Application of Palladium-Mediated $^{18}$F-Fluorination to PET Radiotracer Development: Overcoming Hurdles to Translation

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Abstract

New chemistry methods for the synthesis of radiolabeled small molecules have the potential to impact clinical positron emission tomography (PET) imaging, if they can be successfully translated. However, progression of modern reactions from the stage of synthetic chemistry development to the preparation of radiotracer doses ready for use in human PET imaging is challenging and rare. Here we describe the process of and the successful translation of a modern palladium-mediated fluorination reaction to non-human primate (NHP) baboon PET imaging—an important milestone on the path to human PET imaging. The method, which transforms $[^{18}$F$]$fluoride into an electrophilic fluorination reagent, provides access to aryl-$^{18}$F bonds that would be challenging to synthesize via conventional radiochemistry methods.

Introduction

Positron emission tomography (PET) is a non-invasive imaging technique used to characterize and diagnose disease [1,2]. Historically, PET imaging has been limited by chemistry. There are few convenient and efficient methods to incorporate positron-emitting isotopes into small molecules of imaging interest. Several positron-emitting isotopes are used for PET imaging, including carbon-11, nitrogen-13, oxygen-15, and copper-64, but due to its suitable half-life for synthesis and imaging, fluorine-18 ($^{18}$F) is the most common and most relevant isotope for clinical PET [3,4]. Additionally, fluoride is present in a variety of biologically active molecules and pharmaceuticals [5,6]. However, synthesis with $^{18}$F is difficult due to time restrictions (half-life of $^{18}$F: 110 min) and mass restrictions (scale of synthesis: nanomoles), among others [7]. Thus the general chemical challenges associated with carbon–fluorine bond formation [8,9,10] are exacerbated for $^{18}$F-radiotracer synthesis. Modern methods for the construction of carbon–fluorine bonds are changing the landscape of molecules considered viable for PET [9]. The new chemical methods hold unrealized potential of changing radiotracer design and applications, is important for imaging biological targets of low concentration, like imaging of neurotransmitter receptors in the brain [4]. For pharmacokinetic measurements, specific activity is less critical. 3) $[^{18}$F$]$Fluoride is created by the proton bombardment of $[^{18}$O$]H_2O. While removal of the bulk water is common for synthesis with $^{18}$F, the large hydration energy [12,13,14] and the small amount of fluoride prevents rigorous drying. Hydrated fluoride is less nucleophilic than dry fluoride and therefore cannot
be used in many nucleophilic fluorination reactions. Most recently
developed fluorination reactions do not meet or have yet to meet
one or more of the above considerations. For example, reactions
that require dry solvent or long reaction times are difficult to
transition to radiochemistry, even if yields are high for the
fluorination reactions with \(^{18}\)F, the natural isotopologue.

Once chemistry with \(^{18}\)F is feasible (i.e. a fluorination reaction
can be accomplished with \(^{18}\)F, and a radiochemical yield (RCY)
can be determined), the reaction still may not lead to a practical
synthesis method for molecules useful for PET imaging experi-
ments [15]. Most importantly, radiochemistry techniques must
allow for efficient purification due to time constraints. The
formation of constitutional isomers that are difficult-to-separate
must be minimized during synthesis, and starting materials as well
as byproducts must be removable from the desired sample. In
particular hydro-delhluoro and halo-delhluoro (halo = chloro, bro-
mo, iodo) can be difficult to separate from radiofluorinated
material. Final samples must be radiochemically and chemically
pure and sterile. Additionally, a substantial quantity of radioac-
tivity must be obtained at the end of synthesis; typically 5–10 mCi
per experiment for non-human primates is suitable for imaging
[1]. Automated robotic procedures are utilized to handle this
amount of radioactivity.

Two molecules, targeting the serotonin neurotransmitter
system, were identified and selected for transitioning our new
fluorination method to PET imaging applications [16]. Each
molecule represents one example of the two general functional
uses of PET imaging in drug discovery: 1) labeling a drug to assess
its biodistribution as well as pharmacokinetic and metabolic
profile; and 2) labeling a bioactive molecule to evaluate its
potential as a radiotracer, a marker of a biological system [17]. For
the labeled drug example, paroxetine (1), an antidepressant
selective serotonin reuptake inhibitor (SSRI) medication with
nearly 13 million prescriptions in the US in 2010 [18], was
selected (Figure 1). Despite its widespread use, the pharmacoki-
netic profile and distribution of paroxetine binding in the human
brain are unknown. There has been previous interest in labeling
paroxetine for PET imaging in humans [19], but only paroxetine
derivatives [20], not paroxetine itself, could be labeled with \(^{18}\)F,
presumably due to lack of appropriate chemistry methods. As a
representative molecule for the second class, the 5-HT\(_{2C}\) agonist 2
[21] was selected for evaluation as a potential radiotracer for
imaging 5-HT\(_{2C}\) receptors, an important target in the develop-
ment of new drugs to treat obesity [22] and a target for which no
radiotracer exists (5-HT = 5-hydroxytryptamine = serotonin) [23].
Both molecules would be difficult to radiolabel with \(^{18}\)F using
conventional fluorination strategies.

We recently reported a conceptually different arene radio-
fluorination reaction that relies on C–F bond formation at a
transition metal center [24]. The novelty of the method consists of
\(^{18}\)F-fluoride capture to form an electrophilic fluorination reagent
that could be used for late-stage fluorination. The development
and translation of the method into a reliable, reproducible arene
radiofluorination method that can afford radiolabeled molecules
on a scale suitable for PET imaging in non-human primates
(NHPs) is presented here. The complete method from acquisition
of \(^{18}\)F, through automated synthesis, purification and formulation
for intravenous injection can be performed in less than 100
minutes. The method enabled the first syntheses of \(^{18}\)F-1 and
\(^{18}\)F-2, provided material sufficient in quality and quantity for
PET brain imaging studies in NHPs, and showcased the ability to
obtain \(^{18}\)F-labeled molecules in high specific activity for PET
imaging in NHP that would be challenging to obtain with
conventional fluorination methods. Our results establish that the
palladium-mediated fluorination has the potential to serve as a
research tool for in vivo imaging in drug discovery and radiotracer
development.

Results and Discussion

Development of an Electrophilic Fluorination Reagent
Derived from Fluoride

Radiofluorination with electrophilic \(^{18}\)F is challenging because
the reagent most often used, \(^{18}\)F-fluorine gas (\(^{18}\)F\(_2\)), is difficult
to work with, requires specialized equipment, and is made in low
specific activity [3]. To efficiently liberate \(^{18}\)F from the target, the
sample is purposefully diluted with \(^{19}\)F\(_2\). While progress has
been made to improve the isotopic enrichment of \(^{18}\)F\(_2\) [11], the
specific activity of molecules synthesized from \(^{18}\)F\(_2\) cannot
reach the level of \(^{18}\)F-fluoride because only one of the fluoride
atoms of \(^{18}\)F\(_2\) is \(^{18}\)F. Furthermore, radiochemical yield can be
at most 50% because only one of the fluoride atoms is transferred,
with essentially equal probability. Methods have been devised to
synthesize more practical electrophilic radiofluorination reagents
[25,26,27], but they have all been derived ultimately from \(^{18}\)F\(_2\). Nucleophilic \(^{18}\)F-fluoride is a more practical source of \(^{18}\)F,
because it is widely available and produced without purposeful
dilution with \(^{19}\)F. However, nucleophilic fluorination reactions are
challenging to transition to radiochemistry given hydrated
fluoride’s limited nucleophilicity. A practical electrophilic radio-
fluorination reaction using high specific activity \(^{18}\)F-fluoride may
enable new areas of PET imaging research.

We reported the first high-valent transition metal fluoride
complexes observed to undergo reductive elimination to form arex
fluorides [28,29]. We subsequently discovered that high-valent
palladium fluoride complexes could behave as electrophilic
fluorination reagents, when other reductive processes such as
reductive elimination were inhibited by an appropriate choice of
ligands [24]. But, high-valent transition metal fluorides, including
those described in our original report [28], are typically themselves
synthesized from electrophilic fluorination reagents and not from
fluoride, which would not overcome the specific activity challenge
when dealing with \(^{18}\)F. Therefore, we designed a high-valent
organometallic complex 6 that can capture fluoride via ligand
exchange to form a high-valent transition metal fluoride complex
7, which can serve as an electrophilic fluorination reagent
(Figure 2). The complex was synthesized via benzo[\(h\)]quinoline
cyclopalladation, followed by ligand exchange with potassium
tetracyanopyrrolid borate to afford pallad(II) complex 3 that was
oxidized to high-valent palladium(IV) complex 5 using the \(\lambda\)-
iodane reagent 4. Exchange of the apical 4-cyanopyridine ligand

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Figure 1. Two small molecule aryl fluorides chosen for radio-
fluorination and non-human primate PET imaging presented
here.

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with picoline provided complex 6 that can capture fluoride. Complex 6, which can be synthesized on multi-gram scale, is not unique in its ability to capture fluoride. For example the pyridine (instead of picoline) analog of 6 can capture fluoride as well (see Supporting Information S1). Nevertheless, 6 is currently the complex of choice because the picoline ligand imparts enough electron-donating character to the electron-deficient palladium center for long-term storage, but can quickly be displaced by fluoride.

**Important Characteristics of High-valent Organometallic Complexes 6 and 7**

Complex 6 can capture $^{18}$F from samples of $[^{18}$F$]$_fluoride (Figure 3, Step 1) and then serve as an electrophilic $^{18}$F fluorination reagent (Figure 3, Step 2). Complex $[^{18}$F$]·7$ can be used as the electrophilic reagent, for the fluorination of pyridylsulfonamide-stabilized palladium (II) complexes 9 (see Figure 4) [30], and can provide access to electron-rich, electron-neutral, and electron-poor functionalized aryl fluorides. High-valent palladium complex 7 is likely the first of a class of complexes that exhibit the desired reactivity (capture and electrophilic transfer of fluoride to make C–F bonds).

The ability of 6 and 7 to capture and transfer fluoride is unusual. Complex 7 functions as an electrophilic reagent, similar to conventional electrophilic fluorination reagents previously used [30]. Yet, unequivocally, fluoride is the original source of fluorine. The generation of an electrophilic fluorination reagent from fluoride, without electrochemical methods, is challenging because fluorine is the strongest elemental oxidant known. From an electrostatic standpoint, the electropositive palladium center of fluoride is the strongest elemental oxidant known. From an electrostatic standpoint, the electropositive palladium center of fluoride is needed. Specifically, tridentate coordination is important to stabilize the octahedral geometry for Pd(IV)$^d^6$, but, with minimal structural reorganization, bidentate coordination, of a square planar Pd(II)$^d^8$ complex is possible, the geometry of the complex resulting after fluoride transfer. The ability to access different coordination modes may lower the activation barrier to fluorination. Finally the trflate counterion was chosen because it is non-coordinating so as to not displace fluoride from the palladium center, and it does not contain readily exchangeable fluoride. Common, non-coordinating counterions such as BF$_4^-$ would introduce exogenous $^{19}$F into the sample and lower the specific activity of the final molecule.

![Figure 2. New electrophilic fluorination reagent. Synthesis of high-valent palladium complex 7 that is an electrophilic fluorination reagent derived from complex 6 through fluoride capture. doi:10.1371/journal.pone.0059187.g002](image)

**Synthesis and Radiofluorination of Complex Small Molecules**

Radiolabeled $[^{18}$F$]·7$ can be used as the electrophilic reagent in the palladium-mediated fluorination reaction we have previously disclosed [30] to form radiolabeled ary1 fluorides (Figure 4). The mechanism of fluorination is proposed to occur by oxidation of fluoride from $[^{18}$F$]·7$ to 9 to form a new Pd(IV)$^{18}$F complex, which can then undergo C–F reductive elimination to afford $^{18}$F-labeled ary1 fluoride. Evidence for oxidative fluoride transfer has been established: A high-valent ary1 Pd(IV)$^{18}$F complex could be observed by NMR spectroscopy after oxidation of an ary1 Pd(II) complex, specifically 9 with the ary1 and pyridine substituent linked, with 7 [24]. Complex small molecule substrates can be made in a modular fashion by transmetalation of ary1 boronic acid derivatives with palladium acetate complex 8, synthesized in four steps from commercially available reagents (see Supporting Information S1). Many palladium(II) ary1 complexes, for example 15 and 20 (substrates leading to 1 and 2), can be purified by chromatography on silica gel, are stable to air and moisture, and can be stored for extended periods of time without decomposition (>1 year). Syntheses of palladium ary1 complexes 15 and 20 are shown in Figures 5 and 6.

Palladium complexes 15 and 20 were constructed from commercially available starting materials using a placeholder functional group at the eventual site of fluorination. For syntheses via late-stage fluorination of 1 and 2, ary1 bromides are used as the placeholders then changed to ary1 boronic esters that can undergo transmetalation on to the palladium center in the penultimate step. There are no radiochemistry time constraints associated with the synthesis of the palladium ary1 complexes, because $^{19}$F is only incorporated after the palladium substrates are synthesized. For the non-chemists, fluorination precursors (e.g. 15) are not trivial to make, but could be made available for purchase. Palladium complex 15, the precursor to paroxetine (1) is synthesized via a route similar to a published route [32], but with an ary1 bromide in lieu of an ary1 fluoride, which simplifies access to the desired substrate. Alkylation, acylation, and cyclization reactions starting form of 1-(4-bromophenyl)-3-chloropropan-1-one affords dihydro-pyridone 10 (Figure 5). Asymmetric conjugate reduction using conditions developed by Buchwald [32] provide enantiomERICALLY enriched $\delta$-lactam 11 in 88% ee. Recrystallization of an intermediate later in the synthesis 12 provides material that is $>99\%$ ee. A Claisen
condensation followed by reduction gives alcohol 12. Protecting group exchange followed by Mitsunobu reaction with sesamol and DIAD affords 13 that possesses the full carbon skeleton of paroxetine. Borylation yields 14 and transmetalation provides palladium complex 15.

For the palladium complex 20 leading to 5-HT2C agonist 2, alkylation, olefination, and reduction of 5-bromosalicaldehyde affords allylic alcohol 16 that is used in a Charette asymmetric cyclopropanation reaction [33] to yield cyclopropane 17 in 96% ee (Figure 6). The enantiomeric excess can be upgraded at a later point (18) via recrystallization to >99% ee. Alcohol to amine conversion and deprotection leads to the aryl bromide 18 that undergoes palladium-catalyzed borylation to afford 19. Aryl boronic ester is converted to palladium complex 20 through transmetalation.

We have found that the yield of the “cold” (19F) fluorination reactions for a given substrate roughly correlates with the radiochemical yield of the “hot” (18F) fluorination reactions. Palladium aryl substrates 15 and 20 were fluorinated with the non-radiolabeled (19F) version of palladium complex 6 to afford small molecule aryl fluorides 21 and 22 in 71% and 72% yield, respectively (Figure 7; Equations 1 and 2). Removal of protecting groups reveals paroxetine (1) and the 5-HT2C agonist 2.

Scope and Limitations of Fluorination Reaction

Fluorination of 15 and 20 demonstrates the functional group tolerance of our protocol, and establishes that late-stage fluorination in the presence of a variety of functional group is possible for electron-rich arenes, that are often challenging to prepare with conventional fluorination reactions. Currently, our method does not tolerate nucleophilic, basic functional groups, such as certain amines. While pyridine-substituents are tolerated, amines of higher basicity such as tertiary alkyl amines are not. We have overcome this limitation in some cases through the use of protecting groups such as the Boc groups used for substrates 15 and 20. Additionally, substitution besides hydrogen ortho to the site of fluorination reduces the efficiency of palladium aryl substrate synthesis and fluorination. An additional limitation is the required synthesis of the palladium complexes themselves. While generally stable, the synthesis requires some knowledge of organometallic chemistry.
Practical and Procedural Considerations for the Transition to Radiochemistry

The syntheses of $[^{18}F]^{-1}$ and $[^{18}F]^{-2}$ with $[^{18}F]$fluoride were accomplished with procedural modifications based on the physical and practical differences between fluorination chemistry with $^{18}$F and $^{19}$F. First, $[^{18}F]$fluoride was prepared following common radiochemistry protocols from a sample produced in $[^{18}O]$H$_2$O by a cyclotron [34]. Second, we adapted the procedures for the difference in stoichiometry between reactions with $^{19}$F and $^{18}$F. For high specific activity $[^{18}F]$fluoride reactions only nanomoles of the electrophilic fluorination reagent are synthesized, which remain in the presence of a large excess of 6, the palladium precursor. In practice, we found that it was not feasible or advantageous to rapidly and cleanly isolate $[^{18}F]^{-7}$ from the mixture. Therefore, the $^{18}$F method was modified into a two-step process with the reagent $[^{18}F]^{-7}$ made in situ.

The two-step fluorination reaction can afford radiolabeled aryl fluorides on a scale suitable for PET imaging (Figure 8). Several variables were explored and optimized to accomplish efficient and quick fluorination with $[^{18}F]^{-7}$. Samples of $[^{18}F]$fluoride in H$_2$O with KHCO$_3$ and the common phase transfer reagent, 18-crown-6, were azeotropically dried with MeCN and acetone (Figure 8A). 18-Crown-6 was chosen instead of common cryptand phase transfer reagents (e.g. Kryptofix® 2.2.2), because 5 reacts unproductively with the amine functionality found in Kryptofix® 2.2.2. Initial variability in terms of overall radiochemical yield was often associated with the drying process (“dry-down”) and resolubilizing of fluoride. While the reaction tolerates the presence of water, the efficiency decreases as water concentration increases. Next, palladium complex 5, dissolved in acetone, was added for fluoride capture to form fluorinating reagent $[^{18}F]^{-7}$ in less than 10 minutes. Before fluorination, the reaction mixture is filtered over a pyridine-functionalized resin, JandaJel™, as a quick intermediate purification step, which resulted in higher yields compared to experiments omitting filtration. Filtration removes byproducts that precipitate during the reaction. Pyridine-functionalized solid support is more effective than other filtration methods, which may be due to pyridine capturing 5 by displacing...
the picoline ligand. After filtration, the palladium aryl substrate is added, and the resulting solution was heated at 85°C in a sealed vial.

Generally, radiochemistry methods are first explored using hand manipulations on a small amount of radioactivity (<500 μCi of [18F]fluoride). However, a typical PET imaging experiment in NHPs requires 5–10 mCi of final radiolabeled pure product, which along with the efficiency of the reaction dictates that the starting amount of [18F]fluoride needs to be anywhere from 50 mCi to 1 Ci of radioactivity. Currently, our fluorination method requires starting with 1 Ci to produce enough for NHP PET imaging experiments. Due to safety considerations, automated synthesis protocols were developed using a commercially-available platform and took place inside of a hot cell. Synthesis modules were programmed to run the reaction (azeotropic drying, fluoride capture, filtration, and fluorination; see Supporting Information S1). The automated procedures can now be used to produce tens of mCi of radiolabeled aryl fluorides prior to purification (Figure 8B and 3C).

Post synthesis protocols include 1) filtration through a plug of silica to remove bulk metal contaminants, 2) removal of protecting groups, 3) HPLC purification for isolation, and 4) formulation in a sterile solution for administration. Post synthesis manipulations are currently conducted by hand, though ideally will be automated in the future.

While protecting groups are common in radiochemistry, deprotection reactions must be fast and efficient. For the two radiolabeled molecules in this research, we chose to protect the primary and secondary amines with easily removable t-butoxycarbonyl (Boc) groups. Exposure of [18F]-21 and [18F]-22 to trifluoroacetic acid for two minutes revealed the ammonium salts.

Because the mass of fluorinated material using high specific activity [18F]fluoride is typically less than 10 μg among milligrams of reaction reagents, HPLC can be an efficient and often used tool to remove contaminants and byproducts. We have devised routine HPLC methods that purify [18F]-1 and [18F]-2 with standard semi-preparatory columns in less than 15 minutes. Final samples are chemically and radiochemically pure and have less than 5 parts per billion (ppb) content, well below suggested guidelines for use in humans (<1000 ppb) [36]. The residual amount of palladium was determined by inductively coupled plasma mass spectroscopy (ICP-MS) analysis.

After HPLC purification, the sample is captured with a solid phase extraction column and formulated in a sterile saline solution. Final samples were analyzed for purity, residual solvent, pH, pyrogenicity, and sterility and passed all quality control protocols generally used for PET imaging in NHPs.

Starting with an approximated 1 Ci of radioactivity, our method can be used to produce >10 mCi of formulated product in up to 13 Ci/μmol specific activity at time of injection (TOI), suitable for imaging primates. The entire process from obtaining aqueous samples of [18F] through formulation can be accomplished in less than 100 min.

**Non-human Primate PET Imaging with [18F]-1 and [18F]-2**

Using the procedure outlined above, we prepared [18F]-1 and [18F]-2 for use in four separate MR-PET imaging experiments in baboons (Papio anubis). The objective of these studies was to determine the brain pharmacokinetic profile and distribution of [18F]-1 and [18F]-2 and compare the binding and kinetic profile to scans performed after a drug challenge. This “test-block” procedure provides information about the nature of the radioactive signal that is observed. The initial protocol is designed to determine the contribution of specific and non-specific binding to the PET signal. Specific binding interactions, (i.e. [18F]-1 with SERT [37]; [18F]-2 with 5-HT2C), will undergo competition with drug pretreatment resulting in an overall reduced PET signal; however binding interactions that are non-specific and not in competition with the drug will remain the same. Determining the
extent to which the signal observed is a specific binding interaction is the first and most crucial step in radiotracer validation for receptor systems in the brain; it is also one of the least predictable outcomes in radiotracer development [38]. We chose blocking drugs (citalopram [39] 5.0 mg/kg for SERT; ritanserin [40] 0.1 mg/kg for 5-HT2C) that were known to be safe for administration intravenously [41,42] rather than performing self-saturation experiments with the non-radioactive analogs of \(^{18}\text{F}\)-1 and \(^{18}\text{F}\)-2.

Radiolabeling drug molecules with positron-emitting isotopes has intrinsic value, which is particularly true of central nervous system (CNS) drugs. Inferences about brain penetration and pharmacokinetic profile (PK) in humans are often made through extrapolation from studies in lower mammals and drug concentrations in human plasma, because observing drug concentration at target sites in vivo in humans is challenging.

Many drug molecules, commonly prescribed for the human CNS, are used without any knowledge of human brain PK. Understanding the brain distribution and kinetic profile of drugs or drug candidates in humans can help understanding drug failures and may ultimately provide better predictive tools for success in drug development [43,44]. While there are many reasons why drugs are not often labeled and evaluated with PET, a striking number of molecules cannot be labeled with either of the two most common PET isotopes, carbon-11 and fluorine-18. Paroxetine, a selective serotonin reuptake inhibitor, is a good example of a molecule that was previously considered inaccessible for high specific activity labeling approaches. The challenge of labeling paroxetine (I) was overcome using palladium-mediated fluorination and provided \(^{18}\text{F}\)-1 in suitable yield for initial studies in NHPs.

Figure 8. Palladium-mediated synthesis of \(^{18}\text{F}\)-1 and \(^{18}\text{F}\)-2 using \(^{18}\text{F}\)-7 derived from \(^{18}\text{F}\)fluoride. (A) Synthesis of the electrophilic fluorination reagent \(^{18}\text{F}\)-7 for reaction with palladium aryl complexes. (B) Synthesis of SSRI \(^{18}\text{F}\)-1 on a scale suitable for NHP PET imaging. (C) Synthesis of 5-HT2C agonist \(^{18}\text{F}\)-2 on a scale suitable for NHP PET imaging.
PET Study to Evaluate Biodistribution and Pharmacokinetic Profile

Two MR-PET imaging studies were performed with \([^{18}F]\)-1, a baseline and a challenge with citalopram, a competitive serotonin reuptake inhibitor. The baseline scan revealed modest brain penetration (0.013% ID/cc) corresponding to about 1.7% of the total injected dose accumulating in the brain. Penetration into and accumulation in the brain were rapid (Figure 9), reaching a plateau between 15–30 min. Washout kinetics were markedly slow with a t₁/₂ calculated from the data (35–90 min) of 21.3 h. This is consistent with the plasma half-life range (21–24 h) reported in the monograph for Paxil [45] (one of the trade-names for paroxetine).

The accumulation of \([^{18}F]\)-1 represents predominantly non-specific uptake in the brain. Heterogeneous binding was observed, but was inconsistent with the known SERT distribution from PET studies with validated radiotracers such as \([^{13}C]\)DASB [23]. The lack of specific binding was confirmed by citalopram challenge, in which there was no significant change in the accumulation and distribution of \([^{18}F]\)-1 relative to baseline. Although data analysis with \([^{18}F]\)-1 may be limited by the high degree of non-specific binding, the prevalence of paroxetine used to treat depression warrants evaluation of brain PK and distribution in humans.

PET Study to Evaluate a Potential PET Radiotracer

Our choice to evaluate \([^{18}F]\)-2 as a selective radiotracer for the serotonin 2c (5-HT₂C) receptor was based upon the growing associations between 5-HT₂C and brain-related disorders [46,47,48,49]. Although the associations exist, the 5-HT₂C receptor has previously received less attention in psychopharmacology and neuroimaging than its homolog, 5-HT₂A. To date, selective 5-HT₂C radioligands for autoradiography and 5-HT₂C radiotracers for imaging have not been described, a fact which has no doubt hindered research in neurobiology. Due to the recent development of novel scaffolds for potent and selective ligands of 5-HT₂C, in vivo imaging of 5-HT₂C has become a realistic goal [46]. Very few, if any, of the molecules that selectively target 5-HT₂C are obvious labeling candidates for either carbon-11 or fluorine-18; much less, compounds for which in vivo data support the likelihood for radiotraacer development success. Agonist 2 was chosen from the literature not only because it was well-suited for the chemistry described, but also because of its selectivity profile (40-fold and 14-fold selective for 5-HT₂C over 5-HT₂A and 5-HT₁B, respectively) and its demonstrated efficacy in an animal model of antipsychotic drug activity [21]. To our knowledge, this is the first reported evaluation of a 5-HT₂C-targeted molecule for use in PET.

Two preliminary imaging studies in non-human primates were used to determine whether \([^{18}F]\)-2 exhibited suitable blood-brain penetration, pharmacokinetic profile, and specific binding suitable for use as a 5-HT₂C PET tracer (Figure 10). Data from the baseline study indicate that \([^{18}F]\)-2 has excellent brain penetration with an average brain : plasma ratio of 1.5 at 30 minutes post injection. Analysis of arterial plasma from this experiment suggested that the compound had suitable metabolic stability (nearly 70% of radioactivity in plasma was associated with intact \([^{18}F]\)-2 at 60 min) to warrant continuation to pharmacological challenge studies. For the initial drug challenge, we chose ritanserin, which is a non-selective 5-HT₂A \((E C_{50} = 4.6 \text{ nM})\), 5-HT₂B \((2.2 \text{ nM})\), and 5-HT₅ \((6.6 \text{ nM})\) antagonist [50]. We observed a modest 15% decrease in uptake of \([^{18}F]\)-2 following ritanserin administration. The decrease was observed in the thalamus and throughout the cortex (frontal, medial parietal and temporal), but not in the cerebellum. Decreases in the thalamus and cortex are reasonably correlated with expectations from 5-HT₂C mRNA mapping in the monkey [51] and human brains [52] ex vivo. The lack of blockade in the cerebellum is also consistent with the assumed 5HT₅ receptor distribution. One notable exception is that higher levels of uptake and blockade were anticipated in the choroid plexus. Our preliminary data, which indicate modest specific binding, are encouraging and warrant additional studies to more accurately quantify the reduction in binding potential of \([^{18}F]\)-2 during pharmacological challenge.

Methods

See Supporting Information S1.

Conclusions

We have developed a modern fluorination reaction with large substrate scope that can be used for the synthesis of complex \(^{18}F\)-labeled aryl fluorides suitable for imaging in NHPs. Our synthesis of radiolabeled aryl fluorides employs \(^{18}F\)fluoride, is fast, high-yielding, and affords only one aryl fluoride constitutional isomers and no inseparable byproducts. We have demonstrated that our method can be translated to synthesize radiolabeled aryl fluorides \([^{18}F]\)-1 and \([^{18}F]\)-2 in quantity and quality suitable for PET imaging studies in baboons to obtain pharmacokinetic data. Our method can tolerate a large variety of functional groups, and is suitable for electron-rich and –deficient arenes, which is a

![Figure 9. Distribution and pharmacokinetic profile of \([^{18}F]\)paroxetine (\([^{18}F]\)-1) from MR-PET imaging in non-human primates. (A) Summed images from 5–50 min of the dynamic PET collected following injection of \([^{18}F]\)-1. The images are fused with a structural (T1 weighted) MR image. Although \([^{18}F]\)-1 is a potent and selective ligand for the serotonin transporter (SERT), the observed binding does not match the distribution of SERT. This is indicative of high non-specific binding. The images highlight the overall high blood-brain-barrier penetration of \([^{18}F]\)-1 and heterogeneity of binding observed. (B) Whole-brain average time-activity curves from \([^{18}F]\)-1 from baseline and pretreatment (citalopram) studies. doi:10.1371/journal.pone.0059187.g009](https://doi.org/10.1371/journal.pone.0059187.g009)
challenge for conventional fluorination reactions. Current limitations of the method are the inability to tolerate certain nucleophilic functional groups, most notably tertiary amines, and the substantially lower yields for substrates with ortho substitution. Moreover, our method is not as simple to perform as a conventional nucleophilic aromatic substitution reaction and requires the synthesis of organotransition metal reagents.

We see utility in our method for the predictable synthesis of 18F-labeled molecules that can support drug development, for example, small molecule drug candidates with drug-like structures such as paroxetine can now be labeled. In addition, we provide a tool that enables the synthesis of 18F-labeled molecules for evaluation as potential PET tracers that report on a biological function. PET tracer development is challenging beyond synthesis, as exemplified by the high-nonspecific binding of paroxetine, which makes [18F]paroxetine ([18F]-1) unsuitable for imaging of SERT. But the ability to make more complex and structurally more diverse 18F-labeled molecules more quickly, more reliably, and more efficiently than previously possible, may shift the future challenge toward PET tracer development rather than PET tracer synthesis. New fluorination methods that expand the number and types of molecules that can be radiolabeled blended with advances in tracer development should increase the impact of PET imaging.

In this respect, we are particularly excited about a new method developed in our group, which is based on nickel complexes [53], and may be more easily executed, also by the non-expert.

Supporting Information

Supporting Information S1 Contains details on materials and methods, experimental data for chemistry and radiochemistry, and spectroscopic data. (PDF)

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Author Contributions

Conceived and designed the experiments: ASK CNN JMH TR. Performed the experiments: ASK CNN EL SMC CKM NS. Analyzed the data: ASK CNN JMH TR. Contributed reagents/materials/analysis tools: EL SMC CKM NS. Wrote the paper: ASK JMH TR.

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