to humans.

Regional $V_T$ values appear to be sex independent as comparison baseline scan for $^{18}$F-4 in a 9-year old female rhesus monkey gave numbers similar to those seen in the 7-year old male (Table 2). However, the baseline $V_T$ values obtained with $^{18}$F-2 in the 12-year old female were higher than those from the 7-year old male, which could be due to individual animal variation in
S1R expression, or age effect, as Matsuno et al. reported increased S1R density in aged rhesus monkeys (29).

A comparison of $V_T$ estimates versus scan time demonstrated the bias and regional error associated with shorter scan times (Fig. 5). With 60 min of scan data, $V_T$ values for both $^{18}$F-2 and $^{18}$F-4 were within 10% difference of those derived from the full 240 min data, and within 5% difference when estimated with 90 min of scan data. Tracers $^{18}$F-1 and 3 demonstrated much larger bias and greater errors with shorter scan times, only approaching within 5% difference from the 240 min $V_T$ values at 210 min of scanning time. $V_T$ values for $^{18}$F-1 were underestimated while those of $^{18}$F-3 were overestimated with shorter acquisition times (Fig. 5). For these reasons, $^{18}$F-2 and $^{18}$F-4 gave reliable $V_T$ estimates at 90 min, while $^{18}$F-1 and $^{18}$F-3 required much longer scan times.

Based on their extremely slow tissue kinetics, $^{18}$F-1 and $^{18}$F-3 were deemed unsuitable for PET neuroimaging of S1R in humans, and therefore not selected for the blocking studies. Nondisplaceable volume of distribution ($V_{ND}$) for $^{18}$F-2 and $^{18}$F-4 was estimated from the occupancy plots and used to calculate $BP_{ND}$, with 1TC-derived values displayed in Table 3. These values demonstrate higher specific binding signals for $^{18}$F-4 than $^{18}$F-2. A graphical comparison of $V_T$ as a relative measure of $BP_{ND}$ (27) revealed $BP_{ND}$ rank order of $^{18}$F-3 > $^{18}$F-1 > $^{18}$F-4 > $^{18}$F-2, further supporting the results obtained from the blocking studies. The higher $BP_{ND}$ values for $^{18}$F-1 and $^{18}$F-3 compared to $^{18}$F-2 and $^{18}$F-4 are likely due to their lower $K_i$ values (i.e., higher S1R binding affinity), but higher affinity also contributes to slow and unfavorable binding kinetics in this instance. A comparison of the relative dissociation constants at equilibrium yielded $K_D$ rank order of $^{18}$F-4 > $^{18}$F-2 > $^{18}$F-1 > $^{18}$F-3, which in general are consistent with in vitro $K_i$ measurements (i.e, $K_i$ values for $^{18}$F-4 and $^{18}$F-2 are higher than those of $^{18}$F-1 and $^{18}$F-3), but also reveals some differences between in vivo $K_D$ and in vitro $K_i$ values (Table 1). In addition to individual and species specific variation, these differences between in vitro and in vivo affinities could be due to inter-laboratory variations in methodologies and
techniques, or temperature effects, as *in vitro* $K_i$ measurements were performed at room temperature, while *in vivo* measurements were at body temperature (37 °C). It should be noted, however, that this method of graphical comparison is less useful for $^{18}$F-1 and $^{18}$F-3 because they display nearly irreversible kinetics.

The irreversible nature of binding for $^{18}$F-1 was confirmed in an *in vitro* experiment to measure the individual rate constants ($k_{on}$, $k_{off}$) and the dissociation constants ($K_D$) for $^{18}$F-1 and $^{18}$F-2, which generated $K_D$ value of 0.099 nM for $^{18}$F-2 on cloned human S1R, and $k_{on}$ and $k_{off}$ values of $3.46 \times 10^{-1} \text{min}^{-1}$ and 0.0342 min$^{-1}$, respectively. However, for $^{18}$F-1, $k_{off}$ was extremely slow and could not be reliably measured (See Supplemental Fig. 6 and 7 and Supplemental Table 1).

$^{18}$F-2 and $^{18}$F-4 may offer advantages over previously developed S1R PET tracers. $^{11}$C-SA4503 has been studied in rhesus monkeys (12,29), showing similarly high uptake and regional distributions as $^{18}$F-2 and $^{18}$F-4, and with reasonable subtype selectivity for S1R (S2R/S1R = 103). $^{11}$C-SA4503, however, demonstrates slow washout for the time scale of a $^{11}$C-labelled PET tracer (32) and requires an on-site cyclotron for production. Subsequent studies (33-35) with $^{11}$C-SA4503 showed lower subtype selectivity (S2R/S1R = 13.3-55.0) than previously reported, and when combined with its affinity for VAChT ($K_i = 50 \text{nM}$, VAChT/S1R = 11.3) (34), it may exhibit greater nonspecific binding than $^{18}$F-2 and $^{18}$F-4. A more recent S1R tracer, $^{18}$F-FTC-146, has been evaluated in mice, rats, and squirrel monkeys (36,37). It demonstrated high binding specificity and selectivity ($K_i$ of 0.0025 nM, 364 nM, and 463 nM, respectively, for S1R, S2R, and VAChT), as well as favorable kinetics and promising imaging properties in the squirrel monkey brain (36). Nonetheless, $^{18}$F-FTC-146 has been shown to accumulate in the skull of squirrel monkeys, potentially confounding PET quantitation in the brain and limiting its utility (36). Defluorination and bone uptake of many $^{18}$F-labeled tracers may occur in lower species but not in humans (38), so further studies are warranted for $^{18}$F-FTC-146. Defluorination was not observed for $^{18}$F-2 and $^{18}$F-4. Alongside these possible advantages over previously reported S1R
tracers, $^{18}$F-2 and $^{18}$F-4 show high regional $BP_{ND}$ values, fast kinetics, and the requisite PET imaging characteristics to image and quantitate S1R in the primate brain.

**CONCLUSIONS**

In this report, we compare the binding and kinetic properties of four $^{18}$F-labeled spirocyclic piperidine derivatives in nonhuman primates. Among these tracers, $^{18}$F-2 and $^{18}$F-4 exhibit favorable metabolic profiles, fast brain uptake kinetics, and high specific binding signals in rhesus monkeys. Tracer $^{18}$F-4 has ten-fold higher $f$, three-fold higher $BP_{ND}$, and greater $V_T$ values than $^{18}$F-2 when compared in the same monkey. Both tracers also give reliable estimates of $V_T$ with short (90 min) scan times. Taken together, these data indicate that tracers $^{18}$F-2 and $^{18}$F-4 possess the requisite kinetic and imaging properties as viable PET tracers for imaging S1R in the primate brain, and thus warrant further evaluation in humans.

**DISCLOSURE**

None

**ACKNOWLEDGMENT**

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REFERENCES


FIGURE 1. Structures of previous sigma-1 tracers $^{18}$F-FPS, $^{18}$F-FBP, $^{11}$C-SA4503, $^{18}$F-FTC-146, and spirocyclic piperidine derivatives $^{18}$F-1 to $^{18}$F-4, with in vitro $K_i$ values for S1R (10-15).
FIGURE 2. Syntheses of the four radiotracers. Reagents and conditions: a. $^{18}$F-, Kryptofix 2.2.2, K$_2$CO$_3$, MeCN, 85 °C, 20 min; b. $^{18}$F-, Kryptofix 2.2.2, K$_2$CO$_3$, MeCN, 95 °C, 20 min; c. $^{18}$F-, Kryptofix 2.2.2, K$_2$CO$_3$, MeCN, 80 °C, 10 min; d. Cs$_2$CO$_3$, DMF, 110 °C, 10 min.
FIGURE 3. Plasma analysis of the four radiotracers. (A) Total radioactivity in plasma over time. (B) Time course of parent fraction from the 4 h baseline and 2 h blocking scans with SA4503.
FIGURE 4. Time activity curves of $^{18}$F-1 (A), $^{18}$F-2 (B), $^{18}$F-3 (C), and $^{18}$F-4 (D) from the baseline scans. SUV = standardized uptake value.
FIGURE 5. Time stability of $V_T$ for the four tracers in nonhuman primate brain. Times refer to mid-times of each 10 min acquisition. Data from 60 to 240 min were analyzed in 30 min increments, and $V_T$ is expressed as percentage of the value derived with the complete data set (240 min). Each point is an average $V_T$ from 16 ROIs. Deviation from 100% of mean value indicates bias associated with shorter scanning times, whereas standard deviation indicates regional error associated with shorter scanning times. The bias and error for $^{18}$F-3 at 60 min was too large (>10,000%) to display.
FIGURE 6. PET images (coronal, transverse, and sagittal view) summed from 30-45 min of the baseline scans for $^{18}$F-1 (A), $^{18}$F-2 (B), $^{18}$F-3 (C), and $^{18}$F-4 (D). SUV = standardized uptake value.
FIGURE AND TABLE LEGENDS

TABLE 1
Comparison of binding affinity, selectivity, lipophilicity, and plasma free fraction ($f_p$)

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_i$ (sigma-1)</th>
<th>$K_i$ (sigma-2)</th>
<th>Selectivity</th>
<th>Log $D$</th>
<th>$f_p$</th>
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</thead>
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<tr>
<td>$^{18}$F-1</td>
<td>0.57 nM$^*$</td>
<td>1,650 nM$^*$</td>
<td>2,895$^*$</td>
<td>2.8</td>
<td>2%</td>
</tr>
<tr>
<td>$^{18}$F-2</td>
<td>2.3 nM$^*$</td>
<td>897 nM$^*$</td>
<td>390$^*$</td>
<td>2.8</td>
<td>2%</td>
</tr>
<tr>
<td>$^{18}$F-3</td>
<td>0.79 nM†</td>
<td>277 nM†</td>
<td>351†</td>
<td>2.55</td>
<td>8%</td>
</tr>
<tr>
<td>$^{18}$F-4</td>
<td>2.3 nM†</td>
<td>327 nM†</td>
<td>142†</td>
<td>2.5</td>
<td>18%</td>
</tr>
</tbody>
</table>

$^*$Taken from Holl et al. (15)
†Taken from Li et al. (14)
### TABLE 2
Comparison of 1TC-derived $V_T$ values for the four tracers across different brain regions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Amygdala</th>
<th>Caudate</th>
<th>Cerebellum</th>
<th>Cingulate Cortex</th>
<th>Frontal Cortex</th>
<th>Hippocampus</th>
<th>Occipital Cortex</th>
<th>Putamen</th>
<th>Temporal Cortex</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{18}$F-1 baseline</td>
<td>152.8</td>
<td>180.8</td>
<td>174.9</td>
<td>291.4</td>
<td>215.5</td>
<td>192.8</td>
<td>174.1</td>
<td>199</td>
<td>215.7</td>
<td>127.7</td>
</tr>
<tr>
<td>$^{18}$F-2 baseline</td>
<td>14.6 (31.6)*</td>
<td>13.5 (28.4)*</td>
<td>13.6 (30.2)*</td>
<td>19.6 (37.9)*</td>
<td>17.8 (35.1)*</td>
<td>16.4 (33.5)*</td>
<td>14.6 (30.0)*</td>
<td>14.9 (31.8)*</td>
<td>16.5 (32.6)*</td>
<td>12.2 (30.2)*</td>
</tr>
<tr>
<td>$^{18}$F-3 baseline</td>
<td>574.9</td>
<td>479.3</td>
<td>507.8</td>
<td>1351.9</td>
<td>1099.7</td>
<td>575.8</td>
<td>786.9</td>
<td>530.3</td>
<td>916.8</td>
<td>471</td>
</tr>
<tr>
<td>$^{18}$F-4 baseline</td>
<td>44.4 (42.3)*</td>
<td>36.7 (35.7)*</td>
<td>35.3 (32.7)*</td>
<td>57.9 (56.8)*</td>
<td>46.5 (47.6)*</td>
<td>48.2 (46.5)*</td>
<td>38.2 (31.7)*</td>
<td>38.1 (35.9)*</td>
<td>44.4 (40.6)*</td>
<td>35.3 (38.9)*</td>
</tr>
<tr>
<td>$^{18}$F-2 SA4503 block</td>
<td>6.2</td>
<td>7.1</td>
<td>7.6</td>
<td>8.6</td>
<td>8.3</td>
<td>7.5</td>
<td>8</td>
<td>8.8</td>
<td>8.3</td>
<td>7.2</td>
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<tr>
<td>$^{18}$F-4 SA4503 block</td>
<td>9.4</td>
<td>10.3</td>
<td>9.9</td>
<td>12</td>
<td>11.9</td>
<td>9.8</td>
<td>10.8</td>
<td>11.9</td>
<td>11.5</td>
<td>9.8</td>
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</tbody>
</table>

*baseline $V_T$ values in two different female rhesus monkeys are noted in parentheses for $^{18}$F-2 and $^{18}$F-4
<table>
<thead>
<tr>
<th>Radioligand</th>
<th>Amygdala</th>
<th>Caudate</th>
<th>Cerebellum</th>
<th>Cingulate Cortex</th>
<th>Frontal Cortex</th>
<th>Hippocampus</th>
<th>Occipital Cortex</th>
<th>Putamen</th>
<th>Temporal Cortex</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>18F-2</td>
<td>1.13</td>
<td>0.96</td>
<td>0.98</td>
<td>1.85</td>
<td>1.59</td>
<td>1.38</td>
<td>1.13</td>
<td>1.17</td>
<td>1.40</td>
<td>0.77</td>
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<td>18F-4</td>
<td>3.76</td>
<td>2.94</td>
<td>2.78</td>
<td>5.21</td>
<td>3.99</td>
<td>4.16</td>
<td>3.09</td>
<td>3.08</td>
<td>3.76</td>
<td>2.78</td>
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</tbody>
</table>
Evaluation of Four Sigma-1 PET Tracers: Supplemental

Supplemental Figure 1. Time course for metabolism of $^{18}$F-1 in plasma, showing gamma HPLC chromatograms. The peak at ~11 min is the parent compound, and the rest are metabolites.

HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 60% acetonitrile/40% 0.1 M ammonium formate, flow rate = 1.20 mL/min.
Supplemental Figure 2. Time course for metabolism of $^{18}$F-2 in plasma, showing gamma HPLC chromatograms. The peak at ~11 min is the parent compound, and the rest are metabolites.

HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 60% acetonitrile/40% 0.1 M ammonium formate, flow rate = 1.20 mL/min.
Supplemental Figure 3. Time course for metabolism of $^{18}$F-3 in plasma, showing gamma HPLC chromatograms. The peak at ~11 min is the parent compound, and the rest are metabolites.

HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 45% acetonitrile/55% 0.1 M ammonium formate, flow rate = 1.35 mL/min.
Supplemental Figure 4. Time course for metabolism of $^{18}$F-4 in plasma, showing gamma HPLC chromatograms. The peak at ~10.5 min is the parent compound, and the rest are metabolites.

HPLC conditions: Contents on the capture column were backwashed onto a Phenomenex Luna C18 (2) column (5 µm, 4.6 x 250 mm) eluting with 40% acetonitrile/60% 0.1 M ammonium formate, flow rate = 1.50 mL/min.
Supplemental Figure 5. Receptor occupancy plots for blocking of $^{18}$F-2 and $^{18}$F-4 binding with 0.5 mg/kg of SA4503 in the same adult rhesus monkey. Plot of changes in 1T derived $V_T$ between baseline and 120 min blocking scans vs. 240 min baseline scans for sixteen brain regions for $^{18}$F-2 (A) and $^{18}$F-4 (B). The slope of the regression is the estimated S1R occupancy by SA4503, and the x-intercept is the estimated nondisplaceable volume of distribution ($V_{ND}$) from which $BP_{ND}$ can be calculated.
Association and dissociation studies of $^{18}$F-1 and $^{18}$F-2

Methods

Kinetic studies with rat and human sigma-1 receptors were performed using membrane homogenates obtained from the rat cortex (female SPRD rats, 10-12 weeks old) and HEK293 cells stably transfected with human SIGMAR1 (by courtesy of Olivier Soriani, Institute of Biology Valrose, UNS Université Nice Sophia Antipolis, France), respectively, as well as identical batch of the respective radioligand $^{18}$F-1 [(R)-$^{18}$F-fluspidine] or $^{18}$F-2 [(S)-$^{18}$F-fluspidine]. Association and dissociation experiments were conducted at room temperature in incubation buffer (50 mM TRIS, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$). Non-specific binding was determined by addition of 1 µM haloperidol in the incubation buffer.

Association studies were started with the application of radioligand, and receptor-bound ligand separated from free ligand by filtration (GF-B glass-fibre filter; 48-sample harvester, Brandel, Gaithersburg, MD, USA) after 0.5, 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, and 180 min incubation.

For dissociation studies, receptor preparation and $^{18}$F-1 or $^{18}$F-2 were pre-incubated for 60 min or 180 min, respectively. Dissociation was started by the addition of 30 µM unlabeled 1 or 2 in the incubation buffer. Samples were taken at 0.5, 1, 3, 5, 10, 20, 30, 45, and 60 min after incubation with $^{18}$F-1 or $^{18}$F-2, and additional samples were taken at 90, 120, and 180 min of incubation with $^{18}$F-1. Receptor-bound ligand was separated from free ligand by filtration as described above. Filter-bound radioactivity was measured by gamma-counting (Wallac Wizard 1480, PerkinElmer, Rodgau, Germany) and specific binding at various times calculated.
By non-linear regression analyses (GraphPad Prism 3.0, GraphPad Software Inc., La Jolla, CA, USA), the observed rate constant \( k_{\text{obs}} \) and the dissociation rate constant \( k_{\text{off}} \) were calculated from the association and dissociation experiments, respectively. The association rate constant \( k_{\text{on}} \) was calculated according to \( k_{\text{on}} = (k_{\text{obs}} - k_{\text{off}})/[\text{radioligand}] \), and the \( K_D \) value according to \( K_D = k_{\text{off}}/k_{\text{on}} \). Association half-time (Ass. \( t_{1/2} \)) and dissociation half-time (Diss. \( t_{1/2} \)) were also calculated.

**Results**

The results from kinetic studies of \(^{18}\text{F}-1\) and \(^{18}\text{F}-2\) are presented in Supplemental Figures 6 and 7. Supplemental Table 1 lists the calculated kinetic parameters for \(^{18}\text{F}-1\) and \(^{18}\text{F}-2\). The dissociation rate for \(^{18}\text{F}-1\) was extremely low and could not be reliably measured. As a result, the dissociation constant \( K_D \) could not be determined for \(^{18}\text{F}-1\).

**Supplemental Table 1**: In vitro kinetic parameters of \(^{18}\text{F}-1\) and \(^{18}\text{F}-2\)

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>( \sigma_1 ) receptor type</th>
<th>( k_{\text{on}} ) (M(^{-1})min(^{-1}))</th>
<th>( k_{\text{off}} ) (min(^{-1}))</th>
<th>( K_D ) (nM)</th>
<th>Ass. ( t_{1/2} ) (min)</th>
<th>Diss. ( t_{1/2} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{18}\text{F}-1)</td>
<td>human</td>
<td>0.0318</td>
<td>n.d.</td>
<td>n.d.</td>
<td>22</td>
<td>&gt; 120</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>0.0318</td>
<td>n.d.</td>
<td>n.d.</td>
<td>47</td>
<td>&gt; 180</td>
</tr>
<tr>
<td>(^{18}\text{F}-2)</td>
<td>human</td>
<td>3.46 \times 10^8</td>
<td>0.0342</td>
<td>0.099</td>
<td>7.1</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>5.83 \times 10^8</td>
<td>0.1213</td>
<td>0.208</td>
<td>3.9</td>
<td>5.7</td>
</tr>
</tbody>
</table>

n.d. = non-determinable
Supplemental Figure 6. Association (A, C) and dissociation (B, D) rate measurements of $^8$F-1 in rat cortex homogenates (A, B) and cloned human sigma-1 receptor (C, D).
Supplemental Figure 7. Association (A, C) and dissociation (B, D) rate measurements of $^{18}$F-2 in rat cortex homogenates (A, B) and cloned human sigma-1 receptor (C, D).
PET Imaging Evaluation of Four Novel Sigma-1 Radiotracers in Nonhuman Primates


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