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The preclinical pharmacological study of dopamine transporter imaging agent ^{18}F-FP-β-CIT

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Abstract The paper is to study pharmacologic characteristics of ^{18}F-FP-β-CIT (^{18}F-N-(3-fluoropropyl)-2β- carbomethoxy-3β- (4-iodophenyl)nortropane) as an imaging agent for dopamine transporter. The radiochemical purity of ^{18}F-FP-β-CIT in aqueous solution was over 95% after standing at room temperature for 4h. Biodistribution displayed rapid uptake in rat brain (1.375 %ID/organ at 5min and 0.100 %ID/organ at 180 min) and the striatal uptake was 1.444, 0.731, 0.397, 0.230 and 0.146 %ID/g at 5, 30, 60, 120 and 180 min, respectively. The values of striatum/cerebellum, striatum /frontal cortex and striatum / hippocampus in rat's brain at 30 min were 3.38, 2.17 and 2.40 respectively. The uptake in striatum can be blocked by β-CFT, suggesting that ^{18}F-FP-β-CIT binds to DAT peculiarly. The compound was rapidly cleared from monkey's blood. The striatal uptake was bilaterally decreased in the left-sided lesioned PD rats, compared with normal control. Brain PET imaging studies in normal monkey showed that ^{18}F-FP-β-CIT was concentrated in striatum. The test of undue toxicity showed that the dose received by mice was 1250 times as by human, which indicates that ^{18}F-FP-β-CIT is very safe. So ^{18}F-FP-β-CIT is a promising PET imaging agent for DAT with safety and validity.

Key words Parkinson's disease, Dopamine transporter, Radionuclide imaging, ^{18}F-FP-β-CIT, Biodistribution, Pharmacokinetics

CLC numbers O628.5^{+}1, R962, R742.5, R817

1 Introduction

Dopamine transporter (DAT), a protein located in presynaptic nerve terminals, plays an important role in regulating dopamine neural transmission. DAT is a dopaminergic presynaptic marker affected by Parkinson's disease(PD) and some other neurodegenerative diseases. Non-invasive quantitation of DAT density and affinity is critical to understanding the diseases. With PET or SPECT, ligands binding specifically to DAT are of potential use as *in vivo* imaging agent for studying the diseases and evaluating outcomes of their treatments.

Several cocaine analogues were developed for SPECT and PET studies of the dopamine transporter. The common phenyltropane structure of the cocaine analogues allows labeling with different radionuclides such as ^{123}I, ^{99m}Tc, ^{11}C, or ^{18}F [1~4].

A DAT radioligand, ^{18}F-N-(3-fluoropropyl)-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (^{18}F-FPCIT, Fig.1), was developed to study dopaminergic function. FPCIT has been labeled with ^{123}I for SPECT or ^{18}F for PET. The ^{18}F-labeled FP-β-CIT, with a half-life of 110min, may be of advantage as the conditions to reach a transient equilibrium may be available at a later times. As we reported in a PET study with ^{18}F-FP-β-CIT in a non-human primate (rhesus monkeys) and human subjects [5], the PET image showed

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high radioactivity in the striatum. In this paper, we report the results of the preclinical pharmacologic study.

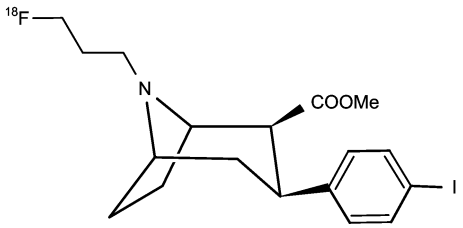


Fig.1 ^{18}F-FP-β-CIT.

2 Materials and methods

2.1 Instruments

Varian SY 5000 HPLC(USA); Packard Cobra automatic gamma counter(USA); Siemens CTI EXACT HR$^+$ PET (USA).

2.2 Reagents

^{18}F-FP-β-CIT was synthesized by our group[6,7], with over 95% of the radiochemical purity. The radiochemical yield at the end of bombardment was 10% with decay correction.

2.3 Animals

Rats (Sprague-Dawley, 180–220 g) and rhesus monkeys were provided by the Center of Experimental Animals of Jiangsu Institute of Nuclear Medicine.

2.4 Determination of stability

The ^{18}F-FP-β-CIT was kept at 25℃ for over four hours, and its radiochemical purity was determined by HPLC at regular intervals.

2.5 Biodistribution in rats and the inhibition of β-CFT

Four Sprague-Dawley rats per group were used for each biodistribution study. 9.2 MBq ^{18}F-FP-β-CIT (0.2 mL) were injected into tail vein of the rats, which were sacrificed under anesthesia (diethyl ether) by cervical dislocation at 5, 30, 60,120 and 180 min post-injection. Regional brain distribution was measured. Samples from different brain regions, i.e. striatum [ST], hippocampus [HP], cerebellum [CB], frontal cortex [FC], occipital cortex [OC], and temporal cortex [TC] were dissected, weighed and counted. The percent injected dose (%ID) per gram of sample was calculated by comparing the sample counts with the count of the diluted initial dose.

In vivo competitive binding in the regional uptake of ^{18}F-FP-β-CIT was investigated by pretreating the rats with β-CFT (5mg/kg, injected intravenously 5 min prior to radiotracer injection), followed by injection of ^{18}F-FP-β-CIT. Similar regional brain distribution was determined as described above.

Four left-sided lesioned PD rats were injected with ^{18}F-FP-β-CIT (0.2 mL, 9.2MBq) via tail veins, and sacrificed 1h later. Similar brain regional distribution was determined as described above.

2.6 Radioactivity kinetics of blood clearance in monkey

The rhesus monkeys (6kg) were injected with 185 MBq of ^{18}F-FP-β-CIT via elbow vein. Blood samples were collected from the other elbow sometime post-injection. Radioactivity of the samples was counted and expressed as percent injected dose per milliliter of blood. A time-radioactivity curve was plotted with the blood samples.

2.7 PET imaging of monkeys

Prior to imaging, the rhesus monkeys were administered intramuscularly with 0.1 g ketamine and 5 mg diazepam. Every 0.5 h after the anesthetization, each monkey was given with an additional 0.05g ketamine. And 100 mg $KClO_4$ was poured into the stomach for blocking the choroid plexus and thyroid. An hour later, 185MBq of ^{18}F-FP-β-CIT was injected into the elbow vein, and PET (Siemens CTI EXACT HR$^+$) imaging was performed at 35, 80, 130, 155 and 230min.

2.8 Undue toxicity test

According to the regulations of Pharmacopoeia of China (2005), the undue toxicity of ^{18}F -FP-β-CIT was determined by observing the death and survival of five mice (17–20 g) within a period of 48h after receiving an injection of 0.5 mL (23MBq) of ^{18}F-FP-β-CIT (50% of the human dose).

3 Results and discussions

3.1 Stability

The radiochemical purity of ^{18}F-FP-β-CIT in aqueous solution was over 95% after being kept at room temperature for 4h.

3.2 Biodistribution in rats

As shown in Fig.2, ^{18}F-FP-β-CIT passed through the blood-brain barrier and localized in striatal region, where dopamine transporters are concentrated. The brain uptake was rapid, being 1.375%ID/organ at 5 min but 0.100 %ID/organ at 180 min. High ST/CB ratios were obtained between 30 and 180 min, and the ratio of ST/CB reached a maximum value of 3.73 at 60 min post-injection. ^{18}F-FP-β-CIT displayed high retention in the ST, and rapid clearance in CB, HP, and CX. Ratios of ST/CB, ST/HP, and ST/CX increased with time (Fig.3).

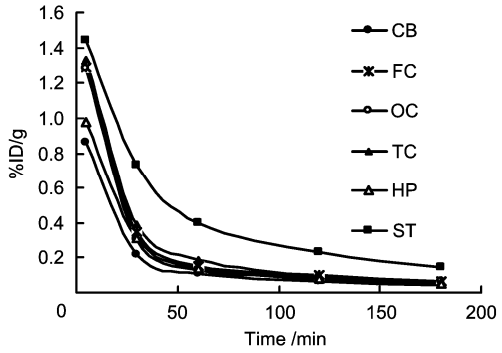


Fig.2 Biodistribution of ^{18}F-FP-β-CIT in rats' brain.

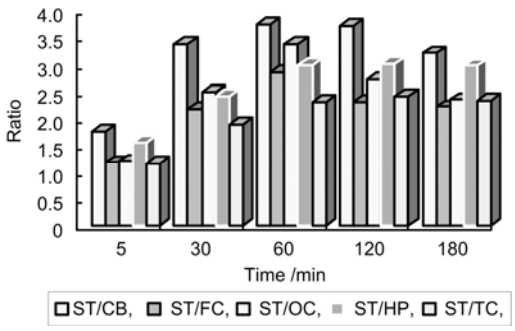


Fig.3 Radioactivity ratios of brain tissues in striatum to different regions at different time in rats.

To further characterize the striatal uptake, the effect of pretreatment with β-CFT on the distribution of ^{18}F-FP-β-CIT was evaluated. The specific uptake in striatum, rather than other regions, was significantly diminished ((ST－CB)/CB ratio decreased from 4.55 to 0.65) by pretreating rats with β-CFT, a dopamine transporter ligand (Fig.4).

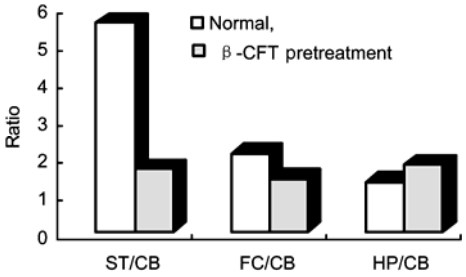


Fig.4 The comparison of the ratios of ST/CB, FC/CB and HP/CB in control and β-CFT pretreated rats (60min).

3.3 Radioactivity kinetics of blood clearance in monkey

The time-radioactivity curve for ^{18}F-FP-β-CIT in monkey blood is shown in Fig.5. The half-life of blood radioactivity elimination was about 10min, a rapid blood clearance of ^{18}F-FP-β-CIT.

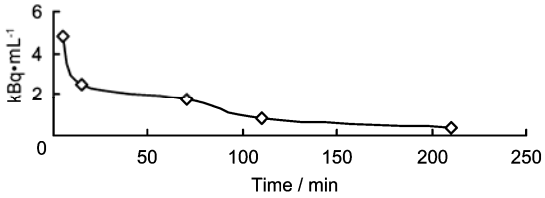


Fig.5 The blood time-radioactivity curve for ^{18}F-FP-β-CIT in monkey.

3.4 Biodistribution in the experimental PD of rats

The striatal uptake was bilaterally decreased in the left-sided lesioned PD rats, compared with normal control (Fig.6). A profound decrease of ^{18}F-FP-β-CIT uptake was found in the lesioned side.

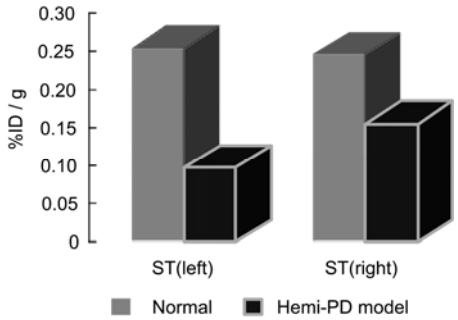
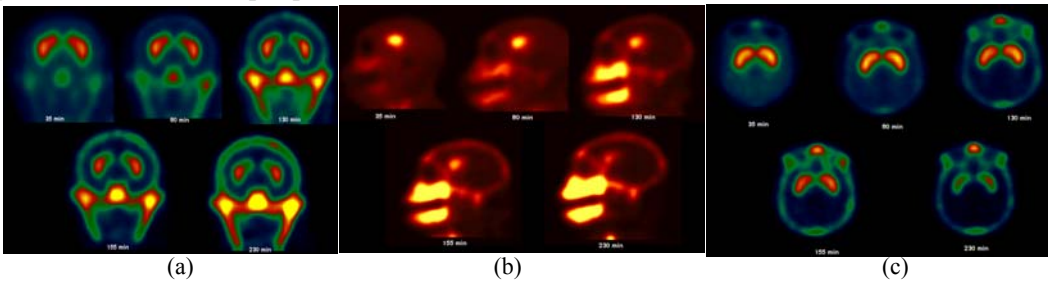


Fig.6 The striatal uptake was bilaterally decreased in the left-sided lesioned PD rats, comparing with normal control.

3.5 Imaging in monkey

Brain PET imaging studies in normal monkey was shown in Fig.7. To facilitate the identification of anatomical location, the coronal, transaxial and sagittal

PET images of normal monkey were compared at 35, 80, 130, 155 and 230min. The striatal uptake was obviously higher than that of the peripheral tissue, and the left and right striatal areas were symmetrical in the coronal and transaxial section images.



(a) (b) (c)

Fig.7 Brain PET imaging in normal monkey.
(a) Transaxial, (b) Sagittal, and (c) Coronal slices.

3.6 Undue toxicity test

After being injected with 50% of the dose of ^{18}F-FP-β-CIT on human (assuming a weight of 50 kg), and raised regularly for 48h, none of the mice died, and no abnormality was observed in all central organs after dissection. The dose per kilogram administered to the mice was 1,250 times greater than that received by humans.

4 Conclusion

^{18}F-FP-β-CIT displays high uptake and selective binding to the dopamine transporter in the striatum comparing to other regions of brain in animals. ^{18}F-FP-β-CIT has potential clinical use for monitoring the change in dopamine transporters associated with various neurodegenerative diseases, such as Parkinson's and similar diseases.

References

1 Abi-Dargham A, Gandelsman M S, De Erausquin G A, *et al*. J Nucl Med, 1996, **37**: 1129-1133.

2 Goodman M M, Keil R, Shoup T M, *et al*. J Nucl Med, 1997, **38**: 119-126.

3 Yung B C K, Dannals R F, Kuhar M J, *et al*. J Nucl Med, 1993, **34**: S197.

4 Kung H F, Kim H J, Kung M P, *et al*. Eur J Nucl Med, 1996, **23**: 1527-1530.

5 Chalyl T, Dhawan V, Kazumata K, *et al*. Nucl Med Biol, 1996, **23**: 999-1004.

6 Fang Ping, Chen Zhengping, Lin Yansong, *et al*. Isotopes (in Chinese), 2001, **14**: 6-10.

7 Chen Zhengping, Wu Chunying, Zhang Zhengwei, *et al*. Chin J Nucl Med (in Chinese), 2003, **23**: 241-243.