Comparative and optimized studies on radiosynthesis of *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine

WANG Ming-Wei, YIN Duan-Zhi*, WANG Yong-Xian, CHENG Deng-Feng, LI Gu-Cai, ZHOU Wei

(Radiopharmaceutical Centre, Shanghai Institute of Applied Physics, the Chinese Academy of Sciences, Shanghai 201800)

Abstract *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET), one of radiolabelled amino acids, is a very promising brain tumor positron emission tomography (PET) imaging agent and holds clinical potential. This paper described out a comparative and optimized radiosynthesis of [^{18}F]FET, concerning three aspects of its two-step preparation method, including reaction components, heating methods and reaction models. As a result, good radiochemical yield (about 45%, no-decay-corrected) and radiochemical purity (more than 95%) were achieved, and total synthesis time of [^{18}F]FET was shortened within 20 min and radiation exposure time also decreased.

Keywords ^{18}F, L-tyrosine, Radiosynthesis, Positron emission tomography

CLC number O621.3

1 Introduction

Over the past few years, clinical interest in metabolic imaging of tumor has been growing. The most typical example is the increasing demand and application of 2-[^{18}F]fluoro-deoxyglucose([^{18}F]FDG) and positron emission tomography (PET). Another interesting target for tumor metabolic imaging is the increased protein metabolism and amino acids transport in cancer cells, which is just the reason for the rapid increasing of studies on radiolabelled amino acids.

Almost all amino acids have been radiolabelled to study their potential imaging characteristics, especially for PET imaging, because the replacement of carbon atom by ^{11}C does not change the chemical, biological and physical features of the molecule itself.[1] However, the physical half-life of ^{11}C is short, only 20.5 min. Comparatively, ^{18}F, which has a longer half-life ($t_{1/2}$ = 109.7 min), matchs better with the synthesis of its labeled compounds and relatively slow process of PET imaging. A number of ^{18}F-labeled amino acids are being investigated and the general tendency in quantity is increasing, of which the most widely studied are tyrosine and its derivatives. Major ^{18}F-labeled tyrosine are as follows: L-2-[^{18}F] fluoro-tyrosine (L-[^{18}F]FT),[2,3] L-3-[^{18}F] fluoro-α-methyl tyrosine ([^{18}F]FMT) [4,5] and *O*-(2-[^{18}F] fluoroethyl)-L-tyrosine ([^{18}F]FET).[6,7]

Of the ^{18}F-labeled tyrosine derivatives, only [^{18}F]FET was synthesized by nucleophilic displacement reaction. The radiochemical yield was 40%, a value much higher than those of L-[^{18}F]FT and [^{18}F]FMT which were both from electrophilic substitution reaction. Moreover, a series of model animal experiments[8-11] and clinically preliminary PET imaging of tumor patients [12,13] about [^{18}F]FET all showed encouraging results. Consequently, it is necessary to further develop means of the synthesis of [^{18}F]FET including shorter synthesis time and a simplified operation process to protect operator from radiation exposure to the maximum. For this purpose, we have carried out a comparative and optimized radiosynthesis of [^{18}F]FET, according to its two-step synthesis procedure, including three aspects such as reaction components, heating methods and reaction

Supported by the Knowledge Innovation Project of Chinese Academy of Sciences (No.KJCX-SW-08) and the National Natural Science Foundation of China (No.30371634)

* Corresponding author. Tel: +86-021-59558507; Fax: +86-021-59554649; E-mail: yinduanzhi@sinap.ac.cn

Received date: 2005-03-15

models.

2 Experimental

2.1 Materials

Aminopolyether Kryptofix 222 (4, 7, 13, 16, 21, 24 hexaoxa-1, 10-diazabicyclo [8.8.8] hexacosan, $K_{2.2.2.}$), dimethyl sulfoxide (DMSO) and dry acetonitrile were purchased from Acros Chemical Co., Ltd., Belgium, L-tyrosine from GL Biochem (Shanghai) Ltd. Other chemicals including sodium hydroxide, potassium carbonate, acetonitrile and chloroform were of spectral analysis grade or HPLC grade, obtained from Shanghai Chemical Company, China. All of the chemicals and solvents was used directly without further purification.

Sep-Pak silica plus cartridge and Sep-Pak Al_2O_3 plus cartridge for solid phase extraction and Mini-Vials were purchased from Waters Corporation, USA and Alltech Associates, Inc. USA, respectively. GF254 silica gel plate based on glass came from Huiyou Silica Development Co., Ltd., Yantai, Shangdong, China.

High performance liquid chromatography (HPLC) system consisting of P680 HPLC pump was equipped with PDA-100 photodiode array detector (Dionex summit HPLC), Bioscan flow-count detector (Bioscan Ins. USA), and analytical Vydac C18 column (10μm, 2.5mm×250mm, Shimadzu Corporation, Japan). Other instruments are Bioscan AR-2000 radio-TLC Scanner (Bioscan Ins. USA), FJ-391A2 radioactivity counter (Beijing Nuclear Instruments Factory, China) and Hitachi MR-8207 microwave oven (Hitachi Ltd. Japan).

2.2 Preparation of [^{18}F]FET

No-carrier-added aqueous $^{18}F^-$ was produced by irradiation of a 1.5 mL water target on a Cyclotron-30 (IBA, Belgium) using a 16.5MeV proton beam on enriched [^{18}O]H_2O by $^{18}O(p, n)^{18}F$ reaction. The activation of $^{18}F^-$ was carried out as described in literatures.[14, 15]

According to literatures[7,16-18], the synthesis route of [^{18}F]FET was slightly modified and determined as in Fig.1.

2.2.1 Step one

To the residue containing the dried complex

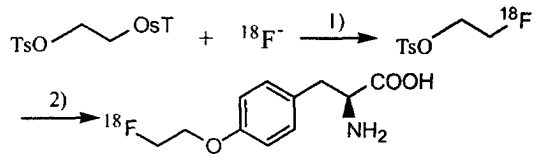

Fig.1 A two-step synthesis procedure of [^{18}F]FET. Reaction conditions: 1) K_{222}/K_2CO_3, $CNCH_3$, oil bath at 90℃ or microwave oven; 2) a solution of L-tyrosine and aqueous NaOH in DMSO or di-Na-salt of L-tyrosine in DMSO; oil bath at 90℃ or microwave oven.

$^{18}F^-/K_{2.2.2.}/K_2CO_3$, a solution of 1,2- ditosyloxyetane (8~10 mg) in CH_3CN (500 μL) was added. The reaction mini-vial was sealed with a Teflon-silicon septum, located in oil bath at 90℃ or in microwave oven until the desired reaction time. Then, the reaction mixture at different reaction time was spotted on GF_{254} silica gel plate with a capillary tube, which was developed in a development chamber containing pure $CHCl_3$ and scanned by radio-TLC Scanner to determine the labeling efficiency. The final reaction mixture was either right dried under N_2 flow or purified with Sep-Pak silica plus cartridge to participate in the next reaction.

The labelled intermediate 2-[^{18}F]Fluoroethyl tosylate was confirmed by both HPLC and radio-TLC mentioned above, and compared with cold reference compound. The mobile phase for HPLC (1mL/min of flow rates) and TLC was CH_3CN/H_2O (*V*/*V*:50/50) and 100% chloroform, respectively, and t_R and R_f was 8.2min and 0.36. The labelling efficiency was over 80% post 10 min for oil bath heating or 1 min for microwave heating and both of radiochemical yields were more than 85% on the average. The radiochemical purity was >97% measured by HPLC and Radio-TLC (Fig.2).

2.2.2 Step two

The solution of L-tyrosine (9.1 mg) and 10% aqueous NaOH (40 μL) in DMSO (500 μL) or di-Na-salt of L-tyrosine in DMSO (500 μL, 18 mg/mL) as reactant was added to the above reaction mini-vial containing either the dried original mixture or separated intermediate in Step one. The vial was closed and heated at the desired temperature in an oil bath or in microwave oven until the required reaction time. The reaction mixture at different reaction time was spotted on GF_{254} silica gel plate with a capillary tube, which was developed in a development chamber containing the mixed solvent of CH_3CN and water

(*V*/*V*: 95/5) and scanned by radio-TLC Scanner to measure the labeling efficiency. The reaction solution passed through one Sep-Pak silica plus cartridge for two-pot reaction model and Sep-Pak silica plus cartridge and Sep-Pak Al_2O_3 plus cartridge connected in series for one-pot reaction model, which was eluted by $CHCl_3$ and phosphate buffer saline (PBS, pH=7.4) in turn.

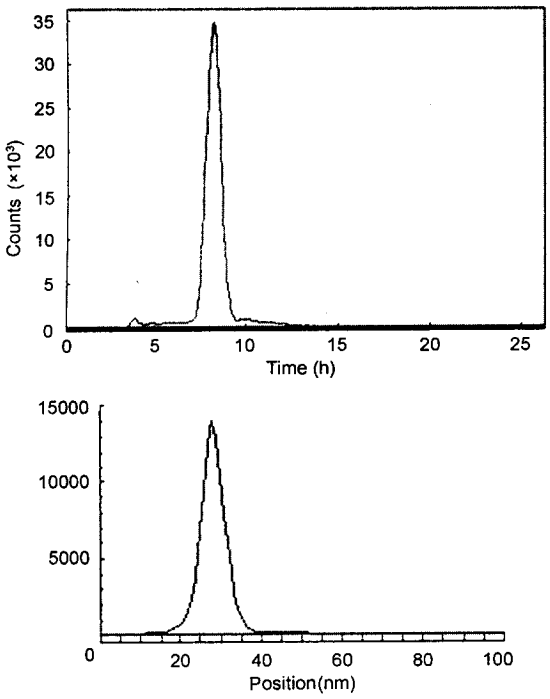

Fig.2 Spectra of HPLC and radio-TLC of the labelled intermediate 2-[^{18}F]Fluoroethyl tosylate from Step one.

The final product [^{18}F]FET was confirmed using HPLC and radio-TLC mentioned above, and compared with cold reference compound. The mobile phase for HPLC (1mL/min of flow rates) and TLC was ethanol/water/acetic acid (*V*/*V*/*V*: 10/87.5/2.5) and CH_3CN/H_2O (*V*/*V*: 95/5), respectively, and t_R and R_f was 4.1min and 0.06. The labelling efficiency was over 80% upon 15 min in oil bath heating or 2 min for microwave heating, and both of radiochemical yields were almost about 62% on the average. The radiochemical purity was >97% measured by HPLC and Radio-TLC (Fig.3).

3 Results and discussion

3.1 Experiments on influence of reaction components

Wester et al.[7] first synthesized [^{18}F]FET by a two-step procedure including nucleophilic ra-

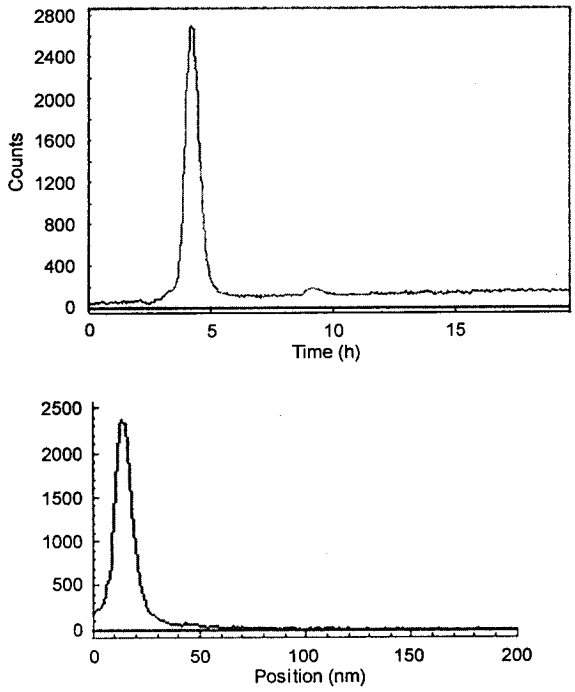

Fig.3 Spectra of HPLC and radio-TLC of the final product [^{18}F]FET from Step two.

diofluorination of 1,2-ditosyloxyethane and fluoroethylation of unprotected L-tyrosine, where the reactant was the solution of di-Na-salt of L-tyrosine in DMSO (reactant I). Recently, the mixture of L-tyrosine and aqueous NaOH in DMSO was taken by Tang Ganghua et al.[16] as the starting materials (reactant II). In theory, above two reaction systems are identical, because the mechanism for both can be attributed to that the free anion of L-tyrosine was selectively fluoroethylated on the site of phenolic hydroxyl. However, in our experiments, it was found that the reaction rate for reactant II was nearly as two times as that of reactant I (Fig.4, curve O vs curve G) when thermal oil bath was employed as heating method. The figure showed that the former needed only 10 min whereas the latter needed more than 20 min when lableling efficiency was equally up to 80%. The labeling efficiency was more than 95% and the reaction was almost completed within 20 min for reactant II, whereas the labeling efficiency was less than 85% within 30 min for reactant I.

For reactant II, di-Na-salt of L-tyrosine was formed *in situ* and there was a small quantity of water, thus the anion of L-tyrosine was free and the reaction site was nude, which aided the reaction. However, for

reactant I, the starting materials was just the solution that solid di-Na-salt of L-tyrosine dissolved in pure DMSO, a dipolar aprotic solvent. Anion in DMSO has a greater tendency to form ion pairs, because of their unsolvated state[19] which is one of the factors in diminishing the reactivity of a nucleophile.[20] Among a typical study on selective *O*-alkylation of L-tyrosine, Solar et al.[21] took also reactant II as the starting materials.

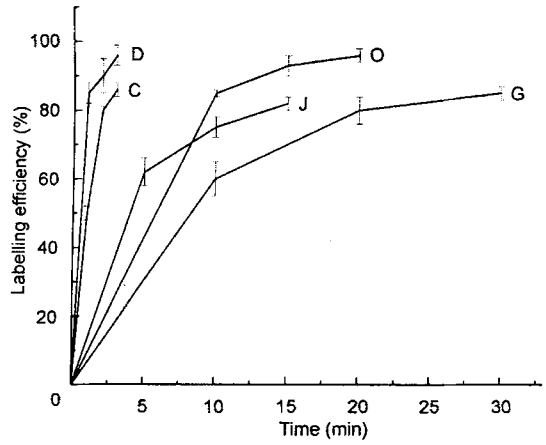

Fig.4 Curves of time vs labeling efficiency for different synthesis methods of [^{18}F]FET.
Note: Curves C and J from Step one, heated by microwave or oil bath, respectively; curves D, O and G from Step two, heated by microwave, oil bath or oil bath, respectively, with the former two using reactant II and the last using reactant I.

3.2 Experiments on influence of heating method

Microwave dielectric heating makes use of electromagnetic energy to directly heat the reaction media. In recent years, microwaves have had a huge impact on how experimental organic or medicinal chemistry is performed.[22,23] Microwave-enhanced radiochemistry can provide a faster, cleaner, more selective and highly atom-efficient methodology. The shorter reaction time coupled with other benefits, such as higher product purity, due to reduced reaction mixture decomposition and the promotion of otherwise sluggish reactions, make it an ideal tool to be explored in radiochemistry with short lived positron-emitters.[24-26]

By so far, the traditional heating methods such as oil bath and water bath are still the most widely used in synthetic chemistry including radiochemistry where physical half-life of radiolabelled nuclide is often considered as a limited factor for the synthetic process. Especially for the shorter half-life of positron-emitter, finding a rapid synthesis route to shorten reaction time is always a pursuit of nuclear chemists in the synthetic field of PET tracers. In the practice of synthesis of [^{18}F]FET, we compared the above-mentioned two heating conditions by microwave and oil bath (Fig.4, Table 1). Microwave heating enhanced the reaction rate by 5~6 times both for Step one (curve C vs curve J) and Step two (curve D vs curve O). Each step could finish within 2~3 min rather than 15~20 min by microwave heating. Thus, the reaction time decreased enormously, which was a meaningful event for the preparation of [^{18}F]FET.

Table 1 Time distribution and total time of different synthesis processes of [^{18}F]FET

			Thermally heated by oil bath		Heated by microwave oven	
			Two-pot method	One-pot method	Two-pot method	One-pot method
Step one	Reaction time		10min	10 min	2 min	2 min
	Separation time		5min	—	5 min	—
Step two	Reaction time	Reactant (II)	15min	15 min	2 min	2 min
		Reactant (I)	30min	30 min	2 min	2min
	Separation time		5min	5 min	5 min	5 min
Total synthesis time			50min for (I)	45min for (I)	14min for (I) or (II)	9 min for (I) or (II)
(not including ^{18}F activation)			35min for (II)	30min for (II)		

Notes: (I) and (II) are representative of reactant I and reactant II, respectively.

3.3 Experiments on influence of reaction model

2-[^{18}F]Fluoroethyl tosylate is a key intermediate for introduction of ^{18}F into target molecule, due to its easy preparation, good stability and wide applicability.[27] In the field of labeling compounds with fluorine-18 for PET, it is widely used as a reagent for *O*-, *N*- and *S*-fluoroalkylations.[7, 14, 15, 27-36] It was reported that it is suitable for the one-pot procedure synthesis.[27, 37, 38]

Generally, the synthesis of [^{18}F]FET adopted two-step reaction without exception. In brief, 2-[^{18}F]Fluoroethyl tosylate was transferred from the first reaction mini-vial into a Sep-Pak cartridge to be separated after its reaction. The eluant was collected into the second reaction mini-vial, then dried and added the reactant to the next step.

It is important to protect operator from the radiation exposure to the maximum, in particular when handling sample by hand. Thereby, we introduced one-pot method to simplify the preparation procedure of [^{18}F]FET, and compared with two-pot in different conditions (Table 1). Total synthesis time of the former was decreased by about 5 min. Loss of radioactivity was diminished because only one reaction mini-vial and one Sep-Pak cartridge was used, which helps to increase radiochemical yield.

4 Conclusion

This paper reported a comparative and optimized radiosynthesis of [^{18}F]FET, according to three aspects of its two-step synthesis method, which include reaction components, heating methods and reaction models. The optimized conditions were as follows: L-tyrosine, aqueous NaOH and DMSO as reaction components, microwave heating, one-pot method as reaction model. As a result, good radiochemical yield of 45% was reached on the average and radiochemical purity was up to 95%, total synthesis time of [^{18}F]FET was shortened to within 20 min and radiation exposure time also decreased.

Acknowledgements

We are very grateful to Amersham Kexing Pharmaceutical Co. Ltd. and engineer Zhang Yongping for production of fluorine-18.

References

1 Vaalburg W, Coenen H H, Crouzel C, *et al*. Int J Rad Appl Instrum B. 1992, **19**: 227-237

2 Coenen H H, Kling P, Stocklin G, *et al*. J Nucl Med, 1989, **30**: 1367-1372

3 Wienhard K, Herholz K, Coenen H H, *et al*. J Nucl Med, 1991, **32**: 1338-1346

4 Inoue T, Tomiyoshi K, Higuichi T, *et al*. J Nucl Med, 1998, **39**: 663-667

5 Inoue T, Shibasaki T, Oriuchi N, *et al*. J Nucl Med, 1999, **40**: 399-405

6 Heiss P, Mayer S, Herz M, *et al*. J Nucl Med, 1999, **40**: 1367-1373

7 Wester H J, Herz M, Weber W, *et al*. J Nucl Med, 1999, **40**: 205-212

8 Weber W A, Wester H J, Grosu A L, *et al*. Eur J Nucl Med, 2000, **27**: 542-549

9 Rau F C, Weber W A, Wester H J, *et al*. Eur J Nucl Med, 2002, **29**: 1039-1046

10 Kaim A H, Weber B, Kurrer M O, *et al*. Eur J Nucl Med, 2002, **29**: 648-654

11 Langen K J, Jaroscha M, Mühlensiepena H, *et al*. Nucl Med Biol, 2003, **30**: 501-508

12 Messing-Jünger A M, Floeth F W, Pauleit D, *et al*. Childs Nerv Syst, 2002, **18**: 445-449

13 Pauleit D, Floeth F, Tellmann L, *et al*. J Nucl Med, 2004, **45**: 374-381

14 Block D, Coenen H H, Stöcklin G. J Label Compd Radiopharm, 1987, **24**: 1029-1042

15 Block D, Coenen H H, Stöcklin G. J Label Compd Radiopharm, 1988, **25**: 201-215

16 TANG Ganghua, WANG Mingfang, TANG Xiaolan, *et al*. Asia J Nucl Med (in Chinese), 2002, **2**: 90-92

17 DANG Yonghong, WANG Shizhen, CHENG Xin, *et al*. Acta Acad Med Sin (in Chinese), 2002, **24**: 370-372

18 Piao Riyang, Cui Ruixue, Du Yikui, *et al*. Chin J Nucl Med (in Chinese), 2000, **20**: 272-273

19 Kingsbury C A. J Org Chem, 1964, **29**: 3262-3270

20 Cavell E. J Chem Soc, 1958, 4217

21 Solar S L, Schumaker R R. J Org Chem, 1966, **31**: 1996-1997

22 Loupy A. Microwaves in organic synthesis. Wiley-VCH: Weinheim, 2002, and references therein

23 Hayes B L. Microwave synthesis chemistry at the speed of light. CEM Publishing: Atthews, 2002, and references therein

24 Elander N, Jones J R, Lu S Y, *et al*. Chem Soc Rev, 2000, **29**: 239-249

25 Jones J R, Lu S Y. In Microwaves in organic synthesis, Loupy A (ed.). Wiley-VCH: Weinheim, 2002: 435–462

26 Elander N, Stone-Elander S. J Label Compd Radiopharm, 2002, **45**: 715-746

27 Chin F T, Musachio J L, Cai L, *et al*. J Label Compd Radiopharm, 2003, **46**: S172

28 Elsinga P H, Kawamura K, Kobayashi T, *et al*. Synapse,

2002, **43**: 259-267

29 Shiue G G, Schirrmacher R, Shiue C Y, *et al*. J Label Compd Radiopharm, 2001, **44**: 127-139

30 Wester H J, Willoch F, Tölle T R, *et al*. J Nucl Med, 2000, **41**: 1279-1286

31 Zhang M R, Tsuchiyama A, Haradahira T, *et al*. Nucl Med Biol, 2002, **29**: 463-468

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

33 Schirrmacher R, Nesseler E, Hamkens W, *et al*. Appl Radiat Isot, 2002, **56**: 511-517

34 Wilson A A, Da Silva J N, Houle S. Appl Radiat Isot, 1995, **46**: 765-770

35 Piel M, Schirrmacher R, Hohnemann S, *et al*. J Label Compd Radiopharm, 2003, **46**: 645-659

36 Chin F T, McCarron J A, Musachio J L, *et al*. J Label Compd Radiopharm, 2003, **46**: S215

37 Skaddan M B, Sherman P S, Kilbourn M R. Nucl Med Biol, 2001, **28**: 753-759

38 Kämäräinen E L, Kyllönen1 T, Nihtilä1 O, *et al*. J Label Compd Radiopharm, 2004, **47**: 37-45