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[^{18}F]-GE-179 positron emission tomography (PET) tracer for *N*-methyl-D-aspartate receptors: One-pot synthesis and preliminary micro-PET study in a rat model of MCAO

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ABSTRACT

Introduction: The objective of this study was to synthesize an *N*-methyl-D-aspartate receptor (NMDAR) radiotracer [^{18}F]-GE-179 in one-pot and evaluate its *in vivo* binding for NMDAR activation after brain ischemia reperfusion injury.
Methods: [^{18}F]-GE-179 was auto-synthesized using a quick one-pot method from a stable disulfide precursor and purified using semi-preparative high-performance liquid chromatography (HPLC) with ethanol/aqueous NaH_2PO_4 as the eluent. Dynamic micro-positron emission tomography (PET)/computed tomography (CT) study of [^{18}F]-GE-179 was successfully performed using a rat model of middle cerebral artery occlusion (MCAO, induced by transient occlusion into the left MCA). A simplified reference tissue model method was used to calculate the [^{18}F]-GE-179 non-displaceable binding potential (BPND) for intracranial NMDAR activation assessment. Immunofluorescence staining of NMDAR NR1 subunit in brain slices containing lesion was also performed.
Results: [^{18}F]-GE-179 was successfully prepared in a yield of 23–30% in a formulation that could be injected directly after dilution. Localized radioactivity accumulation was observed in the animal model of MCAO. Significantly higher ($p = 0.0003$–0.0404) BPND relative to equivalent contralateral was found in the ipsilateral caudate putamen, Acb core/shell, cortex cingulate, amygdala, hypothalamus, and superior colliculus. Immunofluorescence staining showed elevated NMDAR expression in the affected hemisphere.
Conclusions: [^{18}F]-GE-179 was synthesized using a one-pot process with a markedly improved yield. Preliminary *in vivo* micro-PET study visualized excessive NMDAR stimulation successfully in a rodent model of MCAO, which was consistent with results of *in vitro* immunofluorescence staining. The study demonstrates that [^{18}F]-GE-179 is a promising PET probe for the detection of functional NMDAR alterations.

1. Introduction

N-methyl-D-aspartate receptors (NMDARs) are voltage- and ligand-gated ion channels that permit nonselective cation-flux when activated. NMDARs are involved not only in cognitive processes, but also in the pathophysiology of several neurological and neuropsychiatric disorders such as ischemic brain injury [1], epilepsy [2], Parkinson's disease [3], Alzheimer's disease [4], neuropathic pain [5], drug addiction [6], attention deficit hyperactivity disorder [7], and schizophrenia [8]. Among these conditions, stroke is the second leading cause of disability and mortality worldwide [9] and is highly related to acute or prolonged over-activation of NMDARs, which leads to excessive entry of Ca^{2+} and results in glutamate excitotoxicity [10].

Growing evidence suggests that NMDAR antagonists can improve post-injury recovery in animal models of stroke [11]. However, subsequent placebo-controlled clinical trials with NMDAR antagonists have shown either no benefit or detrimental side effects. This discrepancy between animal and human studies requires further investigation of the NMDAR temporal changes following brain damage [12]. Since excessive NMDAR-stimulation related excitotoxicity is regarded as the leading mechanism for stroke-associated neuronal damage [13], detection of NMDAR activation *in vivo* is of great significance.

Positron emission tomography (PET) is a powerful non-invasive tool for the characterization of receptors, enzymes, and other targets *in vivo* [14]. Dozens of PET and single photon emission computed tomography (SPECT) radiotracers labeled by ^{18}F, ^{11}C, ^{123}I, and ^{125}I have been

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developed for targeting NMDARs. Notably, these radio-ligands include [^{11}C]-MK-801, [^{18}F]-MEM, [^{123}I]-CNS-1261, [^{125}I]-CNS-1261, [^{11}C]-CNS-5161, [^{18}F]-GE-179, [^{18}F]-PK-209, and [^{11}C]-GMOM [15–17]. Previously, activation of NMDARs has been successfully visualized using [^{125}I]-CNS-1261 autoradiography in an ischemic rat model with permanent middle cerebral artery occlusion (MCAO) [18]. However, PET imaging outperformed SPECT using ^{125}I or ^{123}I labeled isotopes in terms of image resolution. ^{18}F has a more appropriate half-life (109.8 min) in comparison to ^{11}C (20.5 min) in living organisms. [^{18}F]-GE-179 is the ^{18}F-labeled analogue of [^{11}C]-CNS-5161 with satisfactory *in vitro* evaluation results and *in vivo* kinetic behavior [19,20], which has been already applied to the study of altered NMDAR availability in patients with focal epilepsy [21]. Arguably, [^{18}F]-GE-179 is a highly promising probe for the detection of functional NMDAR alterations *in vivo*.

The only currently available method for the synthesis of [^{18}F]-GE-179 (Fig. 1) has been reported by Robins and colleagues at GE healthcare [20]. Unfortunately, this method requires the use of cyanogen bromide, a highly toxic reagent, for the synthesis of the precursor (as shown in Fig. 1). In addition, the two-step radio-synthesis procedure involves purification of the radioactive intermediate, [^{18}F]-fluoroethyl tosylate **6**, by semi-preparative high-performance liquid chromatography (HPLC) and its subsequent use in the second step after extraction with a C_{18} Sep-Pak cartridge. The entire procedure is highly time-consuming (about 3 h) and furnishes the target product in a very low overall yield (4–9%).

Herein, we report a convenient one-pot synthesis of radiolabeled [^{18}F]-GE-179 in an overall yield of 23–30% ($n = 10$, $\sigma = 0.023$). Micro-PET imaging of [^{18}F]-GE-179 is conducted to display the NMDAR over-stimulation in rat models of MCAO. Magnetic resonance imaging (MRI) and immunofluorescent staining are utilized to confirm the results of the PET imaging study.

2. Materials and methods

2.1. General procedures and materials

All non-aqueous reactions were carried out using oven-dried (110 °C) or heat gun dried glassware under a positive pressure of dry argon unless noted otherwise. Toluene, THF, and dichloromethane were purified by distillation and dried by passage over activated molecular sieves (type 4 Å) under an argon atmosphere. All chemicals were purchased from SCRC, Aladdin, Adamas, J&K, Alfa Aesar, Acros, Aldrich, or TCI and used as obtained unless otherwise stated. Deuterated solvents were purchased from Cambridge Isotope Laboratories. All other commercially available reagents were used without further purification. Unless indicated otherwise, reactions were magnetically stirred and the reaction progress was monitored by thin layer chromatography (TLC) using Huanghai Silica Gel 60 F254 plates and visualized by fluorescence quenching under UV light. In addition, TLC plates were stained using iodine stain if needed. Chromatographic purification of crude products (flash column chromatography) was performed on General-Reagent silica 200–300 mesh. NMR spectra were recorded on a Bruker spectroscope at 400 MHz and 101 MHz for ^{1}H and ^{13}C NMR, respectively. The ^{1}H chemical shift (δ) is reported in parts per million (ppm) relative to the center of the residual solvent resonance (chloroform: δ 7.26, or DMSO: δ 2.50). Data are reported as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, m = multiplet, br = broad signal; coupling constants are reported in Hz. Mass spectra were acquired on an Aglient 6120 Quadrupole HPLC-MS.

^{18}F was produced using an RDS III cyclotron (Siemens, Germany). Semi-automated radio-synthesis of ^{18}F-GE-179 was carried out on an ^{18}F-multifunction synthesizer (Beijing PET Technology Co. Ltd., China) with a computer interface (see Fig. S1). The crude product was extracted on a C18-cartridge (pre-activated with 10 mL ethanol followed by 10 mL water, Waters, Milford, MA, USA), washed with 20 mL water, and eluted with 2 mL ethanol; the ethanol solution was loaded onto a semi-preparative column (μBondpak 7.8 × 300 mm C18 column, Waters, Milford, MA, USA), which was eluted with 1:1 (EtOH/10 mM NaH_2PO_4) at a flow rate of 3 mL/min and monitored by a radio detector fixed on the synthesizer. Radiochemical purity (RCP) of [^{18}F]-GE-179 was determined using an analytical HPLC system with a radio detector using a waters xTerra RP18 Column (5 μm, 3.9 mm × 150 mm, Waters, Milford, MA, USA), which was eluted with 55:45 (acetonitrile (ACN)/10 mM NaH_2PO_4) at a flow rate of 0.8 mL/min. The standard compound, ^{19}F-GE-179, was co-injected and monitored with a UV detector at 254 nm.

MRI was conducted using a 3-T MRI system (Signa Excite HDxt; GE healthcare, Milwaukee, WI) with a commercially available 60-mm diameter gradient coil (Magtron Inc., Jiangyin, China). Small-animal PET/CT images were acquired with a Siemens Inveon PET/CT system (Siemens Medical Solutions, Knoxville, USA).

2.2. Synthetic procedures

2.2.1. Chemical synthesis of [^{19}F]-GE-179

A summary of the synthetic route utilized to prepare [^{19}F]-GE-179 is displayed in Scheme 1.

2.2.2. Synthesis of compound **7**

3-(Methylthio)-aniline **1** (4.26 g, 30.6 mmol) was dissolved in acetone (50 mL) and cooled to 0 °C using an ice bath. Benzoyl isothiocyanate (5.0 g, 30.6 mmol) in acetone (50 mL) was added dropwise to the aforementioned cold mixture, warmed to rt., and stirred overnight. Hexane (40 mL) was added to the reaction mixture and the resultant precipitate was filtered to afford **7**.

Yellow solid (3.97 g, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.61 (s, 1H), 9.08 (s, 1H), 7.89 (d, J = 7.8 Hz, 2H), 7.72 (s, 1H), 7.66 (t, J = 7.4 Hz, 1H),

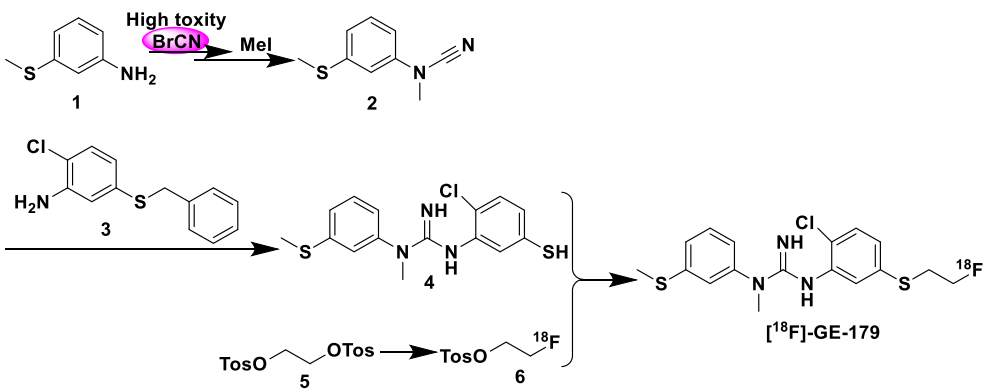

Fig. 1. Synthesis of [^{18}F]-**GE-179** reported by Robins.