Preparation of 6-[^{18}F]fluoro-L-DOPA and its biodistribution in normal and unilateral PD model rats

ZHANG Lan[1], TANG Gang-Hua[1,2], WANG Yong-Xian[1], YIN Duan-Zhi[1]

([1] *Center of Radiopharmaceutical Research, Shanghai Institute of Nuclear Research, the Chinese Academy of Sciences, Shanghai 201800;*

[2] *Nanfang PET Center, Nanfang Hospital, The First Military Medical University, Guangzhou 510515*)

Abstract No-carrier-added 6-[^{18}F]fluoro-L-DOPA (6-FDOPA) was synthesized via a multistep procedure from a commercial available precursor, 6-nitroveratraldehyde. The total synthesis time was 75 min, with a radiochemical yield of (10±3)%, high radiochemical purity (>99%) and high enantiomeric purity (>95%). The biodistributions of 6-FDOPA in normal and unilateral PD model rats were measured. The results from normal rats showed the expected high concentration of radioactivity in striatum and low distributions in cerebrum, cortex and cerebellum. The ratio of the radioactivity in striatum to cerebellum reached a peak value (5.9) at 60 min. In unilateral PD model rats, whose substania nigra of the right side had been damaged by pre-treated with 6-OHDA, the radioactive concentration in striatum of the damaged side was significantly lower than that of the undamaged side or that of both sides in striatum of control groups.

Keywords 6-[^{18}F]fluoro-L-DOPA, Biodistribution, Rats, Unilateral PD model

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1 INTRODUCTION

As a widely used PET tracer, 6-[^{18}F]fluoro-L-DOPA plays an important role in diagnosing many neuropsychiatric diseases such as Parkinson's diseases (PD). Though since early 1980's, many production routes for this radiopharmaceutical have been developed,[1-5] a convenient nucleophilic routes starting from no-carrier-added [^{18}F] fluoride is still attractive. This is based on the following three facts. Firstly, for most of PET centers, production of [^{18}F]fluoride is much easier and more economical than [^{18}F]fluorine gas. Secondly, the final products from nucleophilic routes are no-carrier-added, and thus have a much higher specific activity than carrier-added products from

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Correspondence author: ZHANG Lan: zhangl@sina.com

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electrophilic routes, which is helpful to diagnosis with PET.[6] Lastly, current nucleophilic routes are unsuitable for routine production because of their critical reaction conditions, complicated procedures and long synthesis time.

The latest enantioselective synthesis route reported by Lemaire *et al*[7,8] partially resolved these problems by using chiral phase-transfer catalysts. Via this route, a considerable high radiochemical yield (10%–15%, decay uncorrected) and short synthesis time (110 min) are expectable and the critical reaction conditions can be avoided. Unfortunately, the labeling precursor (6-Trimethylammoniumveratraldehyde Triflate) is not commercially available and further more it is not stable for long time storing. In this paper, an alternative procedure starting from a commercially available precursor (6-nitroveratraldehyde) was described and the traditional nitro for ^{18}F substitution reaction is applied.

6-FDOPA is the analogue of L-DOPA, which is the biosynthesis precursor of dopamine, and behaves similarly *in vivo*. After i.v. administration it is taken up, decarboxylated at the same rate as L-DOPA[9] and stored in the form of ^{18}F-dopamine in striatal tissue.[10] Results from biological studies indicate that the cell bodies of central dopaminergic neurons of mammals are located in the substania nigra and ventral tegmental area, and project to the striatum, frontal, cingulated and olfactory cortices.[11,12] In these neurons dopamine is synthesized from L-DOPA in situ.[13] According to a study with PET in living man, it was shown that the radioactivity is mainly accumulated in the striatum and is also concentrated in the anterior cingulate and frontal cortex.[14]

2 METHODS

2.1 Materials

Cinchonidine, 9-chloromethylanthracene, allyl bromide, $K_{2.2.2}$ (Kryptofix2.2.2) and N-(diphenylmethylene) glycine tert-butyl ester were purchased from Acros (Belgium), and the chiral phase-transfer catalyst O-Allyl-N-(9)-anthracenylcinchonidinium bromide was synthesized according to the procedure reported by Corey.[15] Other chemicals were purchased from Shanghai Chemical Company (China). [^{18}F]fluoride was produced with a GE PET-trace cyclotron by ^{18}O(p, n)^{18}F reaction using enriched ^{18}O-water.

All ^{1}H-NMR were recorded on an AM-400 NMR spectrometer. Mass spectra were recorded on an API-2000 LC-MS-MS system (Applied Biosystems Corp). A LC-10AT HPLC system including a variable wavelength UV detector (Shimadzu, Japan) and a LB 508 Radioflow Detector (EG&G, USA) was used to perform the analysis and purification. A 250 mm×4.6 mm Shim-lack VP-ODS column (Shimadzu, Japan) was used for the analysis of the labeled products (eluted with a mixture of methanol and water (65:35

V/V), flow rate 1 L/min). The final product, 6-FDOPA, was analyzed with the same column (eluted with 0.07 mol/L KH_2PO_4, flow rate 1 mL/min). The enzymeric purity was detected with the same system using a chiral mobile phase (0.017 mol/L L-proline and 0.008 mol/L copper acetate). A 300mm×7.8 mm μBondapak C18 semi-preparative column (Waters, Massachusetts, USA) was used for the purification of the final product (eluted with a mobile phase of 5 mmol/L sodium acetate, 1 mmol/L EDTA, 17 mmol/L acetic acid and 0.57 mmol/L ascorbic acid, flow rate 4.5 mL/min).

Normal rats (Wistar Rats, Clean Grade) used in the experiments were obtained from Experimental Animal Center, First Military Medical University. PD model rats were prepared by localized injection of 6-hydroxyldopamine (6-OHDA) into nigra of the right side and 3 days later, were treated with apomorphine to induce reel (7 r/min, >40 min), the unilateral PD symptom.

2.2 Experimental

2.2.1 Preparation of 6-FDOPA

The no-carrier-added [^{18}F]fluoride was produced by $^{18}O(p,n)^{18}F$ reaction using a small volume of ^{18}O-enriched water(1.5 mL, >95%) target. The target was bombarded with 16.5 MeV proton beam (25 μA) for 50–70 min and yielded about 37 GBq of [^{18}F]fluoride.

7.4 GBq of [^{18}F]fluoride in 200 μL of [^{18}O] water was delivered into a small reaction vial containing 20 mg of $K_{2.2.2}$ and 3 mg of potassium carbonate. In an oil bath (110°C), the mixture was evaporated to dry under nitrogen flow. Then 2×500μL of CH_3CN was added to the residue and evaporated to dry. Next, 15 mg of 6-nitroveratraldehyde in 1 mL of DMSO was added and the vial was capped. The labeling reaction was performed in a household microwave oven (500 W) for 2 min. The reaction mixture was then diluted with 20 mL of HCl (0.5 mol/L), passed through two C-18 sep-pak (Waters) washed with HCl, $NaHCO_3$ and water. Finally it was eluted with CH_2Cl_2 and dried with a small $MgSO_4$ column. About 500 μmol of diiodosilane was prepared in situ according to the well-known procedure[16] and added to the previously obtained radioactive solution. The mixture was stirred for 5 min at RT and then passed through a small silica gel column (1 cm×7.5 cm, 200–300 mesh, eluted with 6 mL of CH_2Cl_2). The resulted elution was collected into a round-bottom flask and mixed with 15 mg of O-allyl-N-(9)-anthracenylcinchonidinium bromide, 40 mg of cesium hydroxide monohydrate and 2.5 mg of Schiff base N-(diphenylmethylene) glycine tert-butyl ester. The mixture was stirred for 5 min at RT and then purified by directly passing a small silica gel column (1 cm×7.5 cm, 200–300 mesh). The solution was evaporated to dry under nitrogen flow in a small reaction vial before 1 mL of HI was added. The hydrolysis reaction was performed in the same

microwave oven for 10 min. The resulted mixture was neutralized with 6 mol/L NaOH, filtrated and then purified with a semi-preparative HPLC column. The HPLC fraction (about 5 mL) was collected into a small vial containing NaCl (50 mg) and ascorbic acid (10 mg). After filtration with a 0.22μ membrane filter (Millex-GS, Millipore, Molsheim, France), the final 6-FDOPA solution was ready for animal injection.

2.2.2 Biodistribution in rats

24 rats were deprived of food for 16 hours before experiments. They were divided into 6 groups and each was administrated with 1 MBq (1 MBq) of 6-FDOPA (i.v.). At 5, 30, 60, 90, 120 and 150 min after injection respectively, a group of rats were killed by decollation and dissected. Distribution of activity in 12 organs or tissues (heart, lung, liver, kidney, spleen, blood, muscle, cerebrum, striatum, hippocampus, frontal cortex and cerebellum) was detected and the values of $\%IDg^{-1}$ were calculated.

12 unilateral PD model rats and 12 normal rats, which have been treated with the same procedure as the model rats except that the 6-hydroxyldopamine was replaced by normal saline, were divided into 3 groups and each was administrated with 1 MBq of 6-FDOPA (i.v.). After 30, 90 and 120 min, respectively, a group of model rats and a group of control rats were killed by decollation and the distribution of activity in striatum, cerebellum, frontal cortex of both sides (left and right) were detected and the values of $\%IDg^{-1}$ were calculated.

3 RESULTS AND DISCUSSION

3.1 Preparation of 6-FDOPA

In Table 1, the radiochemical yield (decay uncorrected) and average synthesis time of each step were summarized.

Table 1 Radiochemical yields and synthesis time of the preparation of 6-FDOPA

Steps	Radiochemical yield(%)	Synthesis time (min)
Removal of water	N/A	5
Labeling and sep-pak purification	25–35	20
Reductive iodination and silica gel column purification	75–85	10
Alkylation and silica gel column purification	75–80	10
Removal of solvent, hydrolysis and HPLC purification	30–35	30
Total	7–13	75

The radiochemical yield of ^{18}F labeling reaction using 6-nitroveratraldehyde as the labeling precursor is about 10 percent lower than that of the reaction when 6-

trimethylammonium veratraldehyde triflate is used. Compared with the later precursor, 6-nitroveratraldehyde needs higher reaction temperature (150°C) and longer reaction time (15 min) when the reaction was conducted with conventional oil-bath heating. Furthermore, the C-18 purification step costs a little more time. The use of microwave oven can largely reduce the reaction time from 15 min to 2 min. The reductive iodination and alkylation under phase-transfer catalysis were very easy to perform conveniently with high yields. The hydrolysis reaction must be performed under a considerable rigorous condition: high temperature and free from oxygen. The use the microwave heating technique in the hydrolysis reaction can save 10–15 min. In our lab, currently available yield of the hydrolysis and HPLC purification is still not as high as expected; this may be partly caused by the use of a 7.8 mm×300 mm semi-preparative HPLC column smaller than that in the literature[4] (9.4 mm×500 mm).

Although the radiochemical yield of ours was lower than that reported[4], this method is still a potential pathway to the routine preparation of 6-FDOPA.

3.2 Biodistribution of 6-FDOPA in Rats

Results of the biodistribution of 6-FDOPA in 6 tissues, in which we are most interested, have been shown in Fig.1.

The results clearly showed the high concentration of radioactivity in striatum and hippocampus, while in the cerebellum, cerebrum and frontal cortex, the activity is much lower. And the clearance of the activity in blood is very fast (80% in 30 min). From 30 to 60 min after injection, the activity in striatum is higher than all the other 11 organs/tissues, while the ratio of the activity in striatum to cerebellum, which is widely accepted as a useful parameter to reflect the integrity of presynaptic dopamine function, reaches a peak (5.9) at 60 min (see Fig.2). This may indicate that there is a "best time" for the 6-FDOPA PET study. Changes in the activity ratios with time, of striatum to cerebellum, striatum to frontal cortex and hippocampus to cerebellum, were shown in Fig.2. It can be observed that the ratio of the activity in striatum to frontal cortex changes with time in a contrary trend to that of striatum to cerebellum from 30 to 90 min. This may be brought about by the different rates of uptaking and clearance of cerebellum and frontal cortex. As to the ratio of hippocampus to cerebellum, it changes in a similar trend to the ratio of striatum to cerebellum, but with about 30 min delay. This may reflect the re-distribution of 6-FDOPA metabolites [^{18}F]OMFD, [^{18}F]DOPAC, [^{18}F]HVA, etc.

Since the rats in this experiment were not been administrated with carbidopa, the inhibitor for aromatic amino acid decarboxylase in extracerebral tissues,[17] the distribution of ^{18}F in extracerebral organs/tissues was high at the early time post-injection,

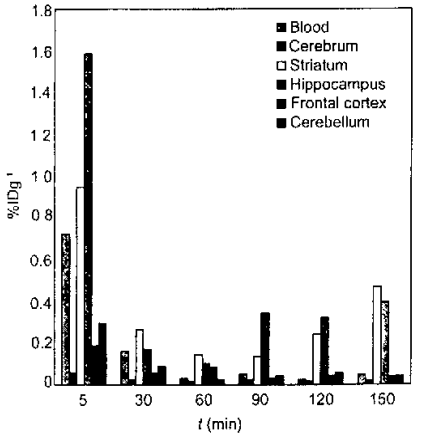

Fig.1 Biodistribution of 6-FDOPA in some tissues of rats

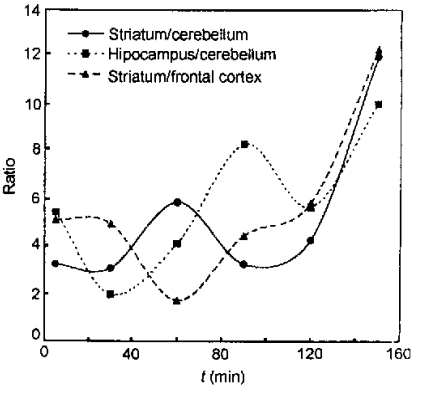

Fig.2 Ratios of radioactivity in striatum, hippocampus, frontal cortex and cerebellum

but over 90% of the activity was cleared out from heart, kidney, liver, spleen and muscle, and over 80% from lung and blood in 30 min. At 5 min post-injection, the %IDg^{-1} value of kidney is about 10 times higher than that of the other extracerebral organs/tissues. This indicates that after administration i.v., 6-FDOPA is mainly metabolized and excreted via kidney. See Fig.3.

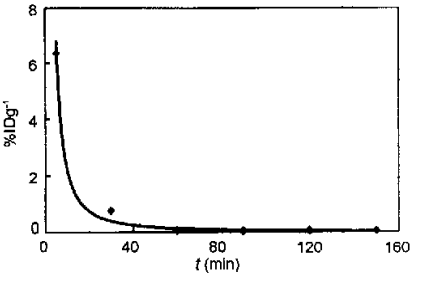

Fig.3 Clearance of radioactivity from kidney

In unilateral PD model rats, the concentration of activity in striatum of the damaged side was significantly lower than that of the undamaged side or that of both sides in striatum of control groups (see Fig.4). This result showed that 6-FDOPA is an efficient PET tracer for examination of integrity of the nigra-striatum dopamine pathway.

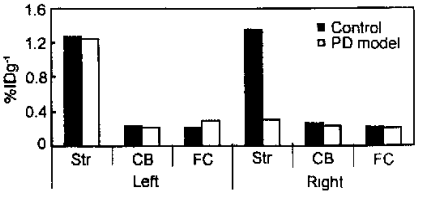

Fig.4 Biodistribution of 6-FDOPA in striatum (Str), cerebellum (CB) and frontal cortex (FC) of unilateral PD model rats at 30 min post-injection

4 CONCLUSION

No-carrier-added 6-[^{18}F]fluoro-L-DOPA was successfully prepared via a multi-step procedure in 75 min. The biodistribution of 6-FDOPA in normal and unilateral PD model rats was measured. The results for normal rats showed an expected high concentration of activity in striatum, low in cerebellum, and high ratio of activity in striatum to cerebellum. For PD model rats, the uptake and maintenance of radioactivity in striatum of the damaged side is much lower that in undamaged striatum. These results revealed the validity of 6-FDOPA prepared in this experiment as a PET tracer for valuing the integrity of presynaptic dopamine system.

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