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ScienceDirect
Nuclear Medicine and Biology 37 (2010) 197–204
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

Uptake of 3-[^{125}I]iodo-α-methyl-L-tyrosine into colon cancer DLD-1 cells: characterization and inhibitory effect of natural amino acids and amino acid-like drugs☆

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Received 20 June 2007; received in revised form 7 October 2009; accepted 31 October 2009

Abstract

Introduction: We examined 3-[^{123}I]iodo-α-methyl-L-tyrosine ([^{123}I]IMT) uptake and inhibition by amino acids and amino acid-like drugs in the human DLD-1 colon cancer cell line, to discuss correlation between the inhibition effect and structure.
Methods: Expression of relevant neutral amino acid transporters was examined by real-time PCR with DLD-1 cells. The time course of [^{125}I]IMT uptake, contributions of transport systems, concentration dependence and inhibition effects by amino acids and amino acid-like drugs (1 mM) on [^{125}I]IMT uptake were examined.
Results: Expression of system L (4F2hc, LAT1 and LAT2), system A (ATA1, ATA2) and system ASC (ASCT1) was strongly detected; system L (LAT3, LAT4) and MCT8 were weakly detected; and B^0AT was not detected. [^{125}I]IMT uptake in DLD-1 cells involved Na^+-independent system L primarily and Na^+-dependent system(s). Uptake of [^{125}I]IMT in Na^+-free buffer followed Michaelis–Menten kinetics, with a K_m of 78 μM and V_{max} of 333 pmol/10^6 cells per minute. Neutral D- and L-amino acids with branched or aromatic large side chains inhibited [^{125}I]IMT uptake. Tyrosine analogues, tryptophan analogues, L-phenylalanine and *p*-halogeno-L-phenylalanines, and gamma amino acids [including 3,4-dihydroxy-L-phenylalanine (L-DOPA), DL-*threo*-β-(3,4-dihydroxyphenyl)serine (DOPS), 4-[bis(2-chloroethyl) amino]-L-phenylalanine and 1-(aminomethyl)-cyclohexaneacetic acid] strongly inhibited [^{125}I]IMT uptake, but L-tyrosine methyl ester and R(+)/S(−)-baclofen weakly inhibited uptake. The substrates of system ASC and A did not inhibit [^{125}I]IMT uptake except L-serine and D/L-cysteine.
Conclusions: [^{125}I]IMT uptake in DLD-1 cells involves mostly LAT1 and its substrates' (including amino acid-like drugs derived from tyrosine, tryptophan and phenylalanine) affinity to transport via LAT1. Whether transport of gamma amino acid analogues is involved in LAT1 depends on the structure of the group corresponding to the amino acid residue. Beta-hydroxylation may confer reduction of transport affinity of tyrosine analogues via LAT1.

Keywords: Amino acid-like drug; Colon cancer cell line; DLD-1; 3-Iodo-α-methyl-L-tyrosine; Inhibitor; L-Type amino acid tranasporter-1

☆ This work was supported by Grants-in-Aid for Scientific Research (#10770451, #14770498, #13557075, #15659283, #16659322 and #17390336) from the Ministry of Education, Science, Sports and Culture of Japan and the Japan Society for the Promotion of Science. Financial support was also provided by the Ibaraki Prefectural University Research Project (9808, 0118, and 0220); Ibaraki Prefectural University Grants-in-Aid of the Encouragement for Young Scientists 2001, 2002, 2004, 2005, 2006, 2008 and 2009; and Japan Atherosclerosis Research Foundation Grant-2008.
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doi:10.1016/j.nucmedbio.2009.10.011

1. Introduction

3-[^{123}I]Iodo-α-methyl-L-tyrosine ([^{123}I]IMT), an artificial amino acid, has been developed as a functional imaging agent for tyrosine transport mechanisms in the brain and pancreas [1–3]. Because amino acids rapidly accumulate in tumor cells for active proliferation, [^{123}I]IMT has also been used clinically for SPECT imaging of tumors, as described by Langen et al. [4–8].

Compared to intracranial tumor cell lines including rat C6 glioma cells [9], human GOS3 glioma cells [10] and 86HG-39 human glioma cells [8], relatively few of the studies of [^{123}I]IMT transport have involved extracranial tumor cell lines. The kinetics of [^{123}I]IMT transport have been studied in human Ewing's sarcoma cells [11]. Furthermore, [^{123}I]IMT transport has been characterized in rat lymphoma cells [12], a porcine kidney epithelial cell line [13], human monocyte-macrophages [14], human small-cell lung cancer [15] and pancreatic carcinoma [16].

In cultured glioma cells of the lines mentioned above, membrane transport of [^{123}I]IMT is dominated by BCH-sensitive transport, i.e., amino acid transport system L, and relatively minor uptake occurs via the Na^+-dependent system [8–10]. System T also mediates [^{123}I]IMT transport into U266 human myeloma cells [17]. However, the gene expression of neutral amino acid transporters has not been fully clarified even in those cell lines.

Some of the system L transporters demonstrate gene expression inducement that is dependent on the cellular conditions, and thus researchers in the fields of cell activation, cellular proliferation and cellular nutrition have become interested in the inhibitors and/or substrates of the system L transporter family [18–20]. The results of such studies are applied to not only cancer imaging [7] or therapeutics [21], but also to the development of a new class of target in immunosuppressant studies [22].

In the present study, we characterized [^{125}I]IMT transport in the human colon cancer cell line, DLD-1, with particular emphasis on the inhibitory effect of amino acid-like drugs. The gene expression of neutral amino acid transporters was confirmed with real-time reverse transcription–PCR (real-time PCR) in DLD-1. We selected the following amino acid-like drugs for investigation: DL-threo-β-(3,4-dihydroxyphenyl)serine [DOPS; amino acid precursor of noradrenaline (NA); elevates brain NA concentrations] [23], 4-[bis (2-chloroethyl)amino]-L-phenylalanine (melphalan; antineoplastic agent that forms DNA intrastrand crosslinks by bifunctional alkylation in 5′-GGC sequences) [21], β-(aminomethyl)4-chlorobenzenepropanoic acid [R(+)-baclofen; GABAB receptor agonist: more active enantiomer; skeletal muscle relaxant, S(−)-baclofen and less active enantiomer of baclofen] [24], 1-(aminomethyl)-cyclohexaneacetic acid (gabapentin; anticonvulsant with unknown mechanism of action, crosses the blood–brain barrier, increases GABA concentrations in the brain and reduces excitatory amino acid neurotransmission) [25], and others as listed in Materials and Methods. In addition, we discuss the correlation between the inhibition effect and the structure of these compounds. To the best of our knowledge, there are no previously published data on the inhibitory effects of such amino acid analogues on [^{125}I]IMT transport.

2. Materials and methods

2.1. Materials and preparation of labeled compounds

Reagent-grade chemicals were acquired from Sigma-Aldrich Japan KK (Tokyo, Japan). Instead of ^{123}I ($T_{1/2}$=13 h) for clinical use, ^{125}I-labeled IMT ([^{125}I]IMT) was used for convenience because of its longer half-life ($T_{1/2}$=60 days). [^{125}I]NaI (8.1×10^7 GBq/mmol) was purchased from Muromachi Chemical Co. (Tokyo, Japan). Human colorectal adenocarcinoma cell line DLD-1 was obtained from the Japanese Collection of Research Bioresources (Tokyo, Japan; catalog number JCRB9094). Plastic tissue culture dishes (diameter, 60 mm; Nalge Nunc International Roskilde, Denmark) and plastic flasks (surface area, 25 cm^2; Nalge Nunc International Roskilde) were used. No-carrier-added [^{125}I]IMT was prepared using the conventional chloramine-T method, as described elsewhere [1–3,13]. [^{125}I]IMT labeling efficiency and radiochemical purities were greater than 80% and 95%, respectively. Specific radioactivity was greater than 8.1×10^{19} Bq/mol.

2.2. Cell cultures

DLD-1 cells were maintained in cells fed with Dulbecco's Modified Eagle's Medium (D-MEM; Sigma-Aldrich Japan) supplemented with 10% FBS and 2 mM glutamine, in a 5% CO_2 humidified atmosphere at 37°C. For the uptake experiments, DLD-1 cells were seeded on 60-mm dishes at a cell density of 5×10^5 cells/dish in 5 ml of complete medium and were used on the third or fourth day after inoculation.

2.3. Measurement of [^{125}I]IMT transport in DLD-1 cells

For the uptake study, we investigated the time course of [^{125}I]IMT uptake (incubation time: 5, 10, 30, 45 and 60 min), contributions of transport systems [in sodium-free medium or sodium-containing medium and sodium-free medium containing 1 mM of 2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH)], concentration dependence (at 3, 10, 30, 100 and 300 mM of IMT) and the effects of natural amino acids (1 mM) on [^{125}I]IMT uptake. In addition, we examined natural amino acid and amino acid-like drug (1 mM) inhibition of [^{125}I]IMT transport into DLD-1 cells in Na^+-containing uptake medium. Kinetic parameters with [^{125}I]IMT uptake were determined based on the results of the concentration-dependence study.

The sodium-containing medium was phosphate-buffered saline, pH 7.4, which was composed of 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM K_2HPO_4, $CaCl_2$ 1.8 mM and MgCl 1 mM. In the sodium-free experiment, NaCl and Na_2HPO_4 were replaced with the same concentrations of choline-Cl and K_2HPO_4, respectively. After removal

of the culture medium, each dish was washed once with 5 ml of incubation medium for 10 min at 37°C. The cells were then incubated with 2 ml of incubation medium containing 18.5 kBq [^{125}I]IMT for various periods of time at 37°C.

For the inhibition experiment, one of several inhibitors was added, and the cells were then incubated for 10 min at 37°C with 18.5 kBq [^{125}I]IMT. The following inhibitors were tested at 1.0 mM: 20 D- or L-amino acids, BCH, R(+)-baclofen, S(−)-baclofen, 1-(aminomethyl)-cyclohexaneacetic acid (gabapentin), 3,4-dihydroxy-L-phenylalanine (L-DOPA), DOPS, 4-[bis(2-chloroethyl)amino]-L-phenylalanine (melphalan), seleno-L-methionine and 2-(methylamino)-isobutyric acid (MeAIB); *N*-methylglycine (sarcosine).

After incubation, the medium was aspirated, and the monolayers were rinsed twice rapidly with 5 ml of ice-cold incubation medium. The cells were solubilized in 1.5 ml of 1N NaOH, and the radioactivity of each aliquot was quantified using a well-type scintillation counter.

2.4. RNA extraction and quantitative real-time reverse transcription–PCR

Total RNA from DLD-1 cells was isolated using the QIAshredder and RNeasy Plus Mini Kit (QIAGEN) according to the manufacturer's description on the third or fourth day after inoculation. cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Mannheim, Germany), using random hexamers priming at 50°C for 1 h, according to the manufacturer's instructions. The primers (Table 1) were designed using the Roche Applied Science website-based Universal ProbeLibrary Assay Design Center (http://www.roche-applied-science.com). Quantitative real-time PCR was performed using LightCycler 2.0 instruments, LightCycler TaqMan Master and the Universal ProbeLibrary (Roche Applied Science) with the following profile: one cycle at 95°C for 10 min, 45 cycles of 95°C for 10 s and 60°C for 25 s, and, finally, one cycle at 40°C for 30 s. PCR products were analyzed by agarose gel electrophoresis, and no nonspecific PCR bands were detected. The relative levels of PCR products were calculated from standard curves established from each primer pair. Expression data were normalized against the amount of glyceraldehyde 3 phosphate dehydrogenase (GAPDH) and actin beta(ACTB) PCR products as the housekeeping genes [26,27].

2.5. Statistical analysis

Data represent the mean±S.D. of five measurements, and each experiment was performed in duplicate. Results were analyzed using Student's *t* test. The $P<.01$ level was considered to denote statistical significance.

3. Results

A representative growth curve of DLD-1 is shown in Fig. 1A. DLD-1 cells 3 to 4 days after inoculation were semiconfluent logarithmic phase cells and were used in this experiment.

Fig. 1B shows the time course of [^{125}I]IMT uptake into DLD-1 cells. Uptake of [^{125}I]IMT increased rapidly during the initial 10 min of incubation and then the intracellular concentration of [^{125}I]IMT reached a steady state after a further 5 min. Fig. 1C shows the contribution of transport system L to total uptake of [^{125}I]IMT. Replacement of sodium with choline reduced [^{125}I]IMT uptake only by a small amount, suggesting that [^{125}I]IMT is a substrate of an Na^+-dependent transport system. Fig. 1D demonstrates the concentration dependence of membrane transport of [^{125}I]IMT in DLD-1 cells. The inset graph is a double reciprocal plot for kinetic analysis. It revealed a high-affinity apparent Michaelis constant of K_m=78 μM, and a maximum transport velocity (V_{max}) of 333 pmol/10^6 cells per minute in Na^+-free buffer.

As shown in Fig. 2A and B, [^{125}I]IMT uptake was inhibited by neutral L- and D-amino acids (Cys, Leu, Ile, Phe, Met, Tyr, His, Trp and Val) in DLD-1 cells. Ala, Ser and Thr

Table 1
Primers for real-time PCR

Gene	Sequences (5′-3′)		Probe no.	GenBank accession number
	Forward	Reverse		
4F2hc	cagaagtggtggcacacg	gtaatcgagacgccccttc	81	NM_001012661.1, NM_001012662.1, NM_002394.4, NM_001012663.1, NM_001012664.1, NM_001013251.1
LAT1	gtggaaaaacaagcccaagt	cacctgcatgagcttctgac	25	NM_003486.5
LAT2	ttgccaatgtcgcttatgtc	ggagcttctctccaaaagtcac	17	NM_012244.2, NM_182728.1
LAT3	gccctcatgattggctctta	ccggcatcgtagatcagc	29	NM_003627.4
LAT4	caagtatggcccgaggaag	caatcagcaagcaggaaacc	3	NM_152346.1
ATA1	tctaatgggaaaggaagagacatt	tgccaaaggtaactatcaccac	64	NM_030674.3, NM_001077484.1
ATA2	cctatgaaatctgtacaaaagattgg	ttgtgtacccaatccaaaacaa	9	NM_018976.3
ASCT1	tttgcgacagcatttgctac	gcacttcatcatagagggaagg	78	NM_003038.2
MCT8	gctgccgttgctttcatt	tgaagtagcgcaggcttagg	17	NM_006517.2
TAT1	ggtgtgaagaaggtttatctacagg	agggccccaaagatgcta	6	NM_018593.3
B0AT1	gcttcccctacctgtgtcag	ggatgaggaacgggatcat	63	NM_001003841.1
ACTB	ccaaccgcgagaagatga	ccagaggcgtacagggatag	64	NM_001101.2
GAPDH	agccacatcgctcagacac	gcccaatacgaccaaatcc	60	NM_002046.3

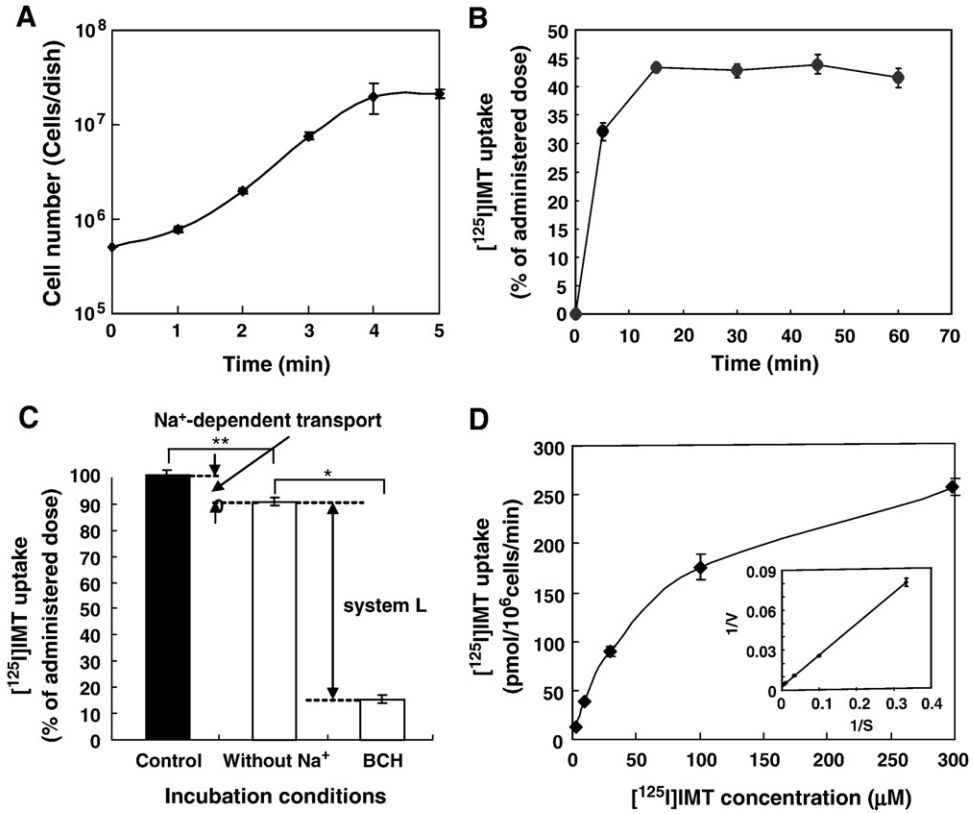

Fig. 1. Uptake mechanisms of 3-[^{125}I]iodo-α-methyl-L-tyrosine ([^{125}I]IMT), an amino acid transport marker, in colon cancer DLD-1 cells. (A) Growth curve of DLD-1. (B) Time course study of [^{125}I]IMT uptake into DLD-1 cells on the third or fourth day after inoculation. The [^{125}I]IMT uptake was plotted against incubation time. (C) Contributions of transport systems to total uptake of [^{125}I]IMT. Closed and open squares represent uptake of [^{125}I]IMT into DLD-1 cells in Na^+-containing or Na^+-free medium, respectively, on the third or fourth day after inoculation. *P<.0001; **P<.005 vs. control. (D) Concentration dependence of membrane transport of [^{125}I]IMT in DLD-1 cells. The uptake was plotted against 3-iodo-α-methyl-L-tyrosine concentration. Uptake of [^{125}I]IMT followed Michaelis–Menten kinetics, with a K_m of 78.0 μM and V_{max} of 333 pmol/10^6 cells per minute in Na^+-free buffer.

had weaker inhibitory effects on [^{125}I]IMT uptake. Most of the L-isomers demonstrated higher inhibitory effects than the D-isomers, particularly without Na^+. On the other hand, the inhibitory effects of D-Phe, D-Met, D-Tyr and D-Asn were higher than those of the corresponding L-isomers in the presence of sodium.

Fig. 3 shows the inhibitory effect of amino acids and amino acid-like drugs on [^{125}I]IMT transport into DLD-1 cells in Na^+-containing uptake medium.

The tested tyrosine analogues, tryptophan analogues, L-phenylalanine and *p*-halogeno-L-phenylalanines, and gamma amino acids (including L-DOPA, DOPS, melphalan and gabapentin) all inhibited [^{125}I]IMT uptake, except for L-tyrosine methyl ester and R(+)-/S(−)-baclofen. L-DOPA inhibited [^{125}I]IMT uptake to a greater extent than DOPS. The substrates of systems ASC and A did not inhibit [^{125}I]IMT uptake, except for L-serine and D/L-cysteine. None of the tested cationic or anionic amino acids inhibited [^{125}I]IMT transport into DLD-1 cells.

The results of representative human neutral amino acid transporter detection in DLD-1 cells by real-time PCR are shown in Table 2. Expression of system L (4F2hc, LAT1 and LAT2), system A (ATA1, ATA2) and system ASC (ASCT1) was strongly detected; system L (LAT3, LAT4) and MCT8 were weakly detected; and B^0AT was not detected by real-time PCR.

4. Discussion

We characterized [^{125}I]IMT uptake and uptake inhibition by amino acids and amino acid-like drugs, using the human colorectal adenocarcinoma cell line, DLD-1, which was isolated by Dexter et al. [28]. Seeding of DLD-1 cells in agar is known to result in clones of two distinct morphologies: clone A and clone D [28]. We used DLD-1 cells 3 to 4 days after inoculation and in semiconfluent logarithmic phase, before the appearance of these clones.

In the time course experiment, uptake of [^{125}I]IMT into DLD-1 cells increased rapidly during the initial 10 min of incubation. [^{125}I]IMT uptake by DLD-1 cells involves primarily system L (about 90%), and [^{125}I]IMT uptake was inhibited by neutral L- and D-amino acids (Cys, Leu, Ile, Phe, Met, Tyr, His, Trp and Val). This inhibition profile is similar

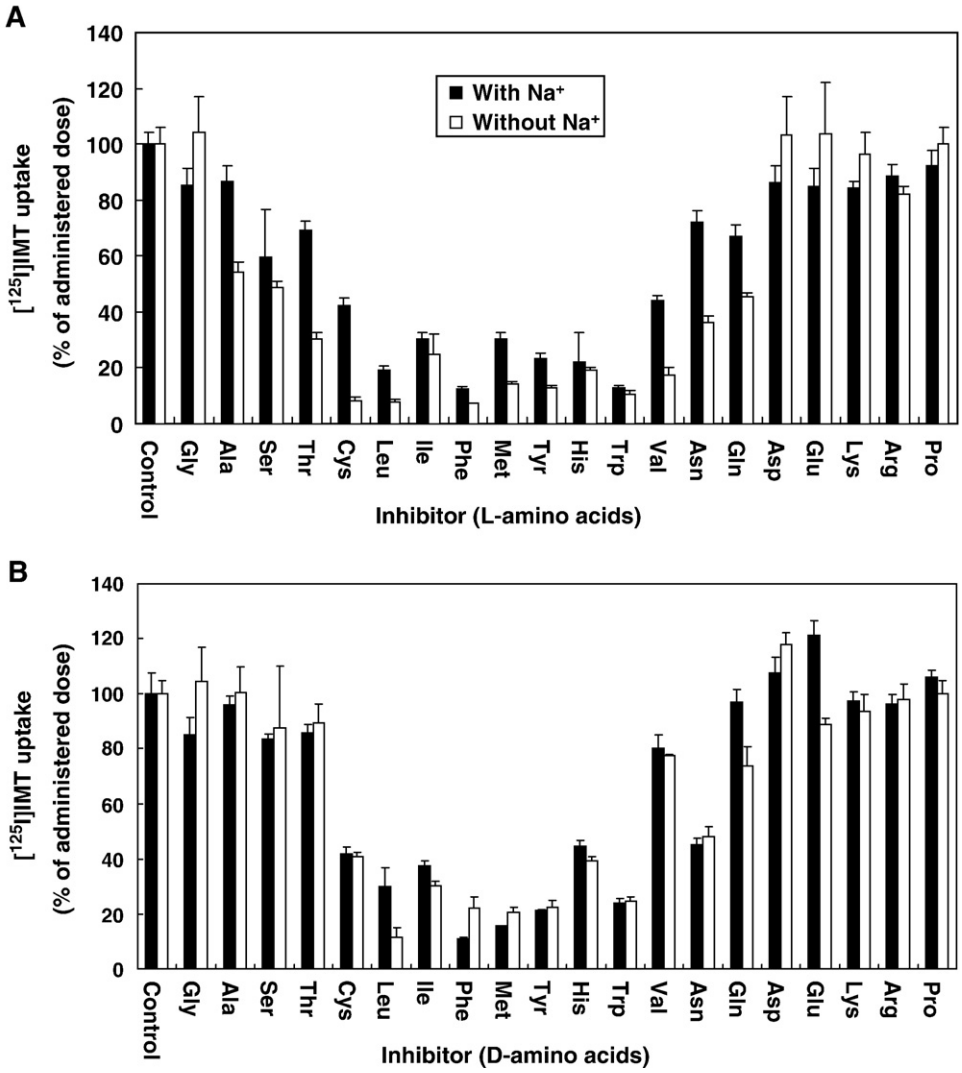

Fig. 2. Effects of natural amino acids (1 mM) on [^{125}I]IMT transport into DLD-1 cells on the third or fourth day after inoculation. (A) L-Amino acids. (B) D-Amino acids. Closed and open columns represent uptake of [^{125}I]IMT into DLD-1 cells in Na^+-containing or Na^+-free uptake medium, respectively.

to our recent report [29] suggesting that LAT1 is the main transporter of [^{125}I]IMT into DLD-1 cells. [^{125}I]IMT appears to be a substrate of an Na^+-dependent transport system but with only small affinity to such systems, because replacement of sodium with choline reduced [^{125}I]IMT uptake only by a small amount. Ala, Ser and Thr had weaker inhibitory effects on [^{125}I]IMT uptake, suggesting [^{125}I]IMT affinity to system ASC [30]. With this partial [^{125}I]IMT uptake inhibition caused by Ser and Cys, participation of LAT2 also appears likely, but LAT2 does not involve [^{125}I]IMT according to our oocyte experiments [31]. On the other hand, system A does not contribute to [^{125}I]IMT uptake into DLD-1 cells because MeAIB, a system A-specific inhibitor, did not demonstrate an inhibitory effect. In DLD-1 cells, the K_m and V_{max} corresponding to system L (78 μM and 333 pmol/10^6 cells per minute) are similar to those described elsewhere in other cell lines [7]. These results do not contradict the major neutral amino acid transporter gene expression profile detected by real-time PCR: system L (4F2hc, LAT1), system A (ATA1, ATA2) and system ASC (ASCT1).

LAT1 is strongly induced depending on the cellular conditions (i.e., cell activation, cellular proliferation and cellular nutrition) [18–20], which is of great interest to those studying not only cancer imaging [7] and therapeutics [21], but also a new class of target in immunosuppressant studies [22]. From this point of view, we conducted a study on inhibition by neutral amino acids and amino acid-like drugs of [^{125}I]IMT transport into DLD-1 cells.

We observed an inhibitory effect on [^{125}I]IMT transport into DLD-1 cells by the following amino acid-like drugs or compounds: DOPS, melphalan and *p*-halogeno-L-phenylalanines. The tested tyrosine analogues, tryptophan analogues and phenylalanine analogues strongly inhibited [^{125}I]IMT transport into DLD-1 cells, except for L-tyrosine methyl ester. These compounds are typical LAT1 selective substrates that are composed of large neutral alpha-amino

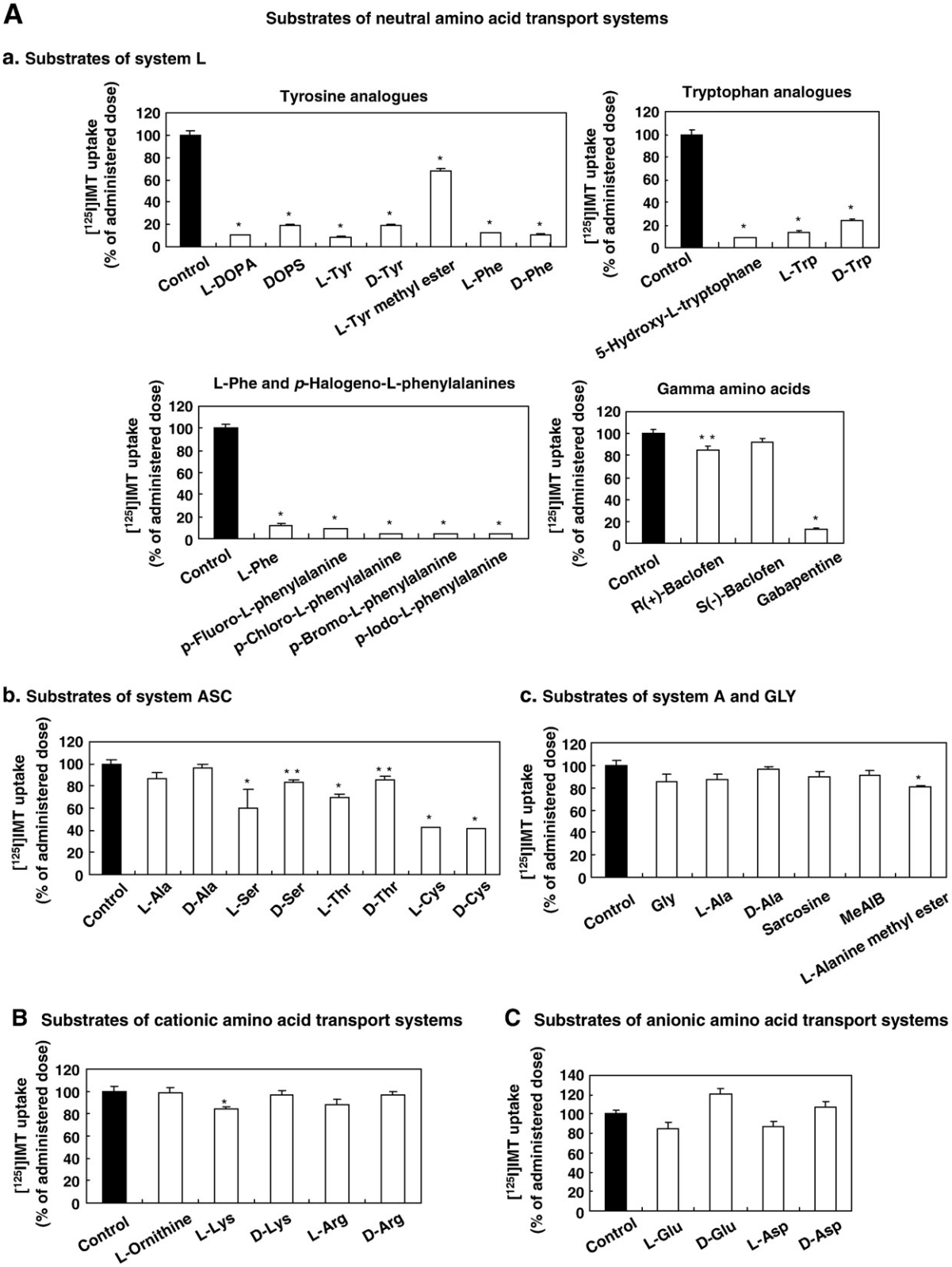

Fig. 3. Inhibitory effect of amino acids and amino acid-like drugs (1 mM) on [^{125}I]IMT transport into DLD-1 cells in Na^+-containing uptake medium on the third or fourth day after inoculation. (A, a) Substrates of neutral amino acid transport systems (tyrosine analogues, tryptophan analogues, L-phenylalanine and *p*-halogeno-L-phenylalanines, and gamma amino acids); (b) substrates of system ASC; (c) substrates of system A. (B) Substrates of cationic amino acid transport systems. (C) Substrates of anionic amino acid transport systems. *P<.001; **P<.01 vs. control.

Table 2
Results of real-time PCR

n=3	Target/GAPDH ratio×1000	Target/ACTB ratio×1000
GAPDH	1000.00±58.19	517.71±30.13
ACTB	1931.58±37.31	1000.00±19.32
4F2hc	109.47± 10.27	56.68±5.32
LAT1	253.95±22.25	131.47±11.52
LAT2	58.95±10.33	30.52±5.35
LAT3	8.84±0.08	4.58±0.04
LAT4	2.36±0.03	1.22±0.01
ATA1	97.63±1.21	50.54±0.62
ATA2	64.53±10.52	33.41±5.45
ASCT1	21.11±4.05	10.93±2.10
MCT8	3.66±0.18	1.90±0.09
TAT1	2.12±0.16	1.10±0.09
B^0AT1	0.00±0.00	0.00±0.00

Values are shown as mean±S.D.

acids with branched or aromatic side chains, an alpha-amino group or alpha carboxyl group, except for L-tyrosine methyl ester [29].

We recently reported that the introduction of hydrophobic iodine into the benzene ring of L-tyrosine results in higher transport affinity, and neither the alpha-methyl group nor the size of the 3-iodinated-L-tyrosine residue was an obstacle to transport via LAT1 [29]. In addition, the possibility arises that beta-hydroxylation confers a reduction of transport affinity via LAT1, based on a comparison of the structure and inhibitory effect of L-DOPA and DOPS on [^{125}I]IMT transport.

Gabapentin, which is not an alpha-amino acid, is a substrate of LAT1, in addition to the alpha-amino acids [29]. Gabapentin, R(+)-baclofen and S(−)-baclofen are gamma amino acid analogues, and, interestingly, we observed a statistically weaker inhibitory effect of R(+)-baclofen and S(−)-baclofen on [^{125}I]IMT transport. Thus, whether gamma amino acid analogue transport is involved in LAT1 may depend on the structure of the group corresponding to the amino acid residue that combines with the beta-carbon.

With regard to the other amino acid transport systems, [^{125}I]IMT appears not to have an affinity for the cationic or anionic amino acid transport systems expressed in DLD-1, as none of the tested cationic or anionic amino acids in this study inhibited [^{125}I]IMT transport into DLD-1 cells. To date, four isoforms belonging to the human cationic amino acid transporter family (CAT family) are known. Human cationic amino acid transporter-1 is almost ubiquitously expressed and probably the most important entity for supplying cells with extracellular arginine, lysine and ornithine [32]. It is expressed endogenously in DLD-1 [32]. The expression profiles of the other cationic amino acid transport systems, system $b^{0,+}$ and system y^+L, are not known. Moreover, the expression in DLD-1 of the human anionic amino acid transport systems, system X^-_{AG} and system X^-_c, is also unknown. Numerous cell lines cultured in vitro express xCT, a component of the system X^-_c transporter family, and thus this system may be expressed in DLD-1 [33] as well, but [^{125}I]IMT may not have an affinity to xCT.

To study the characteristics of in vivo tumor imaging probes, it is essential to consider not only the gene level and cell or tissue level argument, but also the whole-body argument. It is known that nude mice that receive a subcutaneous inoculum of DLD-1 cells develop tumors histologically similar to colonic adenocarcinomas in 10 to 14 days [34]. We plan to study improvements in tumor imaging using colon cancer DLD-1 cells and DLD-1 tumor-bearing mice.

5. Conclusions

We used real-time PCR to detect the gene expression of system L (4F2hc, LAT1 and LAT2), system A (ATA1, ATA2) and system ASC (ASCT1). Weaker expression of system L (LAT3, LAT4) genes and the MCT8 gene was detected, while that of B^0AT was not detected. ^{125}I-IMT uptake into DLD-1 cells was inhibited by the substrates of system L (main) and system ASC (minor), but not of system A. In DLD-1 cells, the K_m and V_{max} corresponding to system L (78 μM and 333 pmol/10^6 cells per minute) were observed in Na^+-free buffer. Thus, [^{125}I]IMT uptake in DLD-1 cells involves primarily LAT1, and its substrates (including amino acid-like drugs derived from tyrosine, tryptophan and phenylalanine) inhibit [^{125}I]IMT uptake. Moreover, whether gamma amino acid analogue transport is involved in LAT1 depends on the structure of the group corresponding to the amino acid residue. The possibility exists that beta-hydroxylation of L-DOPA confers reduction of affinity to [^{125}I]IMT transport via LAT1.

Acknowledgments

We wish to thank Natsumi Mibuka, Yuki Kitamura, Yuzou Taguchi, Miho Aoyama, Hironobu Sakai, Misako Nozaki, Yuko Fujisaku, Mariko Maruyama and Takayoshi Miyakawa of Ibaraki Prefectural University for their excellent technical assistance.

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