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Brain uptake of a non-radioactive pseudo-carrier and its effect on the biodistribution of [^{18} F]AV-133 in mouse brain

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ABSTRACT

Introduction: 9-[^{18} F]Fluoropropyl-(+)-dihydrotetrabenazine ([^{18} F]AV-133) is a new PET imaging agent targeting vesicular monoamine transporter type II (VMAT2). To shorten the preparation of [^{18} F]AV-133 and to make it more widely available, a simple and rapid purification method using solid-phase extraction (SPE) instead of high-pressure liquid chromatography (HPLC) was developed. The SPE method produced doses containing the non-radioactive pseudo-carrier 9-hydroxypropyl-(+)-dihydrotetrabenazine (AV-149). The objectives of this study were to evaluate the brain uptake of AV-149 by UPLC-MS/MS and its effect on the biodistribution of [^{18} F]AV-133 in the brains of mice.

Methods: The mice were injected with a bolus including [^{18} F]AV-133 and different doses of AV-149. Brain tissue and blood samples were harvested. The effect of different amounts of AV-149 on [^{18} F]AV-133 was evaluated by quantifying the brain distribution of radiolabelled tracer [^{18} F]AV-133. The concentrations of AV-149 in the brain and plasma were analyzed using a UPLC-MS/MS method.

Results: The concentrations of AV-149 in the brain and plasma exhibited a good linear relationship with the doses. The receptor occupancy curve was fit, and the calculated ED_{50} value was 8.165 mg/kg. The brain biodistribution and regional selectivity of [^{18} F]AV-133 had no obvious differences at AV-149 doses lower than 0.1 mg/kg. With increasing doses of AV-149, the brain biodistribution of [^{18} F]AV-133 changed significantly.

Conclusion: The results are important to further support that the improved radiolabelling procedure of [^{18} F]AV-133 using an SPE method may be suitable for routine clinical application.

1. Introduction

Nuclear molecular functional imaging based on radiopharmaceuticals is a powerful non-invasive imaging technique used in disease diagnosis. In recent years, increasing numbers of radiopharmaceuticals and molecular imaging probes have been developed to image biochemical, physiological, and pathologic processes *in vivo* by the use of positron emission tomography/computed tomography (PET/CT) and single-photo emission computed tomography/computed tomography (SPECT/CT) scanners [1–5]. Vesicular monoamine transporter type II (VMAT2), which is located on vesicle membranes in neurons, performs a secondary type of transport by storing and packaging the monoamine neurotransmitters into vesicles [6]. A reduction of VMAT2 in the brain was observed in individuals with Parkinson's disease (PD) and other neurodegenerative diseases. Because VMAT2 is less susceptible than dopamine transporter (DAT) to compensatory changes and drugs used to treat PD, some have suggested that it may be superior to DAT as a marker of dopaminergic neurons [7,8]. Imaging targeting VMAT2 alterations would be a sensitive tool for the early diagnosis of PD. The PET tracer [^{11}C](+)-TBZ ((+)-tetrabenazine) was reported for imaging VMAT2 in the early 1990s [9]. [^{11}C](+)-TBZ and its reduced form [^{11}C](+)-DTBZ ((+)-dihydrotetrabenazine) were useful for detecting the reduction of monoamine neuronal function associated with movement disorders [10,11]. Because ^{11}C has a very short half-life ($t_{1/2}$ = 20 min), ^{18} F ($t_{1/2}$ = 110 min)-labeled analogs of DTBZ would be more practical for widespread use.

9-[^{18} F]Fluoropropyl-(+)-dihydrotetrabenazine ([^{18} F]AV-133) is a PET imaging agent targeting vesicular monoamine transporter type II VMAT2 sites in the brain [12–14]. Preliminary clinical studies suggest that [^{18} F]AV-133 can sensitively detect monoaminergic terminal reductions in PD patients [15–17]. [^{18} F]AV-133 is currently under phase III clinical trials to establish its usefulness in the diagnosis of neurodegenerative diseases including dementia with Lewy bodies and Parkinson's disease. To shorten the preparation of [^{18} F]AV-133 and to make it more widely available in clinics, a simple automatic radiosynthesis system was developed [18]. This procedure using a solid phase extraction method (SPE) instead of high pressure liquid chromatography (HPLC) would be faster and more convenient. Compared with HPLC purification, the SPE method produced doses containing the non-radioactive pseudo-

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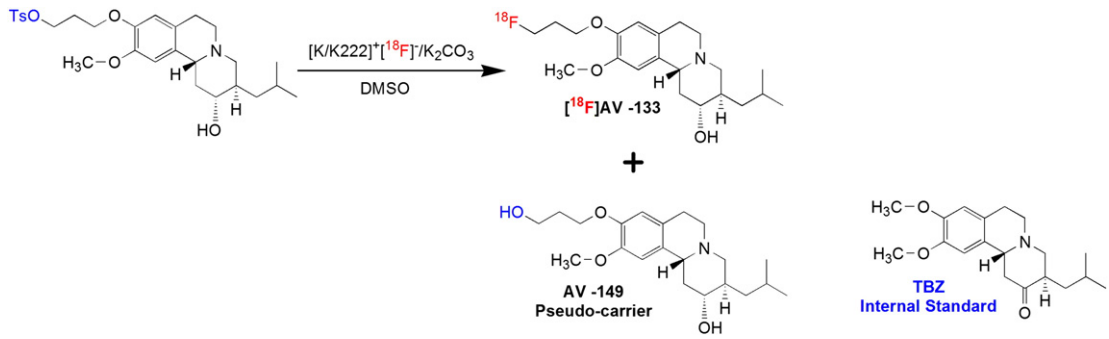

Fig. 1. Chemical structures of [^{18} F]AV-133, AV-149 and TBZ (IS) are shown. Preparation of [^{18} F]AV-133 was achieved by a nucleophilic displacement of the tosylate derivative with [^{18} F] fluoride ion; [^{18} F]AV-133 and its pseudo-carrier, AV-149 were produced.

carrier 9-hydroxypropyl-(+)-dihydrotetrabenazine (AV-149), which came from a hydrolyzed precursor. It yields approximately 200–600 μg/batch prepared in the final production [18]. Fig. 1 shows that [^{18} F]AV-133 achieved by a nucleophilic displacement of the tosylate derivative with a [^{18} F]fluoride ion and AV-149 was produced.

The non-radioactive impurity, AV-149, showed a certain binding affinity for VMAT2 *in vitro* (K_i = 75.8 nM) [12]. Some studies have been performed to evaluate the effect of AV-149 on the imaging of [^{18} F]AV-133 and its pharmacokinetics. The effect of AV-149 on the pharmacokinetics of AV-133 in rat plasma was evaluated by using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method [19]. Zhu et al. [20] conducted PET imaging and *in vitro* autoradiography to study the effect of a pseudo-carrier. However, the ability of AV-149 to cross the blood–brain barrier (BBB) as well as how its presence would likely compete with [^{18} F]AV-133 for the VMAT2 binding sites and affect the brain biodistribution of [^{18} F]AV-133 is unknown.

In this manuscript, *in vivo* brain VMAT2 receptor occupancy measurements of AV-149 in mice were conducted by quantifying the brain regional distribution of the radiolabelled tracer [^{18} F]AV-133, and the concentrations of AV-149 in mice brains were detected using a validated UPLC-MS/MS method. The brain uptake of AV-149 and its effect on the regional biodistribution of [^{18} F]AV-133 in the brain were obtained. The results showed that the brain uptake of AV-149 was lower than [^{18} F]AV-133, and it did not affect the brain biodistribution and regional selectivity of [^{18} F]AV-133.

2. Materials and methods

2.1. Chemicals

[^{18} F]AV-133 was radiolabelled using a labeling reaction reported previously and was purified by HPLC [18]. A radiochemical purity over 95% was obtained. Tetrabenazine (TBZ), which was used as an internal standard (IS), was obtained from Dalian Meilun Biology Technology (Dalian, Liaoning, China). HPLC-grade ammonium acetate was purchased from J. T. Baker (Phillipsburg, NJ.USA). Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Distilled deionized water (18.2 MΩ) was made with an Elga Classic UVF Purification System (Buckinghamshire, UK). All other chemicals and reagents were of analytical grade or better.

2.2. Animals

Male Kunming mice, which were 4 weeks old and weighed between 18 and 22 g, were obtained from the Vital River Laboratory Animal Co. Ltd. (Beijing, China). Mice were kept in an environmentally controlled room (temperature = 20–23 °C, humidity = 50 ± 5%, 12 h light/dark cycle) for 1 week before the experiments. All of the animals were maintained according to Chinese governmental guidelines for the care and use of laboratory animals.

2.3. UPLC-MS/MS method

The previously reported UPLC-MS/MS method was optimized and validated to analyze AV-149 in brains and plasma of mice [19]. The chromatographic analysis was carried out using a Waters Acquity module (Waters, Milford, MA, USA). The chromatographic column was a Waters Acquity ethylene-bridged (BEH™) C18 column (2.1 mm × 50 mm, 1.7 μm particle size, Waters, Milford, MA, USA). The mobile phase chosen was 1 mM ammonium acetate in water (A) and 1 mM ammonium acetate in acetonitrile (B). The gradient program was set to hold at 10% B for the first 0.5 min, increase linearly from 10% B to 90% B in 0.5 min, and then return to 10% B from 1.8 min to 2.0 min. The flow rate was 0.5 mL/min.

Mass spectrometric detection was carried out on a Micromass Quattro micro API mass spectrometer (Waters, Milford, MA) with an electrospray ionization (ESI) interface. The desolvation temperature was maintained at 450 °C, and the source temperature was 110 °C. The capillary voltage was 3.0 kV. Nitrogen was used as the cone gas and the desolvation gas, and the flow rates were 30 L/h and 600 L/h, respectively. Argon was used as the collision gas. Quantification was performed using multiple reaction monitoring (MRM) of the following transitions: *m/z* 364.2 → 318.1, 364.2 → 288.2, and 364.2 → 151.1 for AV-149 with a cone voltage of 40 V and collision energies of 25 eV, 25 eV and 35 eV, respectively; and *m/z* 318.2 → 220.3 and 318.2 → 165.1 for IS (TBZ) with a cone voltage of 20 V and collision energies of 45 eV and 35 eV, respectively. The dwell time was kept at 0.1 s for each transition. Data processing was performed on Masslynx 4.1 software.

2.4. Validation of the analytical method

The optimized UPLC-MS/MS method was validated by a set of parameters that were in compliance with the reference guidelines as defined in FDA documents [21]. These parameters included calibration curves, sensitivity, accuracy, precision, specificity, recovery, matrix effect and stability. Calibration solutions were diluted with blank mouse plasma/brain tissue homogenate to produce the eight concentrations of AV-149 (2.00, 5.00, 10.0, 20.0, 50.0, 100, 400 and 800 ng/mL), and the IS at a concentration of 100 ng/mL. The quality control (QC) solutions were similarly prepared at concentrations of 10.0, 100 and 750 ng/mL for AV-149 by separate weighings of the reference substance. Calibration standards and QC samples were stored at −20 °C and were brought to room temperature before use.

2.5. Sample preparation

Plasma samples and brain tissue homogenate samples were pretreated by liquid–liquid extraction (LLE) with ethyl acetate. To