JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 661–668.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.706

Research Article

Automated commercial synthesis system for preparation of O-(2-[^{18}F]fluoroethyl)-L-tyrosine by direct nucleophilic displacement on a resin column

Ganghua Tang[1,*], Xiaolan Tang[2], Mingfang Wang[1], Lei Luo[1], Manquan Gan[1] and Zuhan Huang[1]

[1] *Nan Fang PET Centre, Nan Fang Hospital, The First Military Medical University, Guangzhou 510515, China*

[2] *Department of Applied Chemistry, South China Agricultural University, Guangzhou 510642 , China*

Summary

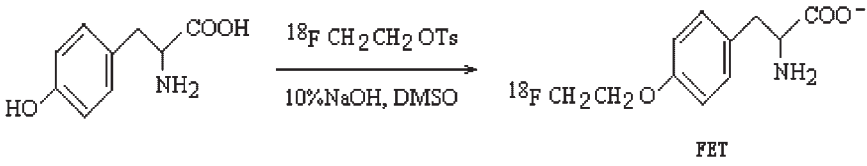
A slightly modified automated commercial synthesis system for preparation of *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET), an amino acid tracer for tumor imaging with positron emission tomography, is described. Direct nucleophilic fluorination of [^{18}F]fluoride with 1,2-di(4-methylphenylsulfonyloxy)ethane on a quaternary 4-(4-methylpiperidinyl)-pyridinium functionalized polystyrene anion exchange resin gave 1-[^{18}F]-2-(4-methylphenylsulfonyloxy)ethane, then [^{18}F]fluoroalkylation of L-tyrosine yielded FET. The overall radiochemical yield with no decay correction was about 8–10%, the whole synthesis time was about 52 min, and the radiochemical purity was above 95%. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine; quaternary 4-aminopyridinium resin; automated synthesis

*Correspondence to: G. Tang, Nang Fang PET Centre, Nan Fang Hospital, The First Military Medical University, Guangzhou 510515, China. E-mail: tghtgh@china.com

Contract/grant sponsor: Presidents' Foundation of Nan Fang Hospital.

Received 26 August 2002
Revised 14 February 2003
Accepted 15 February 2003



FET

Introduction

2-[^{18}F]Fluoro-2-deoxy-D-glucose (FDG) as a tracer of glucose metabolism has been widely used to the diagnosis and treatment evaluation for oncology with positron emission tomography (PET).[1] However, clinical FDG PET studies demonstrated several limitations such as substantial uptake of FDG in tumor, human and experimental inflammatory lesions. Uptake of FDG in acute inflammatory reactions gives rise to false positive results in a considerable percentage of cases.[2]

O-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET), a recently developed amino acid tracer, has shown high uptake in experimental tumors and in patients with brain tumors.[3–5] In addition, almost no uptakes of FET in activated inflammatory cells with experimental acute abscess model and in inflammation within lymph nodes are found. [2,6] The routine synthesis of FET with high radiochemical yield has been reported, however, the separation and purification of FET used HPLC,[3] adding difficulty to performing a fully automated synthesis of FET.

We report here that the PETtrace FDG MicroLab can be applied to the fully automated synthesis of FET.

Results and discussion

Chemistry

The synthetic procedure designing for the preparation of *O*-(2-[^{19}F]fluoroethyl)-L-tyrosine (^{19}FET) is based on the selective *O*-alkylation of tyrosine.[7] Dimethylsulfoxide (DMSO) was particularly effective in promoting base-catalyzed condensation and a lot of alkyl ethers of L-tyrosine were successfully synthesized from *O*-alkylation of tyrosine in this solvent without protecting the amino group.[7] Under the conditions of L-tyrosine dissolved in a mixture of DMSO and aqueous sodium hydroxide and treated with the appropriate alkyl halide at moderate temperature, no significant *N*-alkylation was detected. The

ether ester, major by-product resulting from dialkylation, could be avoided by carrying out the reaction with a slight excess of the amino acid or eliminated by saponification with dilute base before isolation of the product. For this purpose the high yield of ^{19}FET was obtained from the reaction of L-tyrosine dissolved in the solution of 2 equivalent of 10% sodium hydroxide and about 3–10 volume of DMSO at 70–90 i[ae]. The structure of ^{19}FET is confirmed by MS and ^{1}H NMR.

Radiochemistry

Synthesis of FET via a two-step procedure as shown in Figure 1 was similar to that of FDG. First, direct nucleophilic substitution of [^{18}F]fluoride with 1,2-di(4-methylphenylsulfonyloxy)ethane (**1**) on a quaternary 4-(4-methylpiperidinyl)- pyridinium functionalized polystyrene anion exchange resin gave 1-[^{18}F]-2-(4-methylphenylsulfonyloxy)ethane (**2**). Second, [^{18}F]fluoroethylation of L-tyrosine with (**2**) and saponification with dilute sodium hydroxide solution yielded FET (**3**). Hence, commercial PETtrace FDG MicroLab[8] with only minor modifications could be used for the fully automated preparation of FET. The typical chromatograms of FET were consistent with those of the standard ^{19}FET by HPLC analysis (FET retention time R_t = 4.3 min) and TLC analysis (FET R_f = 0.22). After decay of final product (FET injection), product characterization was also confirmed by mass spectrum, MS (EI) m/z: 226 (M^+), furthermore, only little L-tyrosine and no DMSO were detected by HPLC and GC. FET (**3**) was prepared in the overall radiochemical yield of 8–10% with no decay correction and in the radiochemical purity of more than 95% within 52 min synthesis time. No *O*-(2-[^{18}F]fluoroethyl)-D-tyrosine was found by enantiomeric analysis.

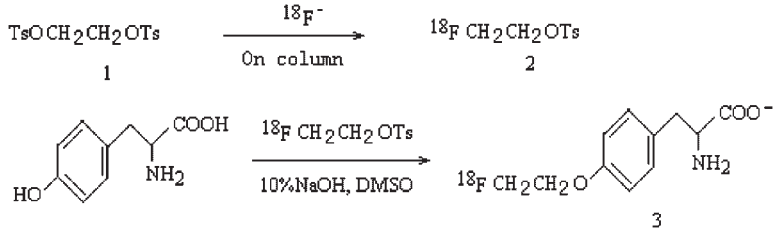


Figure 1. Synthesis of FET by direct nucleophilic exchange on an anion exchange column

The growing importance of FET as a positron-emitting radiopharmaceutical for amino-acid metabolic study makes it necessary to synthesize this compound using a simple method. This work indicates that the fully automated synthesis of FET on commercial PETtrace FDG MicroLab can be prepared routinely for clinical use though radiochemical yield is low. Also, the fully automated synthesis by direct nucleophilic exchange on a quaternary 4-aminopyridinium resin has the following advantages: Firstly, the Sep-Pak cartridges are used to purify and separate the radiolabeled intermediate product and the last product instead of HPLC system,[3] which results in simplification of the operation steps and shortening of the whole synthesis time. Secondly, due to the small overall size of the resin column and heating block, the assembly fits easily into a shielded hot cell. The trapping of [^{18}F]fluoride ion, nucleophilic radiolabeling reagents transport and all radioactive solution transfers done by means of Teflon tubing, helium pressure, remote syringes, or vaccum are performed in shielded state, with no operator handling necessary except to remove the final product vial. These factors keep the radiation exposure to personnel at a minimum. Thirdly, the amount of training required and effort involved in operating this system also are minimal.[8,9] Fourthly, nucleophilic [^{18}F]fluorination procedure on a resin column eliminates some problems which have arisen in other reactions with resolubilized [^{18}F]fluoride ion using counterions such as Kryptofix 222/K^+ [3,10] and tetraalkylammonium salts[11,12] as phase transfer reagents. Loss of [^{18}F]fluoride on reaction vessel walls need not be considered. Furthermore, the use of a resin column eliminates the need to remove phase transfer reagents with toxic chemicals such as Kryptofix 222 and tetraalkylammonium salts.[9]

Experimental

Isotope availability

No-carrier-added aqueous [^{18}F]fluoride ion was produced on a PETtrace cyclotron (GE Co.) by irradiation of a 1.5 ml water target using a 16.5 MeV proton beam on 95% enriched [^{18}O]water by the ^{18}O(p, n)^{18}F nuclear reaction and was transferred to the synthesis module. Typical production: 400–500 mCi (14.8–13.5 GBq) of [^{18}F]F^- at the end of bombardment for a 25 μA, 30 min irradiation.[8]

Experimental apparatus

The fully automated synthesis of FET was performed in a lead-shielded hot cell using a modified PETtrace FDG MicroLab (GE Co.).[8] The PETtrace FDG MicroLab is a computer controlled automated radiochemistry system which will produce FDG from $[^{18}F]F^-$. All chemical transformation and FDG production take part within a disposable process Cassette. One Cassette is required for each batch production run of FDG. The Cassette is able to be used for the synthesis of FET.

Synthesis of O-(2-[^{19}F]fluoroethyl)-L-tyrosine (^{19}FET)

O-(2-[^{19}F]fluoroethyl)-L-tyrosine (^{19}FET) was prepared according to the method described by Solar *et al.*[7] A solution of 0.646 g (3.5 mmol) L-tyrosine in 2.800 g (7.0 mmol) 10% aqueous sodium hydroxide was added to 14 ml of dimethyl sulfoxide and heated in a oil bath to 80°C. To this was added, with stirring, 0.450 g (3.5 mmol) 1-fluoro-2-bromoethylane. Heating and stirring were continued for 2 h and the reaction mixture was then poured into 18 g of crushed ice. The pH was adjusted to about 7.5 and the resulting precipitate was filtered off, washed with water and dried. The crude product was recrystallized from 60% acetic acid to give 0.270 g (1.2 mmol, 34%) of cold standard. ^{1}H NMR ($CDCl_3$) 6.7–7.3 (m, 4 H), 4.7 (t, 2 H), 4.3 (t, 2 H), 3.4 (m, 1 H), 2.8 (m, 2 H) MS (EI) m/z: 226 (M^+)

Automated production of O-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET)

The fully automated radiochemical synthesis of FET was modified from the PETtrace FDG MicroLab by a two-step reaction sequence that consisted of ^{18}F-fluorination of 1,2-ditosyloxyethane and subsequent ^{18}F-fluoroethylation of unprotected L-tyrosine as follows. The generated ^{18}F-fluoride target water was transferred to the target water reservoir on the FDG Cassette, then transferred to one of the syringes and passed through the solid-phase extraction column where the $[^{18}F]F^-$ was trapped. The solid-phase consists of a 2%-crosslinked polystyrene matrix (Merrifields resin, approximately 1.5 meq of active group/g) where the trapping agent is 4-(4-methylpiperidinyl)pyridinium cation. The resin is placed in the column, which was included in the FDG Cassette. The used target water was recovered via a separate collecting vial. The resin-bound $[^{18}F]F^-$ was dried by passing

acetonitrile as the mobile phase instead of water at about 100°C and the target water receiver in the Cassette was rinsed. 1,2-Ditosyloxyethane (10 mg, 0.027 mmol) dissolved in dry acetonitrile (2 ml) was passed through the heated solid-phase extraction column and was reacted with the trapped $[^{18}F]F^-$ on the column. 1-[^{18}F]-2-tosyloxyethane was formed and was transferred to the [^{18}F]fluoroethylation vessel. Then, the acetonitrile was evaporated. The mixture of L-tyrosine (9.1 mg, 50 mol), 100 g/l sodium hydroxide solution (4 mg/40 l, 100 mol) and DMSO (0.5 ml) was added and the reaction mixture was heated at 100°C for 20 to form FET. [^{18}F]Fluoroethylation was halted by stopping the heating and adding 0.15 mol/l phosphate-buffered saline (PBS, pH 7.40) (2 ml). The final product was transferred to the product syringe and further through a silica Sep-Pak cartridge. FET was eluted from the serial silica Sep-Pak cartridge, C-18 Sep-Pak cartridge and Al_2O_3 Sep-Pak cartridge with PBS (pH 0.74). Eluate was passed through 0.22 m sterile filtration to the product vial in a separate radiation shielded container.

Analysis of O-(2-^{18}F-fluoroethyl)-L-tyrosine (FET)

The radiochemical purity was analysed by HPLC on an analytical reverse phase column (RP-18, 150 × 4.6 mm) eluted with ethanol/water/acetic acid (10/87.5/2.5, v/v/v) and 2.5 g/l ammonium acetate (pH 3.0) at a flow of 1 ml/min. The effluent was monitored for UV absorbance and radioactivity (FET retention time *R*f: 4.3 min). Thin-layer chromatography was performed with acetonitrile/water (95/5, v/v) as the solvent system (FET $R_f = 0.22$). The chemical purity was determined by HPLC, TLC and gas chromatography (GC). Enantiomeric analysis was carried out with HPLC using a reversed-phase C18 column and a chiral mobile phase.[13]

Conclusion

A fully automated preparation of *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET) is performed in a lead-shielded hot cell using a modified commercial PETtrace FDG MicroLab. Collection of [^{18}F]fluoride ion from target water, drying and nucleophilic fluorination reaction of [^{18}F]fluoride with 1,2-di(4-methylphenylsulfonyloxy)ethane are all rapidly completed on a quaternary 4-(4-methylpiperidinyl)pyridinium

functionalized polystyrene anion exchange resin column to give 2-[^{18}F]-1-(4-methylphenylsulfonyloxy)ethane. Then, [^{18}F]fluoroalkylation of L-tyrosine with 1-[^{18}F]-2-(4-methylphenylsulfonyloxy) ethane forms FET. The synthesis of FET is performed in the whole synthesis time of about 52 min with overall radiochemical yield about 8–10% (no-decay correction) and radiochemical purity above 95%.

Acknowledgements

This work was supported by the President's Foundation of Nan Fang Hospital, First Military Medical University.

References

1. Heiss P, Mayer S, Herz M, Wester HJ, Schwaiger M, Senekowitsch-Schmidtke R. Investigation of transport mechanism and uptake kinetics of *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine *in vitro* and *in vivo*. *J Nucl Med* 1999; **40**: 1367–1373.
2. Rau FC, Weber WA, Wester HJ, *et al*. *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (FET): a tracer for differentiation of tumour from inflammation in murine lymph nodes. *Eur J Nucl Med* 2002; **29**: 1039–1046.
3. Wester HJ, Herz M, Weber W, *et al*. Synthesis and radiopharmacology of *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine for tumor imaging. *J Nucl Med* 1999; **40**: 205–212.
4. Weber WA, Wester HJ, Grosu AL, *et al*. *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine and L-[methyl-^{11}C]methionine uptake in brain tumours : initial results of a comparative study. *Eur J Nucl Med* 2000; **27**: 542–549.
5. Tang GH, Wang MF, Tang XL, Luo L, Wu HB, Huang ZH. Synthesis and animal experiment of O-(2-^{18}F-fluoroethyl)-L-tyrosine as tumor imaging agent. *Asia J Nucl Med* 2002; **2**: 90-92 (in Chinese).
6. Kaim AH, Weber B, Kurrer MO, *et al*. ^{18}F-FDG and ^{18}F-FET uptake in experimental soft tissue infection. *Eur J Nucl Med* 2002; **29**: 648–654.
7. Solar SL, Schumaker RR. Selective O-alkylation of tyrosine. *J Org Chem* 1966; **31**: 1996–1997.
8. Tang GH, Wang MF, Wu HB, *et al*. Synthesis and tissue distribution in mice of ^{18}F-FDG. *Nuclear Techn* 2001; **24**: 417–420 (in Chinese).
9. Toorongian SA, Mulholland GK, Jewett DM, Bachelor MA, Kilbourn MR. Routine production of 2-deoxy-2-[^{18}F]fluoro-D-glucose by direct nucleophilic exchange on a quaternary 4-aminopyridinium resin. *Nucl Med Biol* 1990; **17**: 273–279.

10. Hamacher K, Coenen HH, Stocklin G. Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *J Nucl Med* 1986; **27**: 235–238.
11. Hamacher K, Coenen HH. Convenient synthesis of nca *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine. *J Label Compd Radipharm* 2001; **44**(Suppl): S855–S856.
12. Hamacher K, Coenen HH. Efficient routine production of the ^{18}F-labelled amino acid *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine. *Appl Radiat Isot* 2002; **57**: 853–856.
13. Tang GH, Zhang L, Tang XL, Wang YX, Yin DZ. Synthesis and determination for enantiomeric purity of 6-fluoro-L-DOPA. *Acta Pharmaceut Sinica* 2001; **36**: 739–742 (in Chinese).

