Nuclear Science and Techniques **20** (2009) 11–16

Animal biodistribution, safety and validation study of dopamine transporter PET imaging agent ^{18}F-FECNT

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Abstract This work was to investigate the pharmacologic characteristics of ^{18}F-FECNT (2β-carbomethoxy-3β-(4-chlorophenyl)-8-(2-[^{18}F]fluoroethyl)nortropane) as a dopamine transporter (DAT) PET imaging agent. Its partition coefficients were determined in *n*-octanol and phosphate buffer (PB) (pH 7.0 and pH 7.4). 6-Hydroxydopamine (6-OHDA) left-sided lesioned Parkinsonian rats were established and validated by rotational behavior tests. Biodistribution in vivo in mice, autoradiography in normal and hemi-Parkinsonian rat brains, and toxicity test were performed. The results showed that partition coefficients were 34.14 (pH 7.0) and 56.41 (pH 7.4), respectively. Biodistribution exhibited rapid uptake and favorable retention in the mice brains. The major radioactivity was metabolized by the hepatic system. The autoradiography showed that ^{18}F-FECNT was highly concentrated in striatum, and that the left and the right striatal uptake were symmetrical in normal SD rat brains. In left-sided lesioned PD rat brains, the striatal uptake of ^{18}F-FECNT bilaterally decreased in comparison with normal rats. No significant uptake was visible in the 6-OHDA lesioned-sided striatal areas. The results demonstrated that ^{18}F-FECNT binds to DAT was specific. Toxicity trial displayed that the acceptable dose per kilogram to mice was 625 times greater than that to human. These indicate that 18F-FECNT is a potentially safe and useful DAT PET imaging agent in the brain.

Key words Dopamine transporter (DAT), PET, ^{18}F-FECNT, Parkinson's disease (PD), Biodistribution, Autoradiography

1 Introduction

The dopamine transporter (DAT), a membrane-bound protein located at the presynaptic dopaminergic nerve terminals, is considered as a marker for functional integrity of dopamine neurons[1,2]. The density change of DAT is consistent with the amount of dopaminergic neurons. Parkinson's disease (PD), a neurodegenerative disease, is characterized clinically by bradykinesia, muscular rigidity and neuropathologically by progressive degeneration of dopamine neurons. Presynaptic striatal DAT density declines with increasing disability in PD patients.

Positron emission tomography (PET)[3] and single photon emission computed tomography (SPECT)[4] imaging with DAT tracers provide valuable tools for *in vivo* diagnosis of neurodegenerative diseases, such as Parkinson's and other similar diseases. It is therefore crucial to develop excellent DAT imaging agents. A series of radiolabeled tropane analogs, e.g. ^{18}F-FP-β-CIT[3,5,6], ^{11}C-CFT[7], ^{99m}Tc-TRODAT-1[8,9], etc., which can specifically bound to DAT, have been successfully applied in clinical *in vivo* evaluation and assessment of dopaminergic neuronal function.

^{18}F is the most widely used radionuclide for radiolabeling PET ligands because its half-life of 110 min allows sufficient time for synthesis, purification and biodisrtibution of the radiopharmaceuticals. ^{18}F-FECNT (2β-carbomethoxy-3β-(4-chlorophenyl)-8-(2-[^{18}F] fluoroethyl) nortropane) (Fig.1)[10-15] is an

Supported by the National Natural Science Foundation of P. R. China (30570518), High Technology Research and Development Program of Jiangsu Province of China (BG2007603) and Science Foundation of Health Department of Jiangsu Province of China (H200401).

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Received date: 2008-10-13

excellent candidate radioligand for *in vivo* imaging the DAT system in human. ^{18}F-FECNT was first reported as the brain PET radioligand of DAT in 2000 by Goodman[10]. It reaches quasi equilibrium 90 min after injection and is rapidly displaced by β-CIT. Davis[13] successfully demonstrated the early diagnosis and grading of disease state of Parkinson's disease in 2003. It has a higher affinity for DAT, and yields the highest target-to-nontarget ratios and has among the nicest kinetics of ^{18}F-radiolabeled DAT ligands.

We have developed a one-step automated synthesis of ^{18}F-FECNT with high radiochemical yield from mesylate precursor[16,17], and preliminary studies were done on biodistribution in mice brain[18]. The results indicated that uptake of ^{18}F-FECNT was specific to DAT. In this paper, we report further preclinical pharmacological characters of ^{18}F-FECNT.

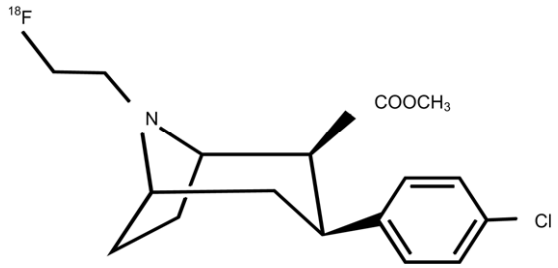

Fig.1 Chemical structure of ^{18}F-FECNT.

2 Materials and methods

2.1 Instruments

Rat brain stereotactic Instrument (Jiangwan-type, Shanghai Alcott Biotech Co. Ltd.), Packard Cobra automatic gamma counter (USA), MNT cryostat microtome (SLEE Technik GMBH, Germany), C431200 Storage phosphor system (Perkin Elmer).

2.2 Regents and Animals

The 6-Hydroxydopamine (6-OHDA) and apomorphine (APO) were purchased from Sigma. ^{18}F-FECNT was prepared by the one-step automated reaction from mesylate precursor as described in Ref.[16,17]. The final ^{18}F-FECNT was obtained with a decay-corrected radiochemical yield of 33±9% and radiochemical purity (RCP) of 98±1%. The radiochemical purity of ^{18}F-FECNT in aqueous solution was greater than 95% after standing for 4 h at room temperature.

Rats (Sprague-Dawley) and mice (NIH) were purchased from Shanghai SLAC Laboratory Animal Center.

2.3 Establish the left-sided 6-OHDA lesioned PD rats[19,20]

SD rats (male, 180–220g) were housed under controlled temperature and light conditions. Food and water were available. The animal were anesthetized with chloral hydrate (400 mg/kg, i. p.), and then placed in the rat brain stereotactic instrument. The incisor bar was adjusted until the heights of lambda and bregma were equal. This flat-skull position was achieved when the incisor bar was lowered 2.4 ± 0.4 mm below horizontal zero. After exposing skull, a hole was drilled in the skull of the rat above the left medial forebrain bundle (MFB). Then, a microsyringe was inserted to the appropriate location of the following coordinates (Bregma –4.0 mm, ML 1.65 mm, DV 8.0 mm), and 4 μL 6-OHDA was infused at 0.5 μL/min. The microsyringe was left in place for 5 min and then slowly withdrawn. The 6-OHDA (5 μg/μL) was dissolved immediately before use in ice-chilled Vit C (0.2 mg/mL Vit C in normal saline) and stored in the dark at 4℃. The control group was infused the same volume saline.

2.4 Rotational behavior tests[21]

Rotational behavior was induced by APO (0.5 mg/kg, i.p.) two weeks after unilateral 6-OHDA lesioning. The rotational data were continuously recorded for 60 min, and analyzed subsequently. Animals that demonstrated minimum per-minute averages of seven clockwise (unlesioned-side) were selected for further study.

2.5 Determination of partition coefficient

The partition coefficients of ^{18}F-FECNT were determined in *n*-octanol and two kinds of phosphate buffer (PB, pH 7.0 and pH 7.4, respectively). For each pH, ^{18}F-FECNT (10 μL, 0.02 MBq) was added to the two-phase system of 3.0 mL *n*-octanol and 3.0 mL PB. The mixture was vortexed for 3×2 min and centrifuged for 5 min at 4,000 rad/min, and then 1.0 mL *n*-octanol and 1.0 mL PB were taken out and counted with a γ-counter. Afterwards, 1.0 mL *n*-octanol was

transferred to another tube containing 3.0 mL PB and 2.0 mL *n*-octanol. The above procedure was repeated for five times. The ratio of the cpm/mL of *n*-octanol to that of PB is the partition coefficient of ^{18}F-FECNT.

2.6 Biodistribution *in vivo* in mice

^{18}F-FECNT (0.2 mL, 3.7 MBq) was injected through tail vein into the mice, which were 18–22 g and divided into six groups (five per group) randomly. The mice were sacrificed by decapitation at 5, 15, 30, 60, 120 and 180 min post-injection. The organs of interest (brain, heart, liver, spleen, lung, kidney, stomach, etc.) were removed and weighed, and radioactivity was counted with the γ-counter. The percent of injected dose per organ (%ID/organ) was calculated by comparing the sample counts with the counts of the dilution-corrected initial injected dose.

2.7 Autoradiography in normal and hemi-Parkinsonian rat brains

Each normal rat or hemi-PD rat was injected with ^{18}F-FECNT (0.2 mL, 18.5 MBq) via tail vein, and sacrificed at 30 min post-injection (two per group). The brains were quickly removed and placed in Cryostat, and frozen at –15℃. After reaching equilibrium at –15℃, the coronal sections were cut (20 μm) consecutively with a cryostat microtome, thaw-mounted on microscope slides, and air dried at room temperature. The slides containing the brain coronal sections were exposed for 1 h before being imaged in Storage phosphor system (C431200). The optical densities (OD) ratios of striatum/cerebellum (ST/CB) were determined with an image analysis system (OptiQuant) developed by Perkin Elmer.

2.8 Abnormal toxicity test

According to the regulations of Pharmacopoeia of China (2005), the abnormal toxicity test of ^{18}F-FECNT was determined by observing the toxic symptoms, change of weight, death and survival of five mice (17–20 g) within 48 h after receiving an injection of 0.5 mL ^{18}F-FECNT (47 MBq, 25 % of the human dose). The control group (five mice) was injected the same volume saline.

3 Results

3.1 Validation of left-sided lesioned PD rats

The clockwise (unlesioned-side) rotation started within 3–10 min and rapidly approached the peak rotational rates at 15–30 min. However, there was no rotational behavior in the control rats.

3.2 Partition coefficient

The partition coefficients were 34.14 and 56.41 at pH 7.0 and pH 7.4, respectively.

3.3 Biodistribution *in vivo* in mice

As shown in Table 1, after being injected into the blood, ^{18}F-FECNT was quickly intussuscepted by tissues. High uptakes were initially observed in liver, kidney and lung, and subsequently decreased. Among the interest organs, liver had the first and the highest uptake. Clearance of all organs was quick, and the remainder of those was low at 180 min post-injection. Uptake of ^{18}F-FECNT in mice brain was 2.22, 1.20, 1.02, 0.78, 0.71, and 0.67 %ID/organ at 5, 15, 30, 60, 120, and 180 min post-injection, respectively (Fig.2).

Table 1 Biodistribution of ^{18}F-FECNT in mice at different minutes after injection (%ID/organ, $\bar{x} \pm sd$, n=5)

Organ	5 min	15 min	30 min	60 min	120 min	180 min
Heart	0.44±0.05	0.33±0.04	0.33±0.06	0.29±0.06	0.30±0.04	0.28±0.07
Liver	7.00±0.19	4.24±0.57	4.10±0.57	3.87±0.28	3.53±0.78	2.83±0.28
Spleen	0.51±0.12	0.47±0.16	0.34±0.04	0.32±0.11	0.34±0.05	0.24±0.05
Lung	1.00±0.09	0.84±0.24	0.78±0.22	0.76±0.23	0.43±0.12	0.36±0.09
Kidney	2.07±0.31	1.43±0.09	1.42±0.09	1.09±0.12	0.89±0.11	0.52±0.09
Stomach	0.67±0.20	0.56±0.15	0.55±0.14	0.42±0.10	0.39±0.07	0.38±0.04
Pancreas	1.04±0.20	0.42±0.12	0.40±0.05	0.22±0.04	0.20±0.04	0.14±0.03
Thymus	0.25±0.02	0.20±0.08	0.23±0.08	0.20±0.05	0.15±0.05	0.14±0.02
Bladder	0.07±0.01	0.11±0.01	0.13±0.04	0.08±0.03	0.12±0.03	0.05±0.02

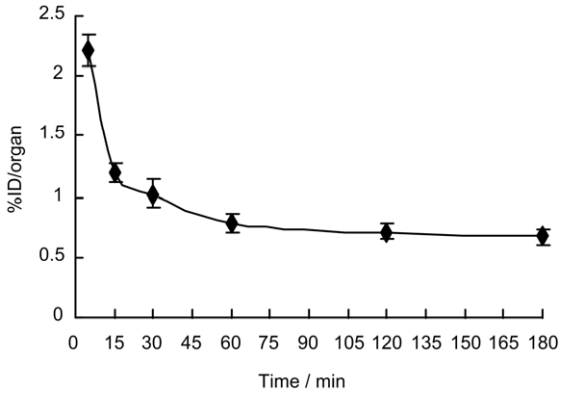

Fig.2 Uptake of ^{18}F-FECNT in mice brain.

3.4 Autoradiography in normal and hemi-PD rat brains

At 30 min post-injection, the regional brain distribution in rats indicated that striatum, the DAT-rich target region, exhibited the most intense radioactivity. However, the uptake of ^{18}F-FECNT in the hypothalamus/thalamus brain regions of high SERT density was low.

In normal SD rat brains, symmetrical uptake of ^{18}F-FECNT was found in the left (L) and the right (R) striatum (ST). They were remarkably higher than that of cerebellum (CB), which contains no dopamine transporters and is used as a background region (Fig.3). The ratios of ST_R/CB, ST_L/CB and ST_R/ST_L were 5.52, 5.42 and 1.02, respectively. Nevertheless, as for the left-sided lesioned PD model rats, the uptake of ^{18}F-FECNT in brain region was evaluated. Compared with normal rats, the striatal uptake of ^{18}F-FECNT was bilaterally decreased, whereas no significant uptake was visible in the 6-OHDA lesioned-sided striatal areas (Fig.4). The average ratios of ST_R/CB and ST_L/CB were 2.57 and 1.05, respectively.

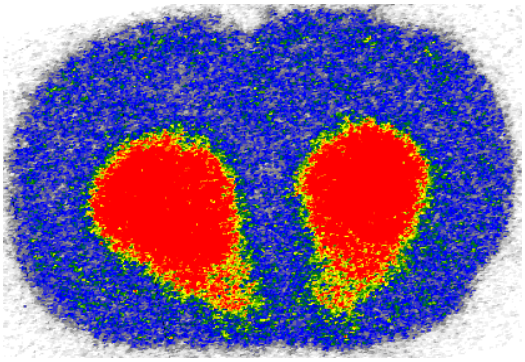

Fig.3 Autoradiography of ^{18}F-FECNT in normal SD rat brains (30 min).

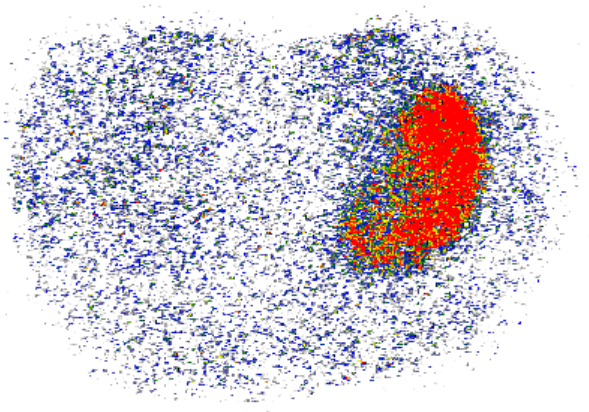

Fig.4 Autoradiography of ^{18}F-FECNT in hemi-PD rat brains (30 min).

In general, autoradiography is simple and fast for detecting the dopamine level. *In vitro* autoradiographic study further verified that the uptake of 18F-FECNT to DAT ((ST/CB)-1) was specific in the rat brains, which can be clearly demonstrated in Fig.5.

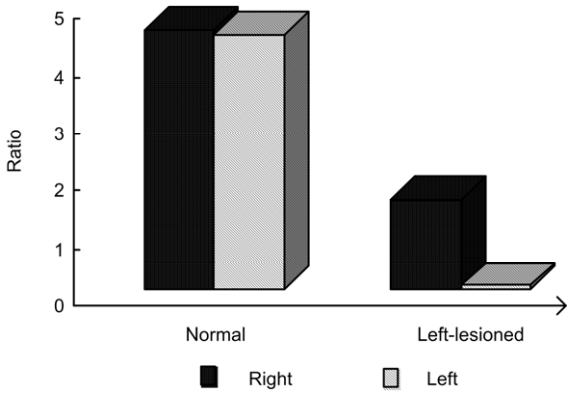

Fig.5 Comparison of the ratios of (ST-CB)/CB in normal and left-lesioned PD rats.

3.5 Abnormal toxicity test

Each mouse was injected with 25% of the human dose of ^{18}F-FECNT. None of the mice died within two days (48 h) of normal feeding, there were no toxic symptoms, the body weight had no significant difference, and no abnormality was observed in all organs after dissection, compared with the control group, even though they had received a dose per kilogram of 625 times as high as a patient's dose (assuming a weight 50 kg). This proved that ^{18}F-FECNT is safe.

4 Discussion

The partition coefficient trial suggested that ^{18}F-FECNT might be more lipophilic at pH 7.4 and be propitious to entry into the brain. The results of biodistribution *in vivo* in mice displayed that the major radioactivity was metabolized by the hepatic system, whereas kidney and bladder were the main excretory organs. The high uptake and favorable retention in the brain confirmed that it is a superior candidate for use as an *in vivo* agent for imaging dopamine transporters. The monoamine neurotoxin 6-OHDA is commonly used to create hemi-parkinsonism in rats[20]. For 6-OHDA, DA neurons are selectively destroyed, with reduction of dopamine in the striatum. The PD rat model were established by sterotaxic microinjection of 6-OHDA into the MFB, it is stable and easy to operate. It has been widely applied in PD researches. The injection of 6-OHDA to the MFB of rats causes remarkably reduced DAT counts on the lesioned-side of the striatum. This lesion is known to induce a complete and irreversible destruction of the nigrostriatal dopamine pathway.

A series of DAT imaging agents in recent development, ^{18}F-FECNT demonstrates excellent selective localization in striatum of brain at the present time. *In vitro* autoradiographic study demonstrated the selective binding of ^{18}F-FECNT to dopamine transporters. The loss of dopamine transporters in the 6-OHDA lesioned rats can be sensitively measured with ^{18}F-FECNT, suggesting the usefulness of this tracer for monitoring the change in dopamine transporters associated with various neurodegenerative diseases, such as PD. Furthermore, initial microPET imaging studies in rats with ^{18}F-FECNT confirmed that the *in vivo* properties of this complex is excellent for imaging dopamine transporters[18].

^{18}F-FECNT has the following advantages over ^{18}F-FP-β-CIT and ^{99m}Tc-TRODAT-1.

Firstly, as the affinity of FECNT is lower than FP-β-CIT for DAT, FECNT probably has a higher rate of dissociation from the DAT binding site, and attains binding equilibrium more rapidly. The imaging time of ^{18}F-FECNT was 1 h earlier than that of ^{18}F-FP-β-CIT. As a result, for the ^{18}F with 110-min half- life, we are able to reduce half of the cost. Moreover, the specific-to-nonspecific binding ratios for ^{18}F-FECNT at transient equilibrium are greater than ^{18}F-FP-β-CIT, for which small variation in dopamine system may be easily assessed.

Secondly, the uptake of ^{18}F-FECNT in brain and striatum is higher than that of ^{99m}Tc-TRODAT-1. However, the application of ^{18}F has been limited in clinical for the higher cost and the relatively scarce source. Applications of ^{99m}Tc-TRODAT-1 were widely reported, because of attractive radionuclide characteristics of ^{99m}Tc, and available kit form. Yet, both the affinity and the selectivity of ^{99m}Tc-TRODAT-1 to DAT are inferior to ^{18}F-FECNT. Additionally, PET with the higher spatial resolution is considered the more informative imaging modality for the quantitative measurement of the density of in vivo neurotransmitter transporters in humans.

5 Conclusion

In summary, ^{18}F-FECNT can penetrate blood-brain barrier and localize in striatum. It is a potentially safe and effective radiotracer for monitoring the change of CNS dopamine transporters related to various neurodegenerative diseases, such as PD.

References

1 Uhl G R, Johnson P S. J Exp Biol, 1994, **196:** 229–236.

2 Wilson J M, Levey A I, Rajput A, *et al*. Neurology, 1996, **47:** 718–726.

3 Li X M, Chen Z P, Wang S P, *et al*. Nucl Sci and Tech, 2007, **18**: 223–226.

4 Hwang W J, Yao W J, Wey S P, *et al*. J Nucl Med, 2004, **45:** 207–213.

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

6 Chen Z P, Wu C Y, Zhang Z W, *et al*. Chin J Nucl Med, 2003, **23**: 241–243 (in Chinese).

7 Dannals D F, Neumeyer J L, Milius R A, *et al*. J Labelled Compd Radiopharm, 1993, **33:** 147–152.

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

9 Kung M P, Stevenson D A, Plossl K, *et al*. Eur J Nucl Med, 1997, **24:** 372–380.

10 Goodman M M, Kilts C D, Keil R *et al*. Nucl Med Biol, 2000, **27:** 1–12.

11 Voll R J, McConathy J, Waldrep M S, *et al*. Appl Radiat Isot, 2005, **63:** 353–361.

12 Deterding T A, Votaw J R, Wang C K, *et al*. J Nucl Med, 2001, **42:** 376–381.

13 Davis M R, Votaw J R, Bremner J D, *et al*. J Nucl Med, 2003, **44:** 855–861.

14 Votaw J R, Byas-Smith M G, Voll R, *et al*. Anesthesiology, 2004, **101:** 1128–1135.

15 Zoghbi S S, Shetty H U, Ichise M, *et al*. J Nucl Med, 2006, **47:** 520–527.

16 Chen Z P, Wang S P, Li X M, *et al*. Appl Radiat Isot, 2008, **66:** 1881–1885.

17 Chen Z P, Wang S P, Li X M, *et al*. J Nucl Med, 2008, **49**(supplement1)**:** 306P.

18 Chen Z P, Wang S P, Li X M, *et al*. Eur J Nucl Med Mol Imaging, 2008, **35**(Suppl 2)**:** S153–S154.

19 Bao X M, Shu S Y. The Stereotaxic Atlas of the Rat Brain. Beijing: People's Health Publishing House, 1991, 48–54 (in Chinese).

20 Niu C S, Fu X M. Chin J Neuromed, 2005, **4:** 521–527 (in Chinese).

21 Zhang J T, Zhang Q Z. Techniques and Methods of Neuropharmacology Research. Beijing: People's Health Publishing House, **2005,** 328–336 (in Chinese).