Synthesis of *O*-(3-[^{18}F]Fluoropropyl)-L-tyrosine (L-[^{18}F]FPT) and Its Biological Evaluation in 9L Tumor Bearing Rat

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1. Introduction

Positron emitting radionuclide labeled amino acid analogs such as [^{18}F]fluorinated tyrosine derivatives recently have been proven to overcome the disadvantages of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG). [^{18}F]FDG is the most widely used radiotracer in tumor diagnosis using PET in clinic, but there have been some limitations that [^{18}F]FDG could not differentiate nonmalignant inflammatory tissue from tumor and showed lower contrast in brain tumor, to normal brain.[1,2] Although L-[^{11}C-methyl]methionine ([^{11}C]-MET) is a useful amino acid PET tracer for brain tumor imaging, its application is only limited to hospitals having own cyclotron due to short half-life of C-11.[3] Recent studies make much progress on simplified synthesis of L-[^{18}F]FET.

L-[^{18}F]FET is known to be useful as an amino acid PET tracer for detection and localization of tumor with a high specificity than other available tracers.[4,5] Moreover, the uptake and image contrast of [^{18}F]FET appear to be very similar to those of [^{11}C]MET in PET studies of human brain tumours. Although other fluorine-18 labeled amino acid derivatives such as fluorocyclobutane-1-carboxylic acid, fluoroproline, fluorophenylalanine, tyrosine, methyl tyrosine, [^{18}F]FBPA (4-borono-2-[^{18}F]fluoro-Lphenylalanine), and L-[^{18}F]FET have been reported, these materials have been shown low radiochemical and synthetic yields. Recently, L-[^{18}F]FPT, like other fluorinated analog of tyrosine, has been studied for tumor imaging but, low radiochemical yield has been reported and occasionally byproduct could occur during reaction.[6-7] Recent systematic studies showed *in vitro* biological stability of fluoroalkyl groups, such as fluoromethyl, fluoroethyl, and fluoropropyl, using rat hepatic microsomes and human serum by Lee *et al.*[8] Although a couple of studies indicate [^{18}F]FPT is superior to FDG in the differentiation of tumor and inflammation and a potential amino acid tracer like [^{18}F]FET for tumor imaging, a direct comparison of [^{18}F]FET and [^{18}F]FPT has not been performed in brain tumor model.

In this study, we developed novel method to promote the radiochemical yield with fluoropropyl tyrosine derivative from the mentioned results and evaluate the feasibility of [^{18}F]FPT as a new brain tumor imaging agent. Biological properties of [^{18}F]FPT were compared with those of [^{18}F]FET in 9L tumor bearing rat. Furthermore, PET imaging of [^{18}F]FPT was acquired in 9L tumor bearing rat.

2. Methods and Results

The precursor for direct [^{18}F]fluorination was synthesized from a commercially available material, *N*-(*tert*-butoxycarbonyl)-Ltyrosine methyl ester. *O*-(3-*tert*-Butyldimethylsilyloxypropyl)-*N*-(*tert*-butoxycarbonyl)-L-tyrosine methyl ester was prepared by the reaction of *N*-(*tert*butoxycarbonyl)-L-tyrosine methyl ester with 3-bromopropoxy-*tert*-butyldimethylsilane in yields of 70%, followed by deprotection of *tert*-butyldimethylsilane group using $nBu_4NF\cdot 3H_2O$ ($TBAF\cdot 3H_2O$). The precursor, tosylated compound, was prepared from *O*-(3-hydroxypropyl)-*N*-(*tert*-butoxycarbonyl)-L-tyrosine methyl ester with *p*-toluenesulfonyl chloride (*p*-TsCl) in the presence of triethylamine (TEA) in yields of 80%. The authentic FPT was synthesized from tosylated compound using $TBAF\cdot 3H_2O$ and 4 N HCl in yields of 44%.

The preparation of [^{18}F]FPT has been achieved by the nucleophilic substitution of precursor (**4**) with [^{18}F]fluoride ion in CH_3CN at 90 °C for 25 min followed by hydrolysis with 4 N HCl at 110 °C for 40 min. The isolation of the labeled compound was performed by HPLC using a semi-preparative column. The further purification was done by loading the purified [^{18}F]FPT through the strong cation exchange resin and collected pure [^{18}F]FPT with the phosphate buffered solution (pH = 7.4). The total time for the preparation of [^{18}F]FPT from EOB to EOS (End-of-synthesis) was 100 min. The quality control of the final product was conducted by HPLC using an analytical column. The biodistribution data of [^{18}F]FPT and [^{18}F]FET in mice bearing 9L tumor are summarized in Table 1 and 2.

Biodistribution data of [^{18}F]FPT was similar to that of [^{18}F]FET, as previously reported. The ratios of tumor-to-blood (T/B), tumor-to-muscle (T/M) and tumor-to-brain (T/Br) for [^{18}F]FET and [^{18}F]FPT at 10, 30, 60 and 120 min postinjection are shown in Figure 1.

Table 1. Biodistribution Data of [^{18}F]FPT in Rats Bearing 9 L Tumor

Organ	Time point (min)			
	10	30	60	120
Blood	0.97 ± 0.62	0.63 ± 0.01	0.83 ± 0.23	0.52 ± 0.02
Liver	1.02 ± 0.11	0.72 ± 0.03	0.76 ± 0.10	0.60 ± 0.02
Lung	0.78 ± 0.08	0.57 ± 0.03	0.63 ± 0.07	0.50 ± 0.01
Spleen	1.10 ± 0.14	0.70 ± 0.05	0.68 ± 0.07	0.57 ± 0.03
Kidney	1.19 ± 0.19	0.83 ± 0.16	0.77 ± 0.09	0.58 ± 0.01
Stomach	0.50 ± 0.11	0.25 ± 0.02	0.29 ± 0.04	0.13 ± 0.01
Intestine	1.24 ± 0.20	0.70 ± 0.29	0.79 ± 0.23	0.57 ± 0.13
Femur	0.44 ± 0.06	0.49 ± 0.02	0.84 ± 0.09	0.92 ± 0.12
Thyroid	0.24 ± 0.07	0.14 ± 0.06	0.18 ± 0.10	0.15 ± 0.06
Tumor (9L)	0.71 ± 0.08	0.89 ± 0.29	1.24 ± 0.15	0.88 ± 0.26
Pancreas	2.46 ± 0.61	1.74 ± 0.17	1.42 ± 0.19	1.25 ± 0.19
Muscle	0.54 ± 0.05	0.54 ± 0.01	0.62 ± 0.07	0.45 ± 0.01
Brain	0.16 ± 0.01	0.20 ± 0.01	0.29 ± 0.03	0.26 ± 0.01

Number of mice/group; n = 3. Data represent as mean ± SD.

Table 2. Biodistribution Data of [^{18}F]FET in Rats Bearing 9L Tumor

Organ	Time point (min)			
	10	30	60	120
Blood	0.84 ± 0.07	0.69 ± 0.10	0.66 ± 0.03	0.69 ± 0.06
Liver	0.74 ± 0.06	0.64 ± 0.08	0.61 ± 0.02	0.62 ± 0.05
Lung	0.78 ± 0.05	0.66 ± 0.09	0.62 ± 0.01	0.61 ± 0.05
Spleen	0.93 ± 0.05	0.81 ± 0.12	0.73 ± 0.04	0.71 ± 0.04
Kidney	0.94 ± 0.12	1.08 ± 0.70	0.69 ± 0.10	0.64 ± 0.07
Stomach	0.45 ± 0.13	0.53 ± 0.20	0.42 ± 0.09	0.48 ± 0.09
Intestine	0.64 ± 0.09	0.58 ± 0.03	0.62 ± 0.08	0.57 ± 0.06
Femur	0.47 ± 0.03	0.45 ± 0.06	0.44 ± 0.01	0.45 ± 0.04
Thyroid	0.17 ± 0.01	0.13 ± 0.04	0.17 ± 0.05	0.19 ± 0.36
Tumor (9L)	0.68 ± 0.15	0.97 ± 0.13	1.19 ± 0.09	1.31 ± 0.54
Pancreas	2.58 ± 0.78	2.98 ± 0.24	3.01 ± 0.76	4.00 ± 0.54
Muscle	0.54 ± 0.06	0.62 ± 0.09	0.63 ± 0.01	0.63 ± 1.89
Brain	0.26 ± 0.02	0.35 ± 0.05	0.42 ± 0.02	0.54 ± 0.18

Number of mice/group; n=3. Data represent as mean ± SD.

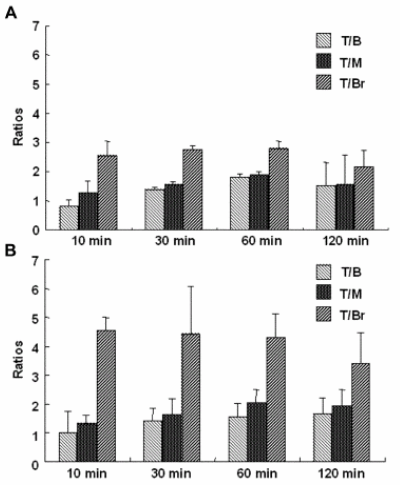

Figure 1. The ratios of tumor-to-blood (T/B), tumor-to-muscle (T/M) and tumor-to-brain (T/Br) of [^{18}F]FPT and [^{18}F]FET in rats bearing 9L tumor. (A) Ratios in [^{18}F]FPT injected rats. (B) Ratios in [^{18}F]FET injected rats at each time point. Data represent as mean ± S.D.

The coronal views of PET images of tumor-bearing rats were obtained 60 min after administration of [^{18}F]FPT as shown in Figure 2. In PET images at 60 min, [^{18}F]FPT was selectively accumulated in 9L tumor of fisher 344 rat. [^{18}F]FPT also accumulated in pancreas.

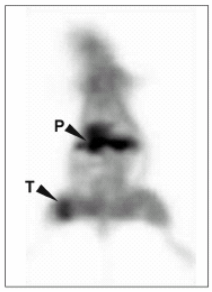

Figure 2. PET image of [^{18}F]FPT in 9L tumor bearing Fisher rat. PET image was obtaned at 60 min postinjection.

In recent reports, amino acid PET tracers showed pancreas uptake in rats and mice, which is in contrast to the observation of low [^{18}F]FET uptake in the pancreas of human. Such discrepancies in amino acid uptake in the pancreas between mice and humans have also been observed for [^{123}I]iodo-α-methyl-L-tyrosine, although the reasons for this difference remain unexplained.[5] These results suggest that [^{18}F]FPT could be used as amino acid tracer for the detection of brain tumor.

3. Conclusion

[^{18}F]FPT was directly prepared from the precursor of *O*-(3-*p*-toluenesulfonyloxypropyl)-*N*-(*tert*-butoxycarbon-yl)-Ltyrosine methyl ester, and the radiochemical yield and radiochemical purity were 40-45% and 95%, respectively. Biodistribution data of [^{18}F]FPT showed similar pattern with those of [^{18}F]FET, but [^{18}F]FPT showed higher tumor-tobrain ratio than [^{18}F]FET. Consequently, [^{18}F]FPT could be used as a new amino acid tracer for brain tumors imaging with PET.

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