Evaluation of F-18-labeled amino acid derivatives and $[^{18}F]$FDG as PET probes in a brain tumor-bearing animal model

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Abstract

2-Deoxy-2-$[^{18}F]$fluoro-D-glucose ($[^{18}F]$FDG) has been extensively used as positron emission tomography (PET) tracer in clinical tumor imaging. This study compared the pharmacokinetics of two $[^{18}F]$-labeled amino acid derivatives, O-2-$[^{18}F]$fluoroethyl-L-tyrosine (L-$[^{18}F]$FET) and 4-borono-2-$[^{18}F]$fluoro-L-phenylalanine-fructose (L-$[^{18}F]$FBPA-Fr), to that of $[^{18}F]$FDG in an animal brain tumor model. 

Methods: A self-modified automated PET tracer synthesizer was used to produce no-carrier-added (nca) L-$[^{18}F]$FET. The cellular uptake, biodistribution, autoradiography and microPET imaging of L-$[^{18}F]$FET, L-$[^{18}F]$FBPA-Fr and $[^{18}F]$FDG were performed with F98 glioma cell culture and F98 glioma-bearing Fischer344 rats.

Results: The radiochemical purity of L-$[^{18}F]$FET was >98% and the radiochemical yield was 50% in average of 16 runs. The uptake of L-$[^{18}F]$FET and L-$[^{18}F]$FBPA-Fr in the F98 glioma cells increased rapidly for the first 5 min and reached a steady-state level after 10 min of incubation, whereas the cellular uptake of $[^{18}F]$FDG kept increasing during the study period. The biodistribution of L-$[^{18}F]$FET, L-$[^{18}F]$FBPA-Fr and $[^{18}F]$FDG in the brain tumors was 1.26 ± 0.22, 0.86 ± 0.08 and 2.77 ± 0.44 %ID/g at 60 min postinjection, respectively, while the tumor-to-normal brain ratios of L-$[^{18}F]$FET (3.15) and L-$[^{18}F]$FBPA-Fr (3.44) were higher than that of $[^{18}F]$FDG (1.44). Both microPET images and autoradiograms of L-$[^{18}F]$FET and L-$[^{18}F]$FBPA-Fr exhibited remarkable uptake with high contrast in the brain tumor, whereas $[^{18}F]$FDG showed high uptake in the normal brain and gave blurred brain tumor images.

Conclusion: Both L-$[^{18}F]$FET and L-$[^{18}F]$FBPA-Fr are superior to $[^{18}F]$FDG for the brain tumor imaging as shown in this study with microPET. © 2005 Elsevier Inc. All rights reserved.

Keywords: $[^{18}F]$FDG; L-$[^{18}F]$FET; L-$[^{18}F]$FBPA-Fr; PET; Autoradiography; Brain tumor

1. Introduction

$[^{18}F]$FDG has been proven to be useful as a positron emission tomography (PET) probe for many kinds of cancers due to the higher demand of glycolysis in the tumor than that of normal counterparts [1–3]. However, $[^{18}F]$FDG PET for the brain lesion imaging is limited for several reasons: firstly, the glucose metabolism in the normal brain tissue is usually higher [4,5] and is difficult to be differentiated from that of the tumor; secondly, the uptake of $[^{18}F]$FDG in the tumor is not specific. Infection, inflammation, granulomatous diseases and many other physiological or pathological conditions have been shown with increased uptake of $[^{18}F]$FDG [5]. Other than the increased demand of glycolysis, amino acid transport as well as protein metabolism is more active in tumor cells [6,7], and the uptake of amino acids in normal tissues is usually lower. In addition, amino acids seem to play a minor role in inflammatory cell metabolism [8], so that radiolabeled amino acids may be the better tumor imaging agents than $[^{18}F]$FDG is [9].

Positron emission tomography with [methyl-$[^{11}C]$]-L-methionine ($[^{11}C]$MET) or L-$[^{11}C]$-tyrosine has been used in many studies. Due to the short half-life of C-11 (20 min), the applications of these tracers usually have to be accompanied with an on-site cyclotron. F-18, on the other hand, with a longer half-life of 110 min and a lower positron...
energy (0.64 MeV), provides higher availability, convenience and better imaging quality for clinical use [10,11]. Although 18F-labeled amino acids are usually not incorporated into proteins, they are still useful for tumor imaging [12,13]. O-(2-[18F]Fluoroethyl)-L-tyrosine (L-[18F]FET), an analogue of tyrosine, is a useful amino acid PET tracer for the detection and localization of tumors with a high specificity, especially for brain tumors [13–15]. 4-Borono-2-[18F]fluoro-L-phenylalanine-fructose (L-[18F]FBPA-Fr), an analogue of phenylalanine, showed high tumor/normal tissue uptake ratio in F98 glioma-bearing rats and could be used as a PET probe for L-p-boronophenylalanine (BPA) in boron neutron capture therapy (BNCT) of the brain tumor and as a tumor-detecting agent as well [12,16–18]. The purpose of this study was to compare the pharmacokinetics of L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG in the same animal, and to evaluate whether F-18-labeled amino acid derivatives could be better alternatives than [18F]FDG for the brain tumor diagnosis with PET.

2. Materials and methods

2.1. General

N-tert-Butyloxy carbonyl-L-tyrosine benzyl ester (N-BOC-L-tyr-OBzl) was purchased from Neosystem (Strasbourg, France). Potassium carbonate, ethylene glycol, p-toluenesulfonyl chloride, anhydrous acetonitrile and other chemicals were purchased from Merck (Whitehouse Station, NJ, USA). QMA Sep-Pak and Sep-Pak Silica Plus cartridges were from Waters (Milford, MA, USA). Ethylene glycol-1,2-ditosylate (1.384 g, 3.73 mmol) and potassium carbonate (20 mg, 0.14 mmol) in anhydrous acetonitrile (10 ml) was heated to reflux for 3 h. The solvent was removed by rotary evaporator. The solid residue was extracted with chloroform (5 ml) and concentrated to about 1 ml. The residue was chromatographed on silica (180 g) with elution of 20% of chloroform in methylene chloride. The eluate was collected and concentrated to give N-BOC-(O-(2-tosyloxyethyl))-L-tyr-OBzl (398 mg, 60.1%) as a white solid. Analytical data of N-BOC-(O-(2-tosyloxyethyl))-L-tyr-OBzl: mp. 85–86; 1H NMR (CDCl3) δ 7.80 (d, 2H, J = 8.4 Hz, Haryl), 7.31 (m, 7H, Haryl), 6.89 (d, 2H, J = 8.4 Hz, Haryl), 6.62 (d, 2H, J = 8.4 Hz, Haryl), 5.15 (d, 1H, 12.2 Hz, CH of benzyl), 5.08 (d, 1H, 12.2 Hz, CH of benzyl), 4.92 (d, 1H, J = 8.0 Hz, NH), 4.54 (m, 1H, CH), 4.33 (t, 2H, J = 4.6 Hz, CH2), 4.07 (t, 2H, J = 4.6 Hz, CH2), 2.99 (d, 2H, J = 5.8 Hz, CH2 of Tyr), 2.43 (s, 3H, CH3 of toluene), 1.39 (s, 9H, CH3 of t-BOC). Elemental analysis calculated for C30H35NO8S: C, 63.27; H, 6.15; N, 2.46. Found C, 63.34; H, 5.62; N, 2.33.

Thin-layer chromatography (TLC) was conducted using an imaging scanner (system 200, Bioscan, USA). High-performance liquid chromatography (HPLC) was conducted using Waters model 600, Waters model 600E pumps, a UV detector (Waters 486 tunable absorbance detector; Waters) and a radiodetector (Flow Count Detector FC-003, Capintec, Bioscan, Washington, DC, USA). Data were collected and analyzed using a computer software (CSW, version 1.7, DataApex). [18F]FDG was prepared according to Lemaire et al. [20] using an automated [18F]FDG synthesis system (Coincidence Technologies, Liège, Belgium) at the National PET/Cyclotron Center in Taipei. Nca L-[18F]FET was synthesized using a self-modified automated PET tracer synthesizer (Nuclear Interface, Münster, Germany).

2.2. Synthesis of L-[18F]FET

2.2.1. Preparation of O-(2-tosyloxyethyl)-N-tert-butyloxy carbonyl-L-tyrosine benzyl ester [N-BOC-(O-(2-tosyloxyethyl))-L-tyr-OBzl]

A mixture of N-BOC-L-tyr-OBzl (450 mg, 1.24 mmol), ethylene glycol-1,2-ditosylate (1.384 g, 3.73 mmol) and potassium carbonate (20 mg, 0.14 mmol) in anhydrous acetonitrile (10 ml) was heated to reflux for 3 h. The solvent was removed by rotary evaporator. The solid residue was extracted with chloroform (5 ml x 3) and concentrated to about 1 ml. The residue was chromatographed on silica (180 g) with elution of 20% of chloroform in methylene chloride. The eluate was collected and concentrated to give N-BOC-(O-(2-tosyloxyethyl))-L-tyr-OBzl (398 mg, 60.1%) as a white solid. Analytical data of N-BOC-(O-(2-tosyloxyethyl))-L-tyr-OBzl: mp. 85–86; 1H NMR (CDCl3) δ 7.80 (d, 2H, J = 8.4 Hz, Haryl), 7.31 (m, 7H, Haryl), 6.89 (d, 2H, J = 8.4 Hz, Haryl), 6.62 (d, 2H, J = 8.4 Hz, Haryl), 5.15 (d, 1H, 12.2 Hz, CH of benzyl), 5.08 (d, 1H, 12.2 Hz, CH of benzyl), 4.92 (d, 1H, J = 8.0 Hz, NH), 4.54 (m, 1H, CH), 4.33 (t, 2H, J = 4.6 Hz, CH2), 4.07 (t, 2H, J = 4.6 Hz, CH2), 2.99 (d, 2H, J = 5.8 Hz, CH2 of Tyr), 2.43 (s, 3H, CH3 of toluene), 1.39 (s, 9H, CH3 of t-BOC). Elemental analysis calculated for C30H35NO8S: C, 63.27; H, 6.15; N, 2.46. Found C, 63.34; H, 5.62; N, 2.33.

Fig. 1. Synthetic method for L-[18F]FET.
2.2.2. [18F]Fluoride fixation and desorption

[18F]HF was produced by the 18O(p,n)18F nuclear reaction by irradiation of 95% 18O-enriched water with a 17-MeV proton beam at 18 μA for 30 min. At the end of irradiation, the [18F]HF was transferred to a transit vial by helium gas pressure and then transferred through a QMA Sep-Pak cartridge, which had been previously conditioned with 0.5 M K2CO3 (10 ml) and water (20 ml). The 18O-enriched water was then recovered. The [18F]fluoride trapped on the cartridge was desorbed by elution with 0.8 ml 40 mM tetra-n-butyl ammonium (TBA) bicarbonate solution of acetonitrile/water (4/1, vol/vol) under helium gas and was sent to the reaction vessel. The TBA [18F]fluoride solution in the reaction vessel was heated at 100°C under reduced pressure for 10 min. The residue was dried again with 2.0 ml anhydrous acetonitrile at 100°C for 7 min (azeotropic evaporation).

2.2.3. [18F]Fluorination

To this dry residue was added a solution of N-Boc-(O-(2-tosyloxyethyl))-L-tyr-OBzl (5 mg, 9.35 μmol) in anhydrous acetonitrile (0.8 ml), and the mixture was heated at 90°C for 10 min. The reaction mixture was dried by evaporation under reduced pressure and with a stream of helium gas.

2.2.4. Solid-phase extraction, deprotection and formulation

After cooling with cold nitrogen stream to room temperature, the dry residue was dissolved in 1.5 ml of dichloromethane. The solution was passed through two Sep-Pak Silica Plus cartridges connected in series and then eluted with 2.5 ml of diethyl ether under helium gas. The eluate was dried again with helium gas at 50°C. 0.3 ml of 1 N HCl was added to the dry residue and heated for 10 min at 100°C to conduct hydrolysis. The reaction mixture was neutralized with 0.3 ml of 1 N NaOH. After adding 1.35 ml distilled water, the solution was passed through a sterile 0.22-μm membrane filter (Milllex-GV, Ref. No. SLGV013SL, Millipore, Bedford, MA, USA) under helium gas to afford the isotonic L-[18F]FET solution with a pH of 6.0–7.0.

2.2.5. Quality assurance

The radiochemical purity of L-[18F]FET was determined using TLC and HPLC. Thin-layer chromatography was performed on an aluminum sheet (Silica gel 60F254, MERCK, Darmstadt, Germany), using 10 mmol/L ammonium acetate and acetonitrile (30/70, vol/vol) as the developing agent. The HPLC was a reversed-phase column (PRP-1, Hamilton, Reno, NV, USA), eluted with ethanol/water/acetic acid (50/40/10, vol/vol).

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**Table 1**

<table>
<thead>
<tr>
<th>Uptake (%ID/g)</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
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<td>Heart</td>
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<td>0.23±0.07</td>
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<td>Stomach</td>
<td>0.44±0.05</td>
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<td>0.21±0.03</td>
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<td>Liver</td>
<td>0.47±0.06</td>
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**Table 2**

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<th>120 min</th>
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</thead>
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<tr>
<td>Heart</td>
<td>0.43±0.10</td>
<td>0.31±0.01</td>
<td>0.29±0.02</td>
<td>0.24±0.11</td>
<td>0.23±0.10</td>
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<tr>
<td>Stomach</td>
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<td>0.29±0.09</td>
<td>0.25±0.08</td>
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<td>Liver</td>
<td>0.72±0.04</td>
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<td>0.17±0.04</td>
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<td>Spleen</td>
<td>0.68±0.06</td>
<td>0.49±0.01</td>
<td>0.39±0.12</td>
<td>0.30±0.11</td>
<td>0.28±0.06</td>
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<tr>
<td>Pancreas</td>
<td>3.19±0.52</td>
<td>2.85±0.24</td>
<td>2.54±0.96</td>
<td>2.15±0.68</td>
<td>2.19±0.52</td>
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<td>Small intestine</td>
<td>0.83±0.15</td>
<td>0.61±0.26</td>
<td>0.45±0.14</td>
<td>0.36±0.14</td>
<td>0.28±0.05</td>
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</table>

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Fig. 2. Cellular uptake of L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG in F98 glioma cells (n = 3; mean±S.D.).
Sterile solution of \( \text{[18F]FBPA-Fr} \) was produced as described previously [17]. The radiochemical purity of \( \text{[18F]FBPA-Fr} \) was 97\% and the specific activity was 11.84 GBq/mmol as determined with HPLC. The radiochemical yield of \( \text{[18F]FBPA-Fr} \) was 20–25\% (444–518 MBq at the end of synthesis, EOS) based on the radioactivity of \( \text{[18F]CH_3COOF} \).

### 2.4. Cell culture and F98 glioma-bearing rat model

F98 glioma cells (a generous gift from Dr. Rolf F. Barth, The Ohio State University, USA) were cultured in DMEM (containing 10\% fetal bovine serum, 100 U/ml penicillin and 100 \( \mu \text{g/ml streptomycin} \)) at 37\°C in a humidified atmosphere of 5\% \( \text{CO}_2 \).

1 \( \times 10^6 \) F98 rat glioma cells (in 10 \( \mu \text{L} \) Hank’s balanced salt solution) were implanted into the left brain of the Fischer344 rat using a protocol as previously described [17].

### 2.5. Accumulation of \( \text{F-18 labeled drugs in F98 glioma cells} \)

The cellular uptake assays of \( \text{L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG} \) in F98 glioma cells were performed as follows.

Cells were preincubated at 37 \°C in six-well plates with 2 ml of culture medium per well. Aliquots of 100 \( \mu \text{l} \) of radiotracer (3.7 MBq/ml) were added into each well and incubated at 37 \°C for 2, 5, 10, 30 and 60 min. Triplicates were carried out for each time point. In order to evaluate the metabolism of these two unnatural amino acid tracers (\( \text{L-[18F]FET} \) and \( \text{L-[18F]FBPA-Fr} \)) during cellular uptake study, 20 \( \mu \text{l} \) of culture medium was sampled at different time points (10, 30, 60 and 120 min) and was passed through a sterile 0.22-\( \mu \text{m} \) membrane filter (Millex-GV, Ref. No. SLGV013SL, Milipore). The radioactive components in the filtrate were analyzed with the TLC method. The identity of components was established by comparison with the reference value of intact radiotracer.

Two milliliters of ice-cold phosphate-buffered saline (PBS) was used to intercept the uptake of tracers. Supernatants were aspirated and the cells were further rinsed twice with 1 ml ice-cold PBS. Cells were then treated with 0.5 ml of trypsin-EDTA, and each well was washed twice with 0.25 ml ice-cold PBS to harvest cells. Cellular uptake of each radiotracer was determined with a gamma scintillation counter.

The cellular uptake of \( \text{[18F]FDG} \) in F98 glioma- and \( \text{[18F]FBPA-Fr} \) during cellular uptake study, 20 \( \mu \text{l} \) of culture medium was sampled at different time points (10, 30, 60 and 120 min) and was passed through a sterile 0.22-\( \mu \text{m} \) membrane filter (Millex-GV, Ref. No. SLGV013SL, Milipore). The radioactive components in the filtrate were analyzed with the TLC method. The identity of components was established by comparison with the reference value of intact radiotracer.

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### Table 3

<table>
<thead>
<tr>
<th>Uptake (%ID/g)</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
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<td>Blood</td>
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<td>Lung</td>
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<td>Liver</td>
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<td>Spleen</td>
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<tr>
<td>Small intestine</td>
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<td>Right brain</td>
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Tumor/Left brain: 1.15 1.26 1.44 1.32 1.23

Tumor/blood: 1.25 3.34 13.85 17.54 28.3

Fig. 3. MicroPET images of Fischer344 male rats with F98 glioma (arrow) in the left brain at 30, 60, 90 and 120 min after intravenous injection of approximately 14.8 MBq of (A) \( \text{L-[18F]FET} \), (B) \( \text{L-[18F]FBPA-Fr} \) and (C) \( \text{[18F]FDG} \). The animals were under isoflurane anesthesia and the image acquisition time was 10 min.
Fig. 4. Brain autoradiograms of the F98 glioma-bearing Fischer344 rats after intravenous injection of 37.0–44.4 MBq of (A) L-[18F]FET, (B) L-[18F]FBPA-Fr and (C) [18F]FDG. The arrow indicates the location of the tumor.

counter. The results were expressed as uptake ratio, which was calculated as the ratio of the intracellular tracer radioactivity to that of the administered radioactivity.

2.6. Biodistributions of radiotracers in F98 glioma-bearing Fischer344 rat model

The animal experiments were approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. The biodistributions of radiotracers (L-[18F]FET, L-[18F]FBPA-Fr, and [18F]FDG) were performed on the 13th day after tumor implantation. Rats were fasted for 8 h before biodistribution studies. Tumor-bearing rats, 250–280 g, anesthetized with ether (catalog no. 100946B; Merck), were injected with 8.15–10.0 MBq/200 μL of radiotracer through lateral tail veins. Three rats of each time point were sacrificed with chloroform (catalog no. 1.02445; Merck) at 15, 30, 60, 90 and 120 min postinjection. Blood samples were obtained by cardiac puncture. Fifteen different tissues (heart, lung, liver, stomach, small intestine, large intestine, urine, bladder, kidney, muscle, spleen, pancreas, left brain, right brain and brain tumor) were excised, and parts of these tissues/organs were weighed and assayed for radioactivity with a gamma scintillation counter.

The uptake of radiotracer in tissues or organs was expressed in counts per minute (cpm) with decay correction and was normalized as %ID per gram tissue according to the following formula:

$$\% \text{ID} / g = \frac{A_0 \times 1000}{\text{Injected dose (μCi)} \times 3.7 \times 10^4 \times \text{Eff} \times \text{organ weight (mg)}}$$

where $\ln(A/A_0) = -0.693/t_{1/2}$, $A$ is the radioactivity (cpm) of tissues or organs measured by gamma counter, $A_0$ is the decay-corrected radioactivity (cpm) of tissues or organs, Eff is the counting efficiency of gamma scintillation counter, $t_{1/2}$ is the half-life of the radioisotope and $t$ is time postinjection.
2.7. MicroPET scanning

MicroPET images of the F98 glioma-bearing rats were obtained using the R4 system (Concorde Microsystems, Knoxville, TN, USA), and this produced 63 image slices over a 7.89-cm axial field of view (FOV), with a slice thickness of about 1.25 mm. The in-plane spatial resolution at the center of the FOV is about 1.6 mm using the ordered-subsets expectation maximization (OSEM) reconstruction method. MicroPET scanning was performed on the 13th day after tumor implantation. Each tumor-bearing rat was anesthetized with isoflurane (Abbott Laboratories, Queensborough, Kent, England) using a vaporizer system (A.M. Bickford, Wales Center, NY, USA) and injected with approximately 14.8 MBq of L-[18F]FET, L-[18F]FBPA-Fr or [18F]FDG in 200 μl of physiologic saline through the lateral tail vein. All injections were bolus injections. Each rat was imaged in the prone position. The rats were scanned for 10 min at each time point (30, 60, 90 and 120 min postinjection) of the radiotracers, with the long axis of the rat parallel to the long axis of the scanner. All images were reconstructed with the OSEM method, with a 128×128 pixel image matrix, 16 subsets, 4 iterations and use of a Gaussian filter. Transmission scanning was performed at 130 min postinjection. Acquisition time was 600 s and images were reconstructed using filtered backprojection.

2.8. Autoradiography

Autoradiography was performed as previously described [17]. Three rats of each group administered with L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG were sacrificed with chloroform at 15, 30, 60, 90 and 120 min post iv injection of radiotracers (37.0–44.4 MBq/0.5 ml). The brains were surgically removed and embedded with Tissue-Tek OCT (optimal cutting temperature) compound (catalog no. 4583, Sakura Finetechnical, Tokyo, Japan) on the round cryostat holder (diameter 2.7 cm) and frozen immediately with dry ice. The embedded samples were cut into 20-μm-thick coronal sections at −20 °C using a cryomicrotome (CM3050, Leica, Bensheim, Germany). Sections thaw-mounted onto microscopic slides were air-dried at room temperature and placed on the imaging plates (IPs) (BAS-SR2040, Fuji Photo Film, Tokyo, Japan) in the imaging plate cassette (2040, Fuji Photo Film) for 18 h. The IPs were assayed with a FLA5000 reader (Fuji Photo Film, Tokyo, Japan).

3. Results

3.1. Synthesis of L-[18F]FET

Starting from N-BOC-(O-(2-tosyloxyethyl))-L-tyr-OBzl, nca L-[18F]FET was synthesized using a self-modified automated PET tracer synthesizer in approximately 1 h with high radiochemical yield (50–55% at EOS, decay corrected) and high radiochemical purity (>98%). The average specific radioactivity of L-[18F]FET was >20 GBq/μmol at EOS. Purification by Sep-Pak Silica Plus cartridge was used instead of preparative HPLC method, which resulted in the simplification of the drug preparation and the shortening of the whole synthesis time.

3.2. Cellular uptake of L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG

The results of cellular uptake of the three drugs are shown in Fig. 2. L-[18F]FET and L-[18F]FBPA-Fr showed similar cellular uptake pattern, both with a fast increasing initial uptake into F98 glioma cells and reached maximum at 10 min (0.475% and 0.115%, individually), followed by a nearly constant tracer concentration in the cells during an incubation period of 60 min. The cellular uptake of [18F]FDG kept increasing over 60 min (1.07% at 60 min). The percentage of intact L-[18F]FET and L-[18F]FBPA-Fr of total radioactivity in the medium assayed by TLC method was more than 90% after incubation for 60 min.

3.3. Biodistributions of three radiotracers in F98 glioma-bearing Fischer344 rats

The biodistributions of L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG in F98 glioma-bearing Fischer344 rats are shown in Tables 1–3. The maximum uptake of drugs in the brain tumors was observed at 90, 60 and 60 min postinjection of L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG (1.49±0.40, 0.86±0.08 and 2.77±0.44 %ID/g, respectively). The tumor-to-left normal brain ratios at 60 min postinjection were 3.15 for L-[18F]FET and 3.44 for L-[18F]FBPA-Fr, but only 1.44 for [18F]FDG. The higher tumor-to-normal brain ratios found for amino acid tracers than that for the glucose tracer may result from the less demand of amino acids in the normal brain. On the contrary, the higher demand of glucose in both the tumor and the normal brain (Table 3) may explain the low tumor-to-left normal brain ratio during the entire study period. In addition, the drug retention in the pancreas was also higher for L-[18F]FET and L-[18F]FBPA-Fr than that for [18F]FDG.

3.4. MicroPET imaging of F98 glioma-bearing Fischer344 rats

In the microPET scan of glioma-bearing rats, significant brain tumor image and high contrast between tumor and normal brain were observed with both L-[18F]FET and L-[18F]FBPA-Fr (Fig. 3). Although the accumulation of L-[18F]FBPA-Fr in the brain tumor was lower than that of L-[18F]FET, the brain tumor still could be clearly
visualized due to the low retention of L-[18F]FBPA-Fr in the normal brain tissue. The high uptake of [18F]FDG in both the brain tumor and the normal brain tissue is shown in Fig. 3. The image of the tumor could not be recognized well from that of the normal brain tissue by [18F]FDG microPET.

3.5. Ex vivo autoradiography

The biodistributions of these F-18-labelled radiotracers in the brain imaged by ex vivo autoradiography are shown in Fig. 4. The uptake of both [18F]FDG and L-[18F]FBPA-Fr in F98 glioma reached the maximum value at 60 min postinjection (Fig. 5) then decreased with time up to 120 min. On the other hand, a slow accumulation of the L-[18F]FET was found in the beginning, i.e., the uptake was low during the first 30 min, increased rapidly afterward and reached the maximum at 90 min postinjection (Fig. 5). However, the highest tumor-to-normal brain ratios for all three radiotracers were found at 60 min postinjection. These data were consistent with the results obtained from the biodistribution studies (Tables 1–3). Autoradiograms showed that the biodistribution patterns of both L-[18F]FET and L-[18F]FBPA-Fr correlated well with the anatomic localization of the tumors. Quantification of the relative uptake of both drugs in tumors from 15 min to 2 h postinjection was significantly higher than that of surrounding normal brain tissues.

4. Discussion

[18F]FDG PET has been accepted as a powerful, noninvasive metabolic imaging method for the diagnosis and staging of cancer [21–23]. Nonetheless, the false-positive findings are also often accompanied with [18F]FDG PET, especially in inflammatory lesions [24]. Nonspecific [18F]FDG uptake was found in granulocytes and macrophages [25]. Some other factors have been reported to affect the [18F]FDG uptake, such as the upregulation of glucose transporter 1 receptors [26,27], the number of viable tumor cells [28], microvessel density and hexokinase expression [29].

Radiolabeled amino acids have been shown to be useful tracers for tumor diagnosis in neuro-oncology as well as for lymphoma [30,31]. We used a self-modified commercial PET tracer synthesizer to prepare the nca L-[18F]FET. Furthermore, a silica cartridge was used to purify the radiolabeled intermediate instead of an HPLC system, such that L-[18F]FET could be prepared in less than an hour after proton bombardment.

The accumulation of radiolabeled amino acid derivatives was less than that of [18F]FDG in F98 glioma cells as shown in the cellular uptake study. Although L-[18F]FET and L-[18F]FBPA-Fr were transported through the amino acid transport system, they could not be further incorporated into proteins [18,32]. Thin-layer chromatography assay of the cultured medium incubated with L-[18F]FET and L-[18F]FBPA-Fr showed that most of the radioactivities...
 (>90%) were contributed by the intact radiodrugs during the study period (1 h). Besides, both amino acid derivatives were shown with fast uptake for the first 10 min, then almost saturated afterward (Fig. 2), suggesting a dynamic equilibrium of intra- and extracellular F-18-labeled amino acid derivatives was reached. On the other hand, [18F]FDG moved across the cell membrane into the cytoplasm with active transport and was converted to [18F]FDG-6-phosphate by hexokinase and trapped in the cytoplasm. This may explain why the radioactivity of [18F]FDG in the cell was continuously increased even up to 60 min of incubation (Fig. 2).

The maximum tumor-to-normal brain ratios were in the order of L-[18F]FBPA-Fr (3.44), L-[18F]FET (3.15) and [18F]FDG (1.44) at 60 min postinjection, which clearly demonstrated the value of F-18-labeled amino acid derivatives as PET tracers in brain tumor diagnosis and imaging. Also, most organs except pancreas were found with low uptake of both L-[18F]FET and L-[18F]FBPA-Fr. In the kidney, only L-[18F]FBPA-Fr was found with high uptake. In addition, the tumor-to-blood ratios at 60 min postinjection were 3.32, 2.97 and 13.85, and the tumor-to-muscle ratios were 4.85, 5.38 and 14.58 for L-[18F]FET, L-[18F]FBPA-Fr and [18F]FDG, respectively. Thus, L-[18F]FET and L-[18F]FBPA-Fr were shown to be promising PET tracers not only for cerebral but for peripheral tumor imaging as well, although [18F]FDG may be more sensitive than F-18-labeled amino acid tracers for the detection of the peripheral tumors.

MicroPET imaging using L-[18F]FET and L-[18F]FBPA-Fr, but not [18F]FDG, could well differentiate benign from malignant brain tissues due to the facilitated transport of amino acids in the tumor cell and the low uptake in the normal brain [33]. The high glucose utilization of normal gray matter limits the detection of brain tumor tissues with microPET and [18F]FDG. L-[18F]FET has been shown to have higher specificity than [18F]FDG and [11C]MET in the differentiation of inflammatory tissues from malignancies in animal models [34,35]. Initial clinical studies using L-[18F]FET PET in human brain tumors also showed similar results to those obtained with [11C]MET PET [14]. Human gliomas assayed by L-[18F]FET PET showed superior delineation as compared to the magnetic resonance imaging using stereotactic biopsies as a reference [36,37]. Patient plasma analysis demonstrated that almost all the radioactivity was attributed to the intact L-[18F]FET. In the urine, about 60–70% of the radioactivity was found to be intact L-[18F]FET with 30–40% metabolites of different fractions. This indicated that some metabolic degradation of L-[18F]FET occurred in the body, and the metabolites were rapidly excreted through the kidneys [38]. High uptake of L-[18F]FET in the pancreas of rats was observed in this study as well as in mice [13,16], but only minor tracer accumulation in human pancreas [13,38]. L-[18F]FET is more convenient for whole-body imaging of malignant lesions owing to its more homogeneous biodistribution and local retention in the body [38].

Since BNCT for the treatment of brain tumors has regained attention during the last two decades, its clinical applications are continuously performed. Boronophenylalanine is one of the two boron delivery agents approved for clinical trials for the treatment of brain tumors [39,40] and melanoma [41]. Determination of tumor boron-10 levels is required for accurate neutron dosimetry during BNCT. L-[18F]FBPA-Fr PET not only could be applied to predict the effectiveness of BNCT using 10B-BPA, but also could be used for the diagnosis of malignancy as well [12,17,18].

5. Conclusion

Both L-[18F]FET and L-[18F]FBPA-Fr showed significant tumor accumulation with high tumor-to-normal brain ratios at 60 min after injection. The results of this study demonstrated in the animal brain tumor model that F-18-labeled amino acid derivatives could be useful PET tracers for the diagnosis of brain tumor and, possibly, peripheral tumors.

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