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Evaluation of *O*-[^{18}F]fluoromethyl-D-tyrosine as a radiotracer for tumor imaging with positron emission tomography☆

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Abstract

O-[^{18}F]Fluoromethyl-D-tyrosine (D-[^{18}F]FMT) has been reported as a potential tumor-detecting agent for positron emission tomography (PET). However, the reason why D-[^{18}F]FMT is better than L-[^{18}F]FMT is unclear. To clarify this point, we examined the mechanism of their transport and their suitability for tumor detection. The stereo-selective uptake and release of enantiomerically pure D- and L-[^{18}F]FMT by rat C6 glioma cells and human cervix adenocarcinoma HeLa cells were examined. The results of a competitive inhibition study using various amino acids and a selective inhibitor for transport system L suggested that D-[^{18}F]FMT, as well as L-[^{18}F]FMT, was transported via system L, the large neutral amino acid transporter, possibly via LAT1. The in vivo distribution of both [^{18}F]FMT and [^{18}F]FDG in tumor-bearing mice and rats was imaged with a high-resolution small-animal PET system. In vivo PET imaging of D-[^{18}F]FMT in mouse xenograft and rat allograft tumor models showed high contrast with a low background, especially in the abdominal and brain region. The results of our in vitro and in vivo studies indicate that L-[^{18}F]FMT and D-[^{18}F]FMT are specifically taken up by tumor cells via system L. D-[^{18}F]FMT, however, provides a better tumor-to-background contrast with a tumor/background (contralateral region) ratio of 2.741 vs. 1.878 with the L-isomer, whose difference appears to be caused by a difference in the influence of extracellular amino acids on the uptake and excretion of these two isomers in various organs. Therefore, D-[^{18}F]FMT would be a more powerful tool as a tumor-detecting agent for PET, especially for the imaging of a brain cancer and an abdominal cancer.

Keywords: *O*-[^{18}F]Fluoromethyl tyrosine; D-isomer; Positron emission tomography (PET); System L transporter; Tumor imaging

1. Introduction

[^{18}F]FDG is the most widely used tracer for tumor detection with PET imaging. However, several limitations with [^{18}F]FDG have been reported, such as a high uptake in normal brain and heart and in inflammatory tissues [1]. In contrast, the accumulation of positron emitter-labeled amino acids was assumed to reflect enhanced amino acid transport, metabolism and protein synthesis. Therefore, these amino acid tracers have been used for detecting tumors especially those in the brain.

Positron emitter-labeled amino acids and their derivatives, such as 1-[^{11}C]methionine [2], methyl-[^{11}C]methionine [2,3], 1-[^{11}C]tyrosine [4], 1-[^{11}C]leucine [5], 1-[^{11}C]phenylalanine [6], 4-[^{18}F]fluoro-phenylalanine [7] and 2-[^{18}F]fluoro-L-tyrosine [8], have been proposed as PET imaging agents. Among these positron emitter-labeled amino acids, [^{11}C]methionine is widely used for tumor imaging with PET. Recently, several amino acid analogs, namely, *O*-[^{11}C]methyl-L-tyrosine [9], *O*-[^{18}F]fluoromethyl-L-tyrosine (L-[^{18}F]FMT) [9], *O*-[^{18}F]fluoroethyl-L-tyrosine [10,11], *O*-[^{18}F]fluoropropyl-L-tyrosine [12,13], [^{11}C]ethyonine [14] and [^{11}C]propionine [14], were synthesized and evaluated as PET imaging agents. These amino acid

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analogues showed relatively low accumulation in normal peripheral tissue (low tissue-to-blood ratio), rapid blood clearance and a rather high amount of label remaining in tumor tissues (high tumor-to-blood ratio).

In contrast to L-isomers of amino acids, D-isomers are considered to behave as unnatural amino acids, like the amino acid analogs mentioned above. In previous reports in the 1980s, in vivo and in vitro experiments using ^{14}C-labeled D-amino acids revealed that the accumulation of D-isomers was higher than that of L-isomers in tumor cells [15,16]. At that time, the potential of D-isomers of amino acids as nuclear imaging agents was mentioned [15–17]. However, the precise mechanism responsible for the higher accumulation of the D-isomers has remained unclear. Recently, the biological functions of D-isomers in the central nervous system [18], developmental biology [19] and some pathological conditions [20,21] were reported, although the precise behavior of D-isomers still remains to be clarified [22].

Amino acid transport across the plasma membrane is mediated via amino acid transporters located on the membrane. Among the amino acid transport systems, system L, a Na^+-independent neutral amino acid transporter system, is the major route for providing cells with large neutral amino acids including branched or aromatic amino acids [23]. Recently, system L amino acid transporters 1 and 2 (LAT1 and LAT2) were isolated, and their characteristics were evaluated [24–26]. LAT1 was shown to be strongly expressed in malignant tumors [27,28] and also expressed in some normal organs such as brain, spleen, placenta and testis [29]. In contrast, the distribution of LAT2 mRNA is ubiquitous [30,31]. We previously reported that the D-isomer of *O*-[^{18}F]fluoromethyl-L-tyrosine (D-[^{18}F]FMT) was highly accumulated in tumor tissue [32,33], although the accumulation of D-[^{18}F]FMT in normal tissues, e.g., brain, kidney and pancreas, was low as was the whole-body background. However, the molecular mechanism of D-[^{18}F]FMT uptake in tumor tissue was not addressed at that time. Since the presence of amino acids in plasma would affect the uptake of this tracer into tissues, the concentrations of amino acids in plasma, in normal and tumor tissues, and in the microenvironment of tumor cells must be considered [34].

In this study, the characteristics and utility of the D-isomer of an artificial amino acid labeled with ^{18}F positron emitter were evaluated; and the behavior of L-[^{18}F]FMT and D-[^{18}F]FMT both in vitro and in vivo was examined.

2. Materials and methods

2.1. Materials

L-Alanine, L-glycine, L-phenylalanine, L-serine, D-leucine and L-leucine were purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). 2-Aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) was obtained from Sigma-Aldrich Japan (Tokyo, Japan). All other reagents were of analytical grade.

2.2. Synthesis of labeled compound

Positron-emitting ^{18}F was produced by ^{18}O(p,n)^{18}F nuclear reaction using the cyclotron (HM-18; Sumitomo Heavy Industry, Japan) at Hamamatsu Photonics PET Center. L- and D-Isomers of [^{18}F]FMT were synthesized by reacting [^{18}F]fluoro-methyl bromide with the corresponding L- and D-tyrosine according to a previous report [32,33]. Enantiomeric purity was analyzed on a CHIOBIOTIC T column (4.6×250 mm; Tokyo Kasei Kogyo). The elution solution was ethanol/water (1:1), and the flow rate was 1 ml/min. The production of [^{18}F]FDG was performed according to the method reported previously [35]. Specific activities of D-[^{18}F]FMT, L-[^{18}F]FMT and [^{18}F]FDG were 115±10, 126±12 and 144±21 GBq/μmol, respectively; and radiochemical purities were 99.6±0.4%, 99.8±0.3% and 100.0±0.0%, respectively.

2.3. Cell culture

C6 glioma cells (ATCC, Rockville, MD, USA) and HeLa cells (RIKEN, Tsukuba, Japan) were grown in Dulbecco's Modified Eagle's Medium (DMEM, Wako) supplemented with 10% fetal bovine serum (Japan Bioserum, Hiroshima, Japan) and appropriate concentrations of antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). The cells were maintained in plastic culture flasks at 37°C in a humidified atmosphere containing 5% CO_2 and kept as monolayers.

2.4. Measurement of uptake by cells in culture

Rat C6 glioma cells and HeLa cells were plated in 24-well culture plates (Corning Japan, Tokyo, Japan) at a density of 2×10^5 cells per well and cultured for 24 h. After the growth medium had been removed, the cells were washed twice with Hank's balanced salt solution (HBSS; 136.6 mM NaCl, 5.4 mM KCl, 4.2 mM $NaHCO_3$, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.44 mM KH_2PO_4 and 0.41 mM $MgSO_4$) and kept in HBSS for 30 min at 37°C to deplete any residual nutrients from the growth medium. Then the HBSS was discarded, and the uptake assay was started by adding a trace amount of D- or L-isomer of [^{18}F]FMT/HBSS (1–3 MBq/ml) to the cultures. After incubation for the selected time period (2, 5, 10, 30 and 60 min), the uptake of labeled compounds was terminated by removing the medium. After the cells had been washed twice with 1 ml of ice-chilled Dulbecco's phosphate-buffered saline (PBS), the cells were lysed in 400 μl of cell lysis solution (0.1 M NaOH, 2% Triton X-100). The radioactivity in the cell lysates was measured by a γ-counter (Aloka ARC-2000). More than three independent experiments, each done in triplicate, were performed.

2.5. Tracer release from cultured cells

Experiments were performed using 24-well culture plates. HeLa cells (2×10^5 cells/well) were incubated with D- or L-isomer of [^{18}F]FMT (1-3 MBq/ml) in HBSS for 30 min at 37°C. Then, the cells were washed three times with HBSS, and all supernatants were discarded. Release experiments were started by the addition of 1 ml HBSS. The supernatant