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Original Article

Implications for the Use of 6-Radioiodinated L-*meta*-tyrosine as a Tumor Imaging Agent: Mouse Biodistribution, Renal Autoradiography, and Colon Cancer DLD-1 Cell Studies

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Abstract

Introduction: ^{125}I-6-iodo-L-*meta*-tyrosine (6-^{125}I-L-*m*Tyr) has been used to measure cerebral aromatic L-amino acid decarboxylase activity but there is no report of its use as a tumor imaging agent. We therefore conducted mouse biodistribution, renal autoradiography and DLD-1 colon cancer cell studies to investigate the utility of 6-^{125}I-L-*m*Tyr as a tumor imaging agent. ***Methods:*** 6-^{125}I-L-*m*Tyr (11.1 kBq/0.1 mL for biodistribution, 670 kBq/0.1 mL for autoradiography) was administered to 6-week-old male ddY mice by injection into the tail vein and the time-course of 6-^{125}I-L-*m*Tyr biodistribution was determined. After five minutes, the kidney was dissected and renal autoradiography was performed. Separately, DLD-1 cells (5.0×10^5) suspended in 5 mL of Dulbecco's Modified Eagles Medium containing fetal bovine serum were cultured in 6 cm diameter culture dishes under typical conditions and time course measurements of the uptake of 6-^{125}I-L-*m*Tyr (18.5 kBq) into the cells were obtained. Furthermore, the transport systems were analyzed by uptake-inhibition experiments using 2-amino-bicyclo[2,2,1] heptane-2-carboxylic acid (a system L–specific inhibitor) and 2-(methylamino)isobutyric acid (a system A–specific inhibitor). ***Results:*** The biodistribution and renal autoradiogram studies demonstrated low accumulation of 6-^{125}I-L-*m*Tyr in normal mouse tissues and rapid clearance from the blood. Membrane transport inhibition studies indicated that approximately 90% of 6-^{125}I-L-*m*Tyr was transported by system L into DLD-1 cells.
Conclusions: The DLD-1 cell experiments showed that metabolically stable 6-^{125}I-L-*m*Tyr accumulates in tumor cells via system L transport and the mouse experiments showed that 6-^{125}I-L-*m*Tyr is excreted rapidly in the urine. 6-^{125}I-L-*m*Tyr can be used as a tumor imaging agent.

Key words: 6-iodo-L-*meta*-tyrosine, system L, neutral amino acid, DLD-1 cells, renal distribution, biodistribution

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1. Introduction

We previously conducted studies on the utility of radioactive iodine–labeled *meta*-tyrosine (*m*-Tyr) as a diagnostic imaging agent for use in single-photon emission computed tomography (SPECT)[1-4] and reported the high metabolic stability and amino acid (AA) transport affinity of L-*meta*-tyrosine (L-*m*Tyr).[1] *m*Tyr was first separated into D- and L-isomers using high-performance liquid chromatography (HPLC), then both isomers were labelled with non-carrier-added ^{125}I. The geometric isomers of radioiodinated L-meta-tyrosine, ^{125}I-6-iodo-L-*meta*-tyrosine (6-^{125}I-L-*m*Tyr) and ^{125}I-4-iodo-L-*meta*-tyrosine (4-^{125}I-L-*m*Tyr), were separated by HPLC from the reaction mixture of labelled and non-carrier-added ^{125}I-L-meta-tyrosine (^{125}I-L-*m*Tyr).[2]

6-^{125}I-L-*m*Tyr (Fig. 1) was reported to bind preferentially to cerebral aromatic L-AA decarboxylase.[2] We proposed that 6-^{125}I-L-*m*Tyr, a new radiopharmaceutical, enters the brain via a stereospecific AA transport system and used SPECT to assess both the AA transport system and to quantify metabolically active L-3,4-dihydroxyphenylalanine (DOPA) decarboxylase. SPECT provides information regarding the functional status of dopaminergic presynaptic neurons.

Tyrosine is an amino acid and is required for protein synthesis during tumor cell growth and proliferation. Consequently, it is likely that radioiodinated tyrosine derivatives, including 6-$^{123/125}$I-L-*m*Tyr, would accumulate in tumors.

In the present study, we focused on 6-^{125}I-L-*m*Tyr and conducted biodistribution and kidney autoradiography studies in mice to assess the basic properties of 6-^{125}I-L-*m*Tyr as a radiopharmaceutical for tumor imaging. Next, we investigated the transport mechanism and accumulation of 6-^{125}I-L-*m*Tyr in DLD-1 colon cancer cells. The frequency of colon cancer has recently increased.[5] In addition, the cellular accumulation of L-[^{14}C(U)]tyrosine, the parent compound used to design the drug 6-^{125}I-L-*m*Tyr, was compared to that of 6-^{125}I-L-*m*Tyr. ^{125}I has a long half-life and is suitable for basic studies whereas ^{123}I-labeled compounds have a shorter half-life and are used clinically.

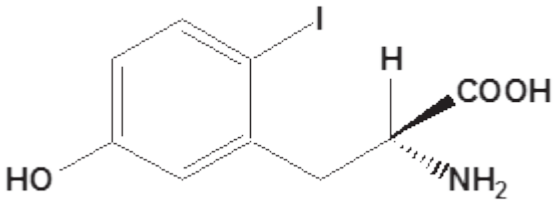

Fig. 1 Structure of 6-iodo-L-*meta*-tyrosine (6-I-L-*m*Tyr).

2. Materials and Methods

The animal experiments were approved by the ethics committees of the universities affiliated with this study.

2.1. Materials

The radiolabel precursor (D,L-*m*Tyr) was purchased from Sigma-Aldrich Japan Co. ^{125}I-NaI was purchased from Amersham Pharmacia Biotech. L-[^{14}C(U)] tyrosine was purchased from American Radiolabeled Chemicals Inc. DLD-1 (human colon adenocarcinoma, JCRB9094) cells were purchased from JCRB. Plastic tissue culture dishes (6 cm diameter, Cat. no. 150288) and plastic culture flasks (surface area 25 cm^2, Cat. no. 163371) were purchased from Nalge Nunc International. 2-Amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH, a system L–specific inhibitor), 2-(methylamino)isobutyric acid (MeAIB, a system A–specific inhibitor), natural L/D-AAs, and reagent grade chemicals were purchased from Kanto Chemical Co., Inc.

2.2. Labeled AAs

L-*m*Tyr was separated from D,L-*m*Tyr by high-performance liquid chromatography (HPLC) using a Crownpak CR(-) chiral column (Daicel Chemical Industries, Ltd.). The separated L-*m*Tyr was labeled with ^{125}I-NaI (8.1×10^{19} [Bq/mol]) and chloramine-T (Kanto Chemical Co., Inc.). Radioiodination of L-*m*Tyr produces two major geometric isomers, 6-iodo- and 4-iodo-L-*m*Tyr,[2] which were separated by HPLC using a Nova-Pak C18 column (Waters Co.). The radiochemical yield was determined using a silica gel thin-layer chromatography kit (Merck). The labeled compounds were used in their noncarrier-added condition.

2.3. Biodistribution Study in Mice

In vivo experiments were performed to investigate the biodistribution of 6-^{125}I-L-*m*Tyr administered to mice. Male ddY mice (6 weeks old, 3-5 per group) were injected with 0.1 mL of saline containing 11.1 kBq of 6-^{125}I-L-*m*Tyr via the tail vein. The animals were anesthetized with ether and sacrificed painlessly by heart puncture at 2, 5, 10, 15 and 30 min after injection, then the brain, heart, lung, liver, pancreas, spleen, kidney, stomach and intestines were removed and weighed, and the blood was sampled. The radioactivity in each organ was determined using a well-type scintillation counter (ARC-300; Aloka). The biodistribution of the radioactivity was determined for each organ as percent dose/organ and on a per-organ-weight basis as percent dose/g.

2.4. In vivo autoradiography

A ddY male mouse weighing 30 g was injected intravenously with 0.1 mL of 6-^{125}I-L-*m*Tyr (670 kBq), then 5 minutes later was anesthetized, sacrificed painlessly, and the kidneys were immediately dissected, washed, placed in an embedding medium and frozen at -15°C for 24 hours. A cryostat microtome (Handex, Shiraimatsu) was used to generate 20 mm axial slices from top to bottom, then the slices were air-dried at -15°C for 24 hours. The slices were placed in contact with an imaging plate (Fuji Photo Film; BASTR2040, 20×40 cm) for at least 24 hours and the resulting images were processed using Bio-Imaging Analyzer (Fuji Photo Film; BAS-2000).

2.5. Time Course of 6-^{125}I-L-mTyr Uptake

DLD-1 cells were cultured to about 80% confluence (3-4 days after inoculation), then the medium was replaced with 5 mL of phosphate-buffered saline (PBS) containing Na^+ (37°C, pH 7.4). The cells were incubated at 37°C for 10 min in a 5% CO_2 atmosphere and then the PBS was replaced with 2.0 mL of uptake solution containing 18.5 kBq of 6-^{125}I-L-*m*Tyr. Incubation was conducted using PBS containing Na^+ uptake solution (NaCl, 137 mM; KCl, 3.7 mM; Na_2HPO_4, 8.0 mM; KH_2PO_4, 1.5 mM; $CaCl_2 \cdot 2H_2O$, 1.8 mM; $MgCl \cdot 6H_2O$, 1 mM). The cells were incubated for 5, 10, 15, 30 and 60 min, the medium was removed, then the cells were washed twice with 5.0 mL of cold PBS and solubilized in 2.0 mL of NaOH (2 N). The radioactivity of each aliquot (500 μL) was counted using an auto-well counter (Aloka, Japan; ARC-380). The results for each set of experimental conditions are reported as the mean of 4 dishes.

2.6. Labeled AA Uptake Inhibition Study

Transport system L in DLD-1 cells was analyzed by removing the culture fluid from the culture dishes, adding 5 mL of 37°C PBS, and incubating the dishes for 10 min at 37°C, 5% CO_2, pH 7.4. The PBS was removed and uptake-inhibition experiments were performed using 6-^{125}I-L-*m*Tyr and the system L-specific and system A-specific inhibitors BCH and MeAIB.[6] The concentration dependence of uptake by transport system L was investigated by adding 0.1, 0.5, 1.0, 5.0 or 10.0 mM BCH to 5 mL of 37°C Na^+-free PBS containing 18.5 kBq of carrier-free 6-^{125}I-L-*m*Tyr dissolved in 137 mM choline-Cl, 3.7 mM KCl, 8.0 mM K_2HPO_4, 1.5 mM KH_2PO_4, 1.8 mM $CaCl_2.2H_2O$ and 1 mM $MgCl \cdot 6H_2O$. The dishes were incubated for 10 min under the conditions described above, then the uptake solution was removed and the surfaces of the adhering cells were rinsed twice with 5 mL of ice-cold PBS. Next, 2 mL of NaOH (2 N) was added and the dishes were left at room temperature for 24 h to solubilize the cells. The radioactivity in an aliquot (500 μL) of each sample was measured.

Transport system A was analyzed in DLD-1 cells using an uptake solution comprising PBS containing Na^+ and the specific transport system inhibitor MeAIB at 0.1, 0.5, 1.0, 5.0 or 10.0 mM to examine the concentration dependence of uptake. The dependence of transport on the specific transport system inhibitor concentration was determined from the radioactivity levels and the transport system was analyzed at the concentration showing the greatest inhibitory effect.

We examined the system L specificity of transport of the labeled AAs in DLD-1 cells. The cells were incubated in 2 mL of incubation medium with/without Na^+ and containing 18.5 kBq of 6-^{125}I-L-*m*Tyr or L-[^{14}C(U)] tyrosine for 10 min at 37°C. Next, the cells were treated with final concentrations of either 5 mM BCH without

Na^+ or MeAIB with Na^+ in the incubation medium (for 6-^{125}I-L-*m*Tyr), or 1 mM BCH without Na^+ or MeAIB with Na^+ in incubation medium (for L-[^{14}C(U)] tyrosine). In medium without Na^+, NaCl was replaced with the same concentration of choline-Cl. We measured the radioactivity associated with the solubilized cells in 2N NaOH using the ARC-380 well-type scintillation counter for 6-^{125}I-L-*m*Tyr and an LS6500 liquid scintillation counter (Beckman Instruments) for L-[^{14}C(U)] tyrosine.

2.7. Statistical Analysis

Data were collated as mean ± standard deviation of 3-5 measurements, and each experiment was performed in duplicate. Results were analyzed using the Student's *t* test. Probability levels of $P<0.001$ were considered to indicate statistical significance.

3. Results

3.1. Biodistribution Study in Mice

6-^{125}I-L-*m*Tyr rapidly moved out of the blood and into the organs following i.v. administration to normal mice (Table 1 A, B) and its accumulation in the pancreas showed *in vivo* kinetics characteristic of AAs. Essentially no radioactivity accumulation was seen in the stomach. There was some accumulation in the intestine and liver on a per-organ basis but it was minimal on a per-organ-weight basis. The physiological accumulation of 6-^{125}I-L-*m*Tyr in normal mouse tissues was low and similar to that of 4-^{125}I-L-*m*Tyr.[2)]

3.2. In vivo autoradiography

Figure 2 show the results of renal autoradiography 5 min after i.v. administration of 6-^{125}I-L-*m*Tyr to normal mice. Low radioactivity was observed in segment 1 (S1) and segment 2 (S2) of proximal tubules located in lesions, similar to that of 4-^{125}I-L-*m*Tyr.[3)] Radioactivity levels in the medulla were very high 5 min after administration. Radioactivity in segment 3 (S3) located in lesions could not be observed due to the high level of radioactivity in vicinal tissue such as renal papilla.

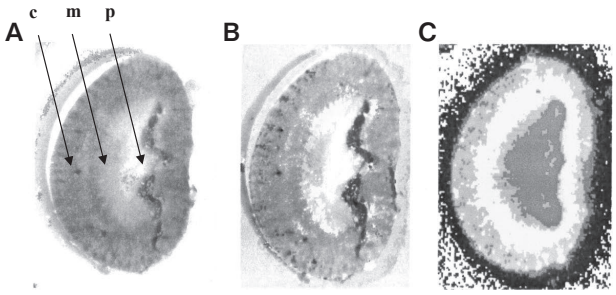

Fig. 2(A) Kidney tissue, (B) fusion image, (C) renal autoradiography at 5 min after i.v. administration. S1 cells are distributed in the periphery of the renal cortex (c) whereas S3 cells are distributed medially, with some S3 cells found in the medulla (m). S2 cells are distributed in the middle of the cortex. A high level of accumulation was observed in renal papilla (p).

3.3. Time Course of 6-^{125}I-L-mTyr Uptake

When the dishes containing DLD-1 cells prepared as described in Section 2.5 were incubated for 10 min with 6-^{125}I-L-*m*Tyr, 9.90±1.00% of the amino acid was taken up into the cells and thereafter the amount of 6-^{125}I-L-*m*Tyr in the cells gradually decreased (Fig. 3). Therefore, transport system analyses using a specific transport system inhibitor were conducted 15 min after the administration of 6-^{125}I-L-*m*Tyr.

3.4. Labeled AA Uptake Inhibition Study

The inhibition of transport system L was dependent on the concentration of the inhibitor, and maximum inhibition was observed at 5 mM (Fig. 4-A). Consequently, we analyzed transport system L using a BCH concentration of 5 mM.

The inhibition of transport system A was not dependent on the concentration of the inhibitor. There were some concentrations at which increased uptake was observed, but there were no statistically significant differences between these concentrations (Fig. 4-B). Therefore, we analyzed transport system A at the MeAIB concentration that provided the greatest inhibitory effect, 5 mM.

The contribution of transport system L to the uptake of 6-^{125}I-L-*m*Tyr was 91.0±3.0%, which was statistically significant ($P<0.001$), whereas the contribution of transport system A to 6-^{125}I-L-*m*Tyr uptake was 9.0±4.0%, which was not statistically significant ($P<0.05$) (Fig. 4-C). Similarly, the contribution of transport system L to the uptake of L-[^{14}C(U)] tyrosine was 97.0±1.0%, which was

Table 1 Biodistribution of 6-^{125}I-L-*m*Tyr in normal mice

A. Biodistribution of 6-^{125}I-L-*m*Tyr in mice. %Dose/g, *%Dose/mL

	2 min	5 min	10 min	15 min	30 min
Blood*	8.919±0.831	5.460±0.766	4.395±0.683	-	2.828±0.362
Brain	0.721±0.082	0.725±0.139	0.637±0.187	0.623±0.096	0.576±0.064
Pancreas	14.949±3.128	21.599±3.606	31.168±11.725	33.819±10.340	6.643±0.947
Spleen	4.640±0.790	3.579±0.537	1.754±0.721	1.275±0.232	1.461±0.041
Stomach	4.529±0.759	3.358±0.521	3.032±0.861	2.998±0.456	2.588±0.374
Intestine	4.034±0.542	3.037±0.722	2.611±0.354	2.351±0.158	1.808±0.293
Liver	9.322±0.943	8.686±1.491	9.327±0.911	9.321±0.724	7.940±2.134
Kidney	19.029±3.119	17.563±1.813	18.885±1.810	15.782±2.583	13.290±2.327
Heart	6.939±1.029	4.122±1.492	3.498±0.452	2.904±0.288	1.784±0.150
Lung	6.951±0.714	5.105±0.504	4.406±0.464	4.019±0.591	3.148±0.342

All values are mean ±SD. n=3-5.

B. Biodistribution of 6-^{125}I-L-*m*Tyr in mice. %Dose/Organ

	2 min	5 min	10 min	15 min	30 min
Blood	3.568±0.333	2.184±0.306	1.758±0.273	-	1.131±0.145
Brain	0.309±0.034	0.311±0.065	0.269±0.083	0.253±0.049	0.246±0.030
Pancreas	2.304±0.652	3.310±1.539	2.245±0.698	2.122±0.465	1.025±0.111
Spleen	0.386±0.050	0.338±0.065	0.176±0.070	0.153±0.028	0.095±0.023
Stomach	1.614±0.282	1.108±0.154	1.046±0.228	0.917±0.209	0.714±0.059
Intestine	8.927±1.876	6.374±1.641	5.438±0.576	4.784±0.664	3.750±0.610
Liver	9.897±1.396	9.401±2.515	10.950±0.742	10.000±0.559	8.766±2.423
Kidney	6.641±0.922	5.995±0.304	7.091±0.915	5.756±1.371	4.727±0.945
Heart	0.735±0.245	0.521±0.184	0.329±0.065	0.274±0.030	0.160±0.007
Lung	1.209±0.191	0.949±0.298	0.686±0.110	0.566±0.275	0.490±0.084

All values are mean ± SD. n=3-5.

statistically significant (P<0.001) and the contribution of transport system A to L-[^{14}C(U)] tyrosine uptake was 8.0±3.0%, which was not statistically significant (Fig. 5). Therefore, the uptake of both L-[^{14}C(U)] tyrosine and 6-^{125}I-L-*m*Tyr occurred via similar transport systems.

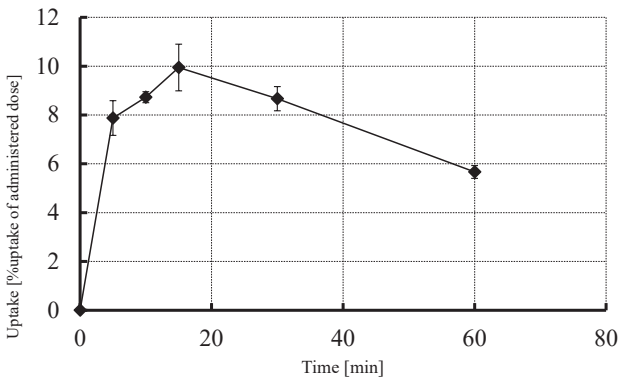

Fig. 3 Time course of 6-^{125}I-L-*m*Tyr uptake by DLD-1 cells (n = 4).

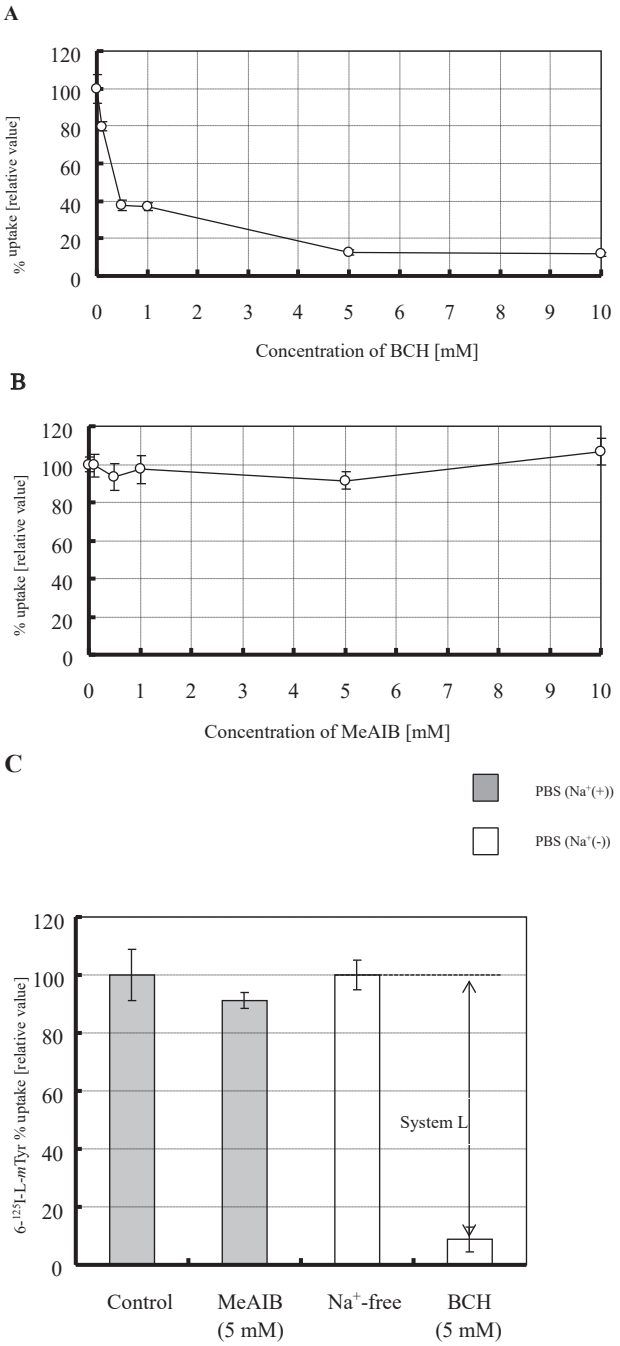

Fig. 4　Concentration-dependent inhibition curves of (A) BCH and (B) MeAIB for 6-^{125}I-L-*m*Tyr. (C): Effects of inhibitors (MeAIB, BCH) and incubation in Na^+-free medium on 6-^{125}I-L-*m*Tyr uptake by DLD-1 cells. Values represent mean ± SD as the percentage of the administered dose (n = 4)).

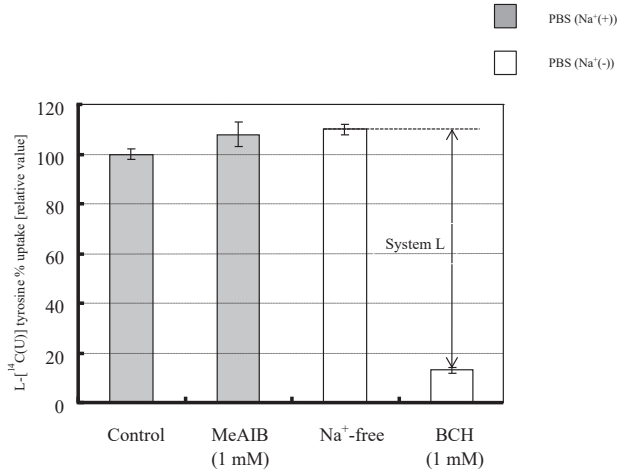

Fig. 5 The effects of inhibitors (MeAIB, BCH) and incubation in Na^+-free medium on L-[^{14}C(U)] tyrosine uptake by DLD-1 cells. Values represent mean ± SD as the percentage of the administered dose (n = 4).

4. Discussion

In designing this study we took into consideration previous reports of the measurement of DOPA decarboxylase activity and the development 6-^{125}I-L-*m*Tyr as a cranial nerve transmission function imaging agent for cerebral dopaminergic presynaptic function.[2] Based on the results of rat brain experiments using inhibitors, we hypothesized that 6-^{125}I-L-*m*Tyr would be useful as a diagnostic imaging agent that reflects both the activity of brain AA transporters and the level of DOPA decarboxylase activity. Therefore, in the present study, we investigated the possibility of using 6-^{125}I-L-*m*Tyr as a tumor imaging agent.

Kloss *et al*. proposed that a suitable radiotracer synthesized using the radioiodination method requires the attachment of radioiodine in such a way that the physiological properties of the compound are not affected.[7] Although this is straightforward for a large molecule such as a protein, it is challenging for small compounds such as tyrosine. The molecular weight of *m*Tyr is about 181 Da and the atomic weight of the stable isotope of iodine is 127 Da. Therefore, the atomic weight of iodine accounts for approximately 41% of the molecular weight of 6-^{125}I-L-*m*Tyr. We anticipated that the addition of iodine at the 6-position of the bulky, hydrophobic benzene ring of *m*Tyr would decrease the affinity of the AA transport system for 6-^{125}I-L-*m*Tyr AA and that iodine in the 4-position of the benzene ring (i.e., 4-^{125}I-L-*m*Tyr) might exhibit different properties for AA transport.

Kloss *et al*. further proposed that the radioiodinated compound should not be broken down quickly by general metabolic pathways.[7] 6-^{125}I-L-*m*Tyr has been reported to be metabolically stable.[2] The accumulation of radioactivity

due to 3-^{123}I-iodo-alpha-methyl-L-tyrosine (^{123}I-IMT) and 4-^{125}I-L-*m*Tyr in the stomach were similar,[3] indicating high resistance of 6-^{125}I-L-*m*Tyr towards de-iodination and suggesting that the use of 4-^{125}I-L-*m*Tyr would prevent undesirable radiation exposure due to radioactive iodine released by de-iodination reactions.

The results of the biodistribution study of 6-^{125}I-L-*m*Tyr summarized in Table 1 indicate rapid blood clearance, maximum tissue uptake, and prolonged retention in the organs, comparable to the biodistribution of other AA analogs such as *O*-(2-^{18}F-fluoroethyl)-L-tyrosine (^{18}F-FET), 3-^{18}F-fluoro-alpha-methyl-L-tyrosine, and ^{123}I-IMT.[3] 6-^{125}I-L-*m*Tyr exhibited higher uptake (% Dose/g) in the pancreas than in the liver and tyrosine is known to accumulate in pancreatic cells and is used for the synthesis of enzymes. The higher accumulation in the pancreas is consistent with the characteristics of AA biodistribution.

There was a higher accumulation of ^{125}I-IMT in all organs studied compared to 4- or 6-radioiodinated L-*meta*-tyrosine. In particular, the accumulation of ^{125}I-IMT in kidney was about 5-fold higher than that of 4- or 6-radioiodinated L-*meta*-tyrosine.[3]

After glomerular filtration, natural AAs are generally largely reabsorbed and most are resorbed by proximal tubules in segment 1 (S1) to S3 cells; consequently, there is little excretion of AAs.[8] In contrast, a large amount of radioactivity due to 6-^{125}I-L-*m*Tyr had moved to the renal papilla 5 min after i.v. administration (Fig. 2). These two observations, of very early excretion of radioactivity through the urine and low radioactivity in the S1 to S3 region, suggest that 6-^{125}I-L-*m*Tyr may not be efficiently reabsorbed, although it was difficult to observe radioactivity in the S3 region, as mentioned above.

We previously reported the accumulation of secreted ^{125}I-IMT, an alpha-methylated Tyr analog, in the cortex of proximal tubule S2 areas, similar to that of ^{99m}Tc-mercaptoacetyltriglycine (MAG3) and p-aminohippuric acid (PAH), which are typical proximal tubule secretion compounds.[3] Probenecid is an organic anion secretion inhibitor that markedly inhibits the accumulation of secreted MAG3, PAH and ^{125}I-IMT in S2 areas.[3] In contrast, 6-^{125}I-L-*m*Tyr did not accumulate significantly in the S2 region of the kidney proximal tubule, suggesting that there are different mechanisms for the renal handling of 6-^{125}I-L-*m*Tyr or 4-^{125}I-L-*m*Tyr and ^{125}I-IMT, possibly due to the alpha-methylation of Tyr.[3]

6-^{125}I-L-*m*Tyr is primarily transported across the membrane via the AA transport system and thus we conducted uptake inhibition experiments targeting this system. Various AA membrane transport systems have been described, including transport system L, transport system A, and transport system ASC. Transport systems A and ASC depend on the presence of Na^+, and they can be distinguished on the basis of specific inhibition by artificial AAs. For example, transport system A is Na^+ dependent, and uptake is inhibited by the artificial AA MeAIB. Similarly, transport system ASC is also Na^+ dependent, but it is not inhibited by MeAIB. Transport system L is independent of Na^+, and it is specifically inhibited by a different artificial AA, BCH.[6,9]

Here we examined 6-^{125}I-L-*m*Tyr uptake into DLD-1 cells to determine the dependence of uptake on the concentration of MeAIB, a specific inhibitor of transport system A, or the concentration of BCH, a specific inhibitor of transport system L. Our aim was to determine what concentration of each of these compounds should be used in inhibition experiments. We found that 6-^{125}I-L-*m*Tyr uptake into DLD-1 cells showed no clear dependence on the concentration of MeAIB and thus we used 5 mM MeAIB in the inhibition experiments (Fig. 4-B). Conversely, the uptake of 6-^{125}I-L-*m*Tyr into DLD-1 cells decreased as the BCH concentration increased and thus we chose a concentration of 5 mM BCH for the uptake inhibition experiments (Fig. 4-A).

The uptake of 6-^{125}I-L-*m*Tyr did not differ significantly whether or not Na^+ was present in the PBS, indicating that neither of the Na^+-dependent transport systems (i.e., systems A and ASC) is involved in 6-^{125}I-L-*m*Tyr uptake by DLD-1 cells. Transport system L is primarily responsible for 6-^{125}I-L-*m*Tyr uptake (Fig. 4-C), as supported by real-time PCR results demonstrating expression of the LAT1, LAT2, LAT3, and LAT4 transporter genes of transport system L and of the 4F2hc coupling factor in DLD-1 cells (data not shown).[10-14] Similarly, the cellular accumulation of L-[^{14}C(U)]tyrosine,

the parent compound used to design the drug 6-^{125}I-L-*m*Tyr, is primarily due to transport system L (Fig. 5).

Based on the results and discussion above, we suggest that 6-radioiodinated L-*meta*-tyrosine (e.g.; 6-^{123}I-L-*m*Tyr, 6-^{124}I-L-*m*Tyr) may be useful as a tumor imaging agent for the following reasons. After i.v. administration to normal mice, 6-^{125}I-L-*m*Tyr was rapidly transported out of the blood and into the tissues (Table 1 A, B), supporting its usefulness as a molecular imaging agent for tumors. Also, the accumulation of 6-^{125}I-L-*m*Tyr in the pancreas is characteristic of the *in vivo* kinetics of AAs. Almost no radioactivity accumulation was observed in the stomach, indicating the high resistance of 6-^{125}I-L-*m*Tyr to de-iodination. This suggests that it will be possible to avoid undesirable radiation exposure of the thyroid, stomach, and other organs due to radioactive iodine released by de-iodination reactions. There was some accumulation of radioactive iodine in the intestine and liver on a per-organ basis, but it was minor on the per-organ-weight basis. Accumulation in normal mouse tissues was low overall, and the low accumulation in brain and lung suggests that 6-^{125}I-L-*m*Tyr may be effective for evaluating tumors in these organs.

5. Conclusion

Experiments in mice confirmed that 6-^{125}I-L-*m*Tyr is highly resistant to de-iodination and the *in vivo* accumulation of 6-^{125}I-L-*m*Tyr in normal mouse tissues is low. The concentration of 6-^{125}I-L-*m*Tyr in various organs and tissues decreased rapidly following administration to normal mice. We confirmed that DLD-1 cells accumulate 6-^{125}I-L-*m*Tyr via transport system L. We suggest that 6-^{125}I-L-*m*Tyr may be highly useful as a new radiopharmaceutical for the molecular imaging of tumors because it is highly resistant to de-iodination, it has high affinity for tumors specifically expressing transport system L, and its physiological accumulation in normal tissue is low. We aim to examine the utility of 6-^{125}I-L-*m*Tyr for the molecular imaging of tumors using SPECT.

Conflict of interest

The authors declare that they have no conflict of interest.

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和文抄録

^{123}I-6-iodo-L-*meta*-tyrosine (6-^{123}I-L-*m*Tyr)は，脳の芳香族L-アミノ酸脱炭酸酵素活性測定に使用できると報告されたが，腫瘍診断薬としての使用可能性の報告はない。我々は^{125}I-標識体を用い，腫瘍診断薬としての6-^{123}I-L-*m*Tyrの有用性を基礎的に調べるために，6週齢雄性ddY系マウスの体内分布，腎臓オートラジオグラフィー，DLD-1大腸癌細胞により集積に関わるアミノ酸輸送系に関する検討を行った。体内分布から，正常マウス組織における6-^{125}I-L-*m*Tyrの低集積性および血液から尿への迅速なクリアランスが示された。DLD-1膜輸送阻害実験では，約90%がL系輸送を介して腫瘍細胞に蓄積することが示された。 6-^{125}I-L-*m*Tyrは，腫瘍診断薬として使用することができると考えられる。