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Characterization of 4-[^{18}F]-ADAM as an imaging agent for SERT in non-human primate brain using PET: a dynamic study

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Abstract

Introduction: Serotonin transporter (SERT) has been associated with many psychiatric diseases. This study investigated the biodistribution of a serotonin transporter imaging agent, *N,N*-dimethyl-2-(2-amino-4-^{18}F-fluorophenylthio)benzylamine (4-[^{18}F]-ADAM), in nonhuman primate brain using positron emission tomography (PET).

Methods: Six and four *Macaca cyclopis* monkeys were used to determine the transit time (i.e., time necessary to reach biodistribution equilibrium) and the reproducibility of 4-[^{18}F]-ADAM biodistribution in the brain, respectively. The sensitivity and specificity of 4-[^{18}F]-ADAM binding to SERT were evaluated in one monkey challenged with different doses of fluoxetine and one monkey treated with 3,4-methylendioxymethamphetamine (MDMA). Dynamic PET imaging was performed for 3 h after 4-[^{18}F]-ADAM intravenous bolus injection. The specific uptake ratios (SURs) in the midbrain (MB), thalamus (TH), striatum (ST) and frontal cortex (FC) were calculated.

Results: The distribution of 4-[^{18}F]-ADAM reached equilibrium 120–150 min after injection. The mean SURs were 2.49±0.13 in MB, 1.59±0.17 in TH, 1.35±0.06 in ST and 0.34±0.03 in FC, and the minimum variability was shown 120–150 min after 4-[^{18}F]-ADAM injection. Using SURs and intraclass coefficient of correlation, the test/retest variability was under 8% and above 0.8, respectively, in SERT-rich areas. Challenge with fluoxetin (0.75–2 mg) dose-dependently inhibited the SURs in various brain regions. 4-[^{18}F]-ADAM binding was markedly reduced in the brain of an MDMA-treated monkey compared to that in brains of normal controls.

Conclusion: 4-[^{18}F]-ADAM appears to be a highly selective radioligand for imaging SERT in monkey brain.

Keywords: Brain; Serotonin transporter; PET; Nonhuman primate or monkey; Biodistribution; Transit time

1. Introduction

Serotonin is a brain neurotransmitter with an important regulatory role in many normal central nervous system and peripheral nervous system functions including sleep, mood, cognition and endocrine regulation [1,2]. Abnormal levels of secreted serotonin in neuron synapses may cause many neuropsychiatric disorders including depression, Parkinson's disease, Alzheimer disease, schizophrenia, drug addiction and eating disorders [1,3,4]. Serotonin transporter (SERT) is located on the membrane surface of neurons, It regulates the extracellular serotonin concentration by the reuptake of serotonin from the synaptic cleft into the presynaptic nerve

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cell and plays a key role in serotonergic signaling [5]. SERT is also the main target of selective serotonin reuptake inhibitors (SSRIs) [6,7] and several drugs of abuse such as cocaine and 3,4-methylenedioxymethamphetamine (MDMA) [8]. Therefore, it is expected that non-invasive modalities such as single-photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI), etc., can be used to assess the status of SERT and the progress of diseases.

Highly selective and specific radioligands are needed for high-quality PET or SPECT images. Many radioligands have been developed for PET to study the SERT, such as ^{11}C-labeled citalopram [9], fluoxetine [10] or related derivatives and ^{18}F-labeled paroxetine [11] and fluoxetine. However, the target-to-background ratios of those radioligands were not high enough to satisfy PET imaging requirements [12]. In 1995, Szabo et al. [13]successfully applied [^{11}C](+)McN-5652 to assess SERT density in human brain. At that time [^{11}C](+)McN-5652 had been considered a promising agent for studying SERT, but its nonspecific binding was high enough to affect the accuracy of SERT quantification in areas of the brain with relatively low SERT density. Besides, the pharmacokinetics of its uptake by the brain were not optimal, extending the time required to get a stable estimate of SERT density. Moreover, the metabolism of [^{11}C](+)McN-5652 was rapid and the free fraction in the plasma was quite low. All the above mentioned limitations made [^{11}C](+)mcN-5652 difficult to use in clinical studies [14,15].

^{11}C-DASB (*N,N*-dimethyl-2-2-amino-4-cyanophenylthiobenzylamine) has been found to be a highly suitable tool for probing SERT in humans with PET [16]. However, the short half-life of ^{11}C-labeled tracers is just 20 min, which limits its application to those medical centers possessing an onsite cyclotron and skilled radiochemistry team. Recently, we developed a novel SERT imaging agent.

N,N-dimethyl-2-(2-amino-4-[^{18}F]-fluorophenylthio)-benzylamine (4-[^{18}F]-ADAM) [17]. The utility of this radioligand has been further validated in paroxetine-treated rats, a 5,7-dihydroxytryptamine-lesioned rat model, and a *p*-chloroamphetamine-induced 5-hydroxy-tryptophan depletion rat model [18]. This radioligand coupled with micro-PET was also used to study the effect of fluoxetine on MDMA-induced SERT loss in rat brain and was found to have validity as a probe for monitoring SERT status. We also found that 4-[^{18}F]-ADAM was a safe radioligand to use in rats and Formosan rock monkeys [19]. However, the test/retest reproducibility of brain distribution of SERT using 4-[^{18}F]-ADAM as probe has not been reported yet. Therefore, the first aim of this study was to determine time to equilibration and thereby the optimal image acquisition window. The second aim of this study was to evaluate the reproducibility of determining SERT distribution with 4-[^{18}F]-ADAM. Finally, we used fluoxetine, a serotonin selective reuptake inhibitor (SSRI), and MDMA, a serotonergic neurotoxin, to explore the sensitivity and specificity of 4-[^{18}F]-ADAM binding in the brain of *Macaca cyclopis*.

2. Materials and methods

2.1. Radiopharmaceuticals preparation

The 4-[^{18}F]-ADAM used in this study was synthesized in an automated synthesizer as previously reported [17,20]. Briefly, the precursor, *N,N*-dimethyl-2-(2-nitro-4-trimethylammoniumtrifluoromethanesulfonylphenylthio) benzamide, in dimethyl sulfoxide, was added to dried potassium ^{18}F-fluoride/Kryptofix $_{2.2.2}$ (Merck, Darmstadt, Germany) (K-^{18}F/$K_{2.2.2}$), and the solution was heated at 120°C for 10 min. The intermediate, *N,N*-dimethyl-2-(2-nitro-4-^{18}F-fluorophenylthio)benzylamine, was purified using a C_{18} Sep-Pak cartridge (Waters, Milford, MA) and reduced with $Cu(OAc)_2$-$NaBH_4$ in EtOH. The final product was purified using a high-performance liquid chromatography system [C_{18}, 10×250 mm, Luna (Phenomenex, Aschaffenburg, Germany); CH_3CN:0.1 mol/L HCO_2NH_4 (30:70) containing 0.3%, by volume, of acetic acid; 5 ml/min]. The fraction containing 4-[^{18}F]-ADAM was collected, evaporated to dryness, formulated in saline and filtered through a 0.22-μm filter (Millipore, Bedford, MA, USA) into a multi-injection vial. The radiochemical yield of 4-[^{18}F]-ADAM synthesized was 14.8±4.0% in a synthesis time of 120 min. analytical high-performance liquid chromatography analysis showed that the radiochemical purity was >98% and the specific activity was about 3 Ci/μmol.

2.2. Animal preparation

The animal study protocol was approved by the Institutional Animal Care and Use Committee of National Defense Medical Center. Four male and two female age-matched Formosan rock monkeys (*Macaca cyclopis*) weighing 4–7 kg (5.5±1.13) were employed in experiments to determine the dynamic biodistribution of 4-[^{18}F]-ADAM. Two male and two female monkeys were used in test re-test studies involving 4-[^{18}F]-ADAM/PET. One male monkey and another male monkey were utilized for the fluoxetine and MDMA challenge studies, respectively, to explore the sensitivity and specificity of 4-[^{18}F]-ADAM binding.

The interval between the test and retest studies was two weeks. For the fluoxetine challenge study, the monkey was fed fluoxetine orally to mimic the clinical usage of fluoxetine in humans. Fluoxetine 0.75, 1 and 2 mg/kg was administered 6 h before 4-[^{18}F]-ADAM / PET imaging was performed. In MDMA challenge studies, the dose regimen of MDMA was modified from that used in a previous study [21]. The monkey was injected subcutaneously with 5 mg/kg MDMA twice a day, for four consecutive days to damage the serotonergic systems. This particular dosage regimen was proved, which would lead to moderate to severe lesion of serotonergic systems [22,23]. After 1 year of MDMA treatment, the 4-[^{18}F]-ADAM / PET imaging studies were performed 4 times to investigate the specificity of the 4-[^{18}F]-ADAM binding in the monkey's brain. The interval of each scan was 2 weeks.