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Radiochemical synthesis and *in vivo* evaluation of [^{18}F]AZ11637326: An agonist probe for the α7 nicotinic acetylcholine receptor

Hayden T. Ravert [a], Peter Dorff [b], Catherine A. Foss [a], Ronnie C. Mease [a], Hong Fan [a], Christopher R. Holmquist [a], Eifion Phillips [b], Dennis J. McCarthy [b], J. Richard Heys [b], Daniel P. Holt [a], Yuchuan Wang [a], Christopher J. Endres [a], Robert F. Dannals [a], Martin G. Pomper [a,*]

[a] *Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins Medical School, 1550 Orleans St., 492 CRB II, Baltimore, MD 21287–0014*
[b] *AstraZeneca, Wilmington, DE 19850*

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ABSTRACT

Introduction: The alpha-7 nicotinic acetylcholine receptor (α7 nAChR) is key in brain communication and has been implicated in the pathophysiology of diseases of the central nervous system. A positron-emitting radioligand targeting the α7 nAChR would enable better understanding of a variety of neuropsychiatric illnesses, including schizophrenia and Alzheimer's disease, and could enhance the development of new drugs for these and other conditions. We describe our attempt to synthesize an α7 nAChR-selective radiotracer for positron emission tomography (PET).

Methods: We prepared the high-affinity (K_d = 0.2 nM) α7 nAChR agonist, 5′-(2-[^{18}F]fluorophenyl)spiro[1-azabicyclo-[2.2.2]octane]-3,2′-(3′H)furo[2,3-b]pyridine, [^{18}F]AZ11637326, in two steps, a nucleophilic fluorination followed by decarbonylation. We studied [^{18}F]AZ11637326 in rodents, including mice lacking α7 nAChR, and in non-human primates.

Results: [^{18}F]AZ11637326 was synthesized in a non-decay-corrected radiochemical yield of 3% from the end of synthesis (90 min) with a radiochemical purity >90% and average specific radioactivity of 140 GBq/μmol (3,781 mCi/μmol). Modest rodent brain uptake was observed (2–5% injected dose per gram of tissue, depending on specific activity), with studies comparing CD-1 and α7 nAChR null mice indicating an element of target-specific binding. Blocking studies in non-human primates did not reveal specific binding within the brain.

Conclusion: Despite the high affinity and target selectivity of AZ11637326 for α7 nAChR in vitro and encouraging rodent studies, receptor-mediated binding could not be demonstrated in non-human primates. Further structural optimization of compounds of this class will be required for them to serve as suitable radiotracers for PET.

1. Introduction

The alpha-7 nicotinic acetylcholine receptor (α7 nAChR) is a pentameric, cationic, ligand-gated calcium channel that is abundant within the central nervous system where it mediates a variety of functions including modulation of other transmitter systems, plasticity and other aspects of communication within the brain [1,2]. Dysregulation of the α7 nAChR has been implicated in schizophrenia, a variety of dementing disorders, inflammation and, in the periphery, cancer [3–6]. Because of its importance, efforts are directed toward the development of therapeutic agents targeting the α7 nAChR. For example, a recent clinical trial demonstrated that the partial α7 nAChR agonist TC-5619 proved efficacious in treating cognitive dysfunction and negative symptoms in patients with schizophrenia [7]. Agonists, antagonists and positive allosteric modulators are currently under development for the treatment of disorders in which the α7 nAChR has been implicated [8–13].

A radiotracer can be used not only to study pharmacokinetics in relevant model systems, but if developed in positron-emitting form it can be used to study biodistribution, target selectivity and for dose-finding (occupancy) studies in human subjects. While the α4β2 nAChR has been an amenable target to efforts at developing a viable radiopharmaceutical, with many studies having appeared in human subjects [14], the α7 nAChR has proved less tractable. Reasons include the relatively low α7 nAChR concentration in target areas of the brain, namely, on the order of 2–30 fmol/mg protein in humans [2]. By contrast α4β2 nAChR, which are present at concentrations of 40–80 fmol/mg have required extremely potent radioligands, on the order of K_d = 150 pM, to be useful in clinical studies [2]. Because

* Corresponding author. Tel.: +1 410 955 2789; fax: +1 443 817 0990.
E-mail address: mpomper@jhmi.edu (M.G. Pomper).

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target site concentration and required affinity for imaging are inversely related, an even higher affinity is likely to be necessary for imaging α7 nAChR target sites in human brain.

Several reviews have been published that outline efforts at developing radiotracers targeting the α7 nAChR [1,2]. The focus of these efforts has been on the orthostatic acetylcholine binding site and includes compounds bearing the quinuclidine or anabaseine nuclei, among others [15–17]. Notably, a compound based on the 1,4-diazabicyclo[3.2.2] nonane nucleus, [^{11}C]CHIBA-1001, has been studied in conscious monkeys, with dosimetry and biodistribution reported in human subjects [18–20]. Nevertheless, neither [^{11}C]CHIBA-1001 nor any other α7 nAChR-targeted radiotracer has yet demonstrated clinically useful binding specificity, generally indicated by receptor blockade in non-human primate studies. Availability of the X-ray crystal structure of the acetylcholine binding protein [21] has not facilitated the development of potent radiotracers for the α7 nAChR.

The tritiated spirofuropyridine, 5′-[2-fluoro-3,4,5-[^{3}H]phenyl]-spiro[1-azabicyclo-[2.2.2]octane]-3,2′-(3′H)furo[2,3-b]pyridine, ([^{3}H]AZ11637326) has been exhaustively characterized in rodent with respect to brain penetration, localization, biodistribution and binding selectivity, performed in vitro and ex vivo using autoradiography [22,23]. Salient results include demonstration of adequate brain penetration and binding selectivity to the known α7 nAChR target sites of hippocampus and frontal cortex, determined through dose-dependent blockade using the known, high-affinity α7 nAChR antagonist methyllycaconitine (MLA). The α7 nAChR shares approximately 30% sequence homology with the 5-HT$_3$ receptor [2,24], which provides an impediment to the development of selective agents for the former. The lack of selectivity of the first-generation quinuclidine radiotracers, which demonstrated only two-fold selectivity of α7 nAChR over 5-HT$_3$ receptor, may have accounted in part for our inability to demonstrate significant specific binding of these compounds [15]. By contrast, [^{3}H]AZ11637326 demonstrates over 100-fold selectivity for α7 nAChR over 5-HT$_3$ receptor [23]. This, in addition to a LogD of 2.1, molecular weight of 310 Da., K_d of 0.2 nM and promising binding selectivity in rodents, suggested development of 5′-(2-[^{18}F]fluorophenyl)spiro[1-azabicyclo-[2.2.2]octane]-3,2′-(3′H)furo[2,3-b]pyridine ([^{18}F]AZ11637326) for eventual clinical use. Here we report the radiosynthesis and in vivo study of [^{18}F]AZ11637326 in rodent and non-human primate brain.

2. Materials and methods

2.1. General chemistry

[^{18}F]Fluoride was produced by 18 MeV proton bombardment of a high pressure [^{18}O]H_2O target (GE high pressure silver target) using a GE PETtrace biomedical cyclotron (GE Healthcare, Waukesha, WI). All reagents were pure by American Chemical Society or high-performance liquid chromatography (HPLC) criteria. The para-nitrobenzaldehyde precursor, 5′-(2-nitro-5-formyl-phenyl)-spiro[1-azabicyclo-[2.2.2]octane]-3,2′(3′H)-furo[2,3-b]pyridine **1**, was synthesized at AstraZeneca, as previously described [25]. Dimethylsulfoxide (DMSO) was distilled under vacuum from barium oxide prior to use. Reverse phase HPLC analysis and purification were performed with two Waters 610 HPLC pumps, a Waters 440 fixed wavelength (254 nm) UV detector (Waters Corporation, Milford, MA), and a Bioscan Flow Count NaI radioactivity detector (Bioscan, Inc., Washington, DC). All HPLC chromatograms were recorded with the Varian Galaxie™ chromatography system (Varian, Inc., Walnut Creek, CA). The semi-preparative two step gradient was performed on a Luna (Phenomenex, Torrence, CA) CN column (10 × 250 mm) using 90% THF:10% $CHCl_3$ at 5 mL/min for 10 min then THF containing 0.5% trifluoroacetic acid (TFA). The reverse phase HPLC was performed using a Hamilton (Hamilton Company, Reno, NV) 10 μm PRP-1 column (7.0 × 305 mm) and 25:75 acetonitrile:water made with 0.1 M ammonium formate at a flow rate of 7 mL/min. Chemical and radiochemical purity were determined using an XTerra (Waters Corp.) 3.5 μm C-18 column (4.1 × 100 mm) using 30:70 acetonitrile:water made 0.1 M with ammonium formate at a flow rate of 3 mL/min. A Capintec 15R dose calibrator (Capintec, Inc., Ramsey, NJ) was used for all radioactivity measurements.

2.2. Radiochemistry

[^{18}F]Fluoride was collected on an anion exchange resin column (D & W, Inc. Oakdale, TN). The column was eluted with 0.5 mg of K_2CO_3 in 0.3 mL water (Fluka-Sigma-Aldrich Corp., St. Louis, MO) into the first glassy carbon reaction vessel on the double vessel synthesis module (Tracerlab™, GE Healthcare, Waukesha, WI). A solution of 5 mg of Kryptofix® 222 in 1 mL acetonitrile was added. The mixture was heated to 55 °C under vacuum and argon flow for 2 min then 100 °C for 1.5 min. The vacuum and argon were stopped. The temperature was raised to 130 °C at which time 0.25 mL of DMSO was added and the vessel sealed. After heating and stirring for 3 min, the vessel was cooled to 50 °C and 2 mg of **1** in 0.25 mL DMSO was added. The vessel was heated to 130 °C for 20 min. After cooling to 50 °C, 2 mL of dioxane was added. The dioxane/DMSO mixture was transferred to the second glassy carbon reaction vessel, which contained 20 mg of Wilkinson's catalyst [$RhCl(PPh_3)_3$, 99.99% purity]. The mixture was heated to 130 °C for 10 min. The reaction mixture was then cooled to 35 °C with vacuum and argon flow (to remove as much dioxane as possible). After cooling, the mixture was pushed into a 5 mL V-vial. The solution was diluted with 9:1 THF:$CHCl_3$ (2 mL) and injected onto the CN column at 5 mL/min of flow. The solvent was switched at 10 min to THF containing 0.5% TFA and the crude product was collected (15–19 min at 5 mL/min). This solution was evaporated to a small homogeneous volume under argon flow and upon warming to 35 °C, 1.5–2 mL of water was added and the mixture was injected onto the semi-preparative Hamilton C-18 HPLC column for final purification ([^{18}F]AZ11637326: R_T = 7.9 minutes, k′ = 6.4). The [^{18}F]AZ11637326 was collected in a rotary evaporator and the solvent was evaporated to near-dryness. Sterile saline was added and the solution sterile filtered (Millipore GV, 25 mm, 0.2 μm) (EMD Millipore, Billerica, MA) into a sterile evacuated vial prior to animal studies. An aliquot of the solution was removed to determine chemical and radiochemical purity by analytical HPLC ([^{18}F]AZ11637326: R_T = 3.7, k′ = 2.7).

2.3. Biodistribution and dose saturation of [^{18}F]AZ11637326 in CD-1 and α7 nAChR null mice

All animal procedures were performed in accordance with the regulations of the Johns Hopkins Animal Care and Use Committee. Male CD-1 mice (Charles River Laboratories, Wilmington, MA) aged 3 months and α7 nAChR null mice (AstraZeneca, Wilmington, DE) were injected with 3.7 MBq (100 μCi) of [^{18}F]AZ11637326 (4.48 GBq/μmol; 121 Ci/mmol; 28 nmol/kg IV via the tail vein) in 150 μL of sterile saline vehicle. Mice were sacrificed (4 per time point) at 5, 15, 30, 60, and 120 min post-injection by cervical dislocation. The α7 nAChR null mice were sacrificed only at 30 min post-injection. Selected brain regions, fat and bone were dissected, and were then weighed and assayed for radioactivity content in an automated gamma counter (LKB Wallac 1282 Compugamma Universal Gamma Counter, Perkin Elmer, Boston, MA). A diluted standard dose was also assayed to determine percent injected dose per gram of tissue (%ID/g).

Mice also underwent pre-treatment with MLA, nicotine and ondansetron in order to test for binding specificity. Male CD-1 mice aged 4 months (35 g average weight, 4 mice per group) were injected with either 1 mg/kg of MLA (Sigma-Aldrich) with radiotracer in saline, 2 mg/kg of ondansetron with radiotracer in saline, or were pre-