Available online at www.sciencedirect.com
ScienceDirect
Nuclear Medicine and Biology 37 (2010) 189–196
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

Stimulation of ^{125}I-3-iodo-α-methyl-L-tyrosine uptake in Chinese hamster ovary (CHO-K1) cells by tyrosine esters

Naoto Shikano[a,*,1], Masato Ogura[a], Jun-ichi Sagara[a], Syuichi Nakajima[a], Masato Kobayashi[b], Takeshi Baba[a], Naoto Yamaguchi[a], Yukio Iwamura[a], Nobuo Kubota[a], Keiichi Kawai[b]

[a]*Department of Radiological Sciences, Center for Medical Sciences and Center for Humanities and Sciences, Ibaraki Prefectural University of Health Sciences, Inashiki-gun, Ibaraki, Japan*
[b]*Division of Health Science, Graduate School of Health Sciences, Kanazawa University, Kanazawa, Ishikawa, Japan*

Received 24 March 2009; received in revised form 17 September 2009; accepted 2 October 2009

Abstract

Introduction: Transport of the amino acid analog ^{123}I-3-iodo-α-methyl-L-tyrosine, which is used in clinical SPECT imaging, occurs mainly via L-type amino acid transporter type 1 (LAT1; an amino acid exchanger). As LAT1 is highly expressed in actively proliferating tumors, we made a preliminary investigation of the effects of amino acid esters on enhancement of ^{125}I-3-iodo-α-methyl-L-tyrosine (IMT) uptake via LAT1 in Chinese hamster ovary (CHO-K1) cells.

Methods: Because the sequence of the CHO-K1 LAT1 gene is not available, we confirmed LAT1 expression through IMT (18.5 kBq) uptake mechanisms using specific inhibitors. L-Gly, L-Ser, L-Leu, L-Phe, L-Met, L-Tyr, D-Tyr, L-Val and L-Lys ethyl/methyl esters were tested in combination with IMT. Time-course studies over a 3-h period were conducted, and the concentration dependence of L-Tyr ethyl and methyl esters (0.001 to 10 mM) in combination with IMT was also examined. For a proof of de-esterification of L- and D-Tyr ethyl and methyl esters in the cells (by enzymatic attack or other cause), the concentration of L- and D-Tyr was analyzed by high-performance liquid chromatography of the esters in phosphate buffer (pH 7.4) and cell homogenates at 37°C or under ice-cold conditions.

Results: Inhibition tests suggested that LAT1 is involved in IMT uptake by CHO-K1 cells. Co-administration of 1 mM of L-Tyr ethyl or methyl ester with IMT produced the greatest enhancement. The de-esterification reaction was stereo selective and temperature dependent in the homogenate. De-esterification kinetics were very fast in the homogenate and very slow in the phosphate buffer.

Conclusions: The L-Tyr ethyl or methyl esters were the most effective enhancers of IMT uptake into CHO-K1 cells and acted by trans-stimulation of the amino acid exchange function of LAT1. This result suggests that de-esterification in the cells may be caused by enzymatic attack. We will use IMT and L-Tyr ethyl or methyl esters to examine LAT1 function in tumor cells or tissues in vivo.

Keywords: Amino acid transport; Amino acid ester; Chinese hamster ovary cells; 3-iodo-α-methyl-L-tyrosine; In vitro study

1. Introduction

The rapid accumulation of amino acids in tumor cells enables active proliferation, and this fact has been exploited for the clinical imaging of tumors using ^{123}I-labeled 3-iodo-α-methyl-L-tyrosine by SPECT [1–5]. ^{125}I-3-iodo-α-methyl-L-tyrosine (IMT) has also been developed as a functional imaging agent for analysis of the tyrosine (Tyr) transport mechanisms in the brain and pancreas [6–8]. Understanding transport systems of amino acids forms the underlying basis for developing enhanced imaging systems.

The transport of neutral amino acids involves several distinct systems with overlapping substrate specificities [9]. The main Na^+-dependent transport systems are systems A and ASC (defined below), while the main Na^+-independent transport system is system L. System L is defined by its Na^+ independency and inhibition by 2-amino-bicyclo[2,2,1] heptane-2-carboxylic acid (BCH). System A is defined by its Na^+ dependency and inhibition by 2-(methylamino)

* Corresponding author. Department of Radiological Sciences, Ibaraki Prefectural University of Health Sciences, Inashiki-gun, Ibaraki 300-0394, Japan. Tel.: +81 298 40 2217; fax: +81 298 40 2317.
E-mail address: sikano@ipu.ac.jp (N. Shikano).
[1] Current address: Division of Health Science, Graduate School of Health Sciences, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, Ishikawa 9200-942, Japan.

0969-8051/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nucmedbio.2009.10.003

isobutyric acid (MeAIB), while system ASC is a Na^+-dependent transport system, which is intolerant to *N*-methylation of substrates. While systems L and A have specific inhibitors, system ASC does not [9].

At the molecular level, the genes of the proteins that transport substances are classified into various solute carrier (SLC) families according to the standards established by the Human Gene Nomenclature Committee [10–12]. System L is divided into two classes [13]: system L1 belonging to the SLC7 family and system L2 belonging to the SLC43 family. There are two known isoforms of system L1, namely, L-type amino acid transporter (LAT) types 1 and 2 (LAT1 and LAT2). The SLC7 family comprises two classes: the LAT family and the cationic amino acid transporter family [10–12]. To express transport function, LAT1 and LAT2 of system L1 require an additional protein, the heavy chain of 4F2 antigen (CD98; 4F2hc/SLC3A2), to form a heterodimeric functional complex. By obligatory exchange mechanisms, LAT1 and LAT2 selectively transport neutral amino acids. On the other hand, members of system L2, LAT3 and LAT4, do not require 4F2hc. These transporters possess broad substrate selectivity for various neutral amino acids. The expression patterns and the substrates of these transporters partly overlap among the systems but do differ [10–12].

It is noteworthy that LAT1 is conspicuously up-regulated in many tumors and transformed cell lines, which is consistent with the observed increase in uptake of amino acids needed for rapid cell growth and proliferation [14,15].

In the future, we aim to develop a technique for IMT and amino acid ester enhancement of amino acid uptake via the amino acid exchanger LAT1 in tumor tissue or cell samples from patients as an alternative to the current immunohistochemical quantification of transporter proteins. As a preliminary investigation was needed to develop this experimental system, we examined the mechanisms of IMT uptake in Chinese hamster ovary (CHO-K1) cells. Although transport of IMT has been better studied in other cell lines [16–20] than in CHO-K1, we chose this cell line as it has been well characterized [21] and would thus enable biological comparisons of the transport properties of artificial amino acid tracers. We modified the methods of Shotwell et al. [21] to examine the CHO-K1 cells. Because it should be possible to detect the isoform of the system L transporter that mediates IMT transport by assessing the inhibition profiles of the 20 D- or L-amino acids, we conducted an inhibition study using 20 naturally occurring amino acids including the mother amino acid, Tyr. To confirm the expression of LAT1 in human cells, RT-PCR should be used to detect the heavy chain of LAT1 mRNA, but homologues of CHO cells have not yet been sequenced. We investigated the effects of amino acid methyl/ethyl esters in combination with IMT on IMT accumulation in CHO-K1 cells. To confirm the extent of de-esterification, we analyzed the concentration of amino acids produced through hydrolysis of the esters in the cell homogenates.

2. Materials and methods

2.1. Materials and preparation of labeled compounds

CHO-K1 cells were purchased from the Riken Bioresource center (Tsukuba, Japan). Reagent-grade α-methyl-L-tyrosine was acquired from Sigma-Aldrich Japan K.K. (Tokyo, Japan). Instead of ^{123}I-labeled IMT, which is for clinical SPECT studies, ^{125}I-labeled IMT (IMT) was used. ^{125}I-NaI (8.1×10^7 GBq/mmol) was purchased from Muromachi Chemical Co. (Tokyo, Japan). Chloramine-T and other chemicals of reagent grade were purchased from Kanto Chemical Co. (Tokyo, Japan). No-carrier-added IMT was prepared by the conventional chloramine-T method, using α-methyl-L-tyrosine as a precursor, as previously reported [12–14].

2.2. Cell culture

Cell line studies were performed using a modification of the methods described by Shotwell et al. [21], as follows. CHO-K1 cells were maintained by serial passage in 25-cm^2 cell culture flasks. Cells were fed with Dulbecco's modified Eagle's medium (Sigma-Aldrich Japan K.K.) supplemented with L-glutamine (2 mM) and 10% fetal bovine serum without antibiotics in an atmosphere of 5% CO_2 and 95% air at 37°C (pH 7.4). Subculturing was performed every 5 days using 0.02% EDTA and 0.05% trypsin.

2.3. Measurement of IMT transport in CHO-K1 cells

CHO-K1 cells were seeded on 60-mm-diameter plastic tissue culture dishes at a cell density of 5×10^5 cells/dish in 5 ml of complete medium and were used on the fourth day after inoculation (semi-confluent phase cells). Uptake into cells of ^{11}C-methionine and IMT has been reported to differ considerably depending on whether the cells are in the growth phase or the confluent phase [11].

After removal of culture medium, each dish was washed once with 5 ml of incubation medium, which was Dulbecco's phosphate-buffered saline for 10 min at 37°C (pH 7.4). Uptake was measured in 2 ml of Na^+-containing incubation medium or in Na^+-free incubation medium as the normal control with 18.5 kBq of non-carrier-added IMT for 10 min at 37°C. The incubation medium was phosphate-buffered saline (PBS; pH 7.4) containing 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_2, 1 mM $CaCl_2$ and 0.5 mM MgCl, with pH adjusted by the addition of HCl or NaOH solution. In the Na^+-free medium, NaCl and Na_2HPO_4 normally present in PBS were replaced with choline chloride and K_2HPO_4, respectively.

For the inhibition study, the following inhibitors (at a concentration of 1.0 mM) were tested: MeAIB (a system A-specific inhibitor), BCH (a system L-specific inhibitor), *p*-aminohippurate (PAH, an organic anion transporter specific inhibitor), tetraethylammonium chloride (TEA, an organic cation transporter specific inhibitor), 2,4-dinitrophenol (DNP, an inhibitor of anaerobic mitochondrial energy

production), and sodium azide (NaN_3, an inhibitor of aerobic energy production). We also investigated the effect of low temperature (4°C) [20,22–25].

To identify the system L transporter isoform that mediates IMT transport, test inhibition was conducted with each of the 20 D- or L-amino acids (final concentration, 1.0 mM) [21], and ^{125}I radioactivity was determined.

2.4. Screening effective amino acid esters and optimizing concentration and incubation duration for enhanced IMT transport

To identify effective amino acid esters and their optimal concentration and incubation duration for enhanced IMT transport, cells were incubated in Na^+-containing medium at 37°C for 1 h (except in the time-course analysis) with 18.5 kBq of non-carrier-added IMT and one of the following: (1) 1.0 mM of the ethyl/methyl ester chloride salts L-Gly, L-Ser, L-Leu, L-Phe, L-Met, L-Tyr, D-Tyr, L-Val and L-Lys; (2) 1 mM of the ethyl and methyl esters of L-Met and L-Tyr for 5, 15, 30, 45, 60, 120 or 180 min for time-course analysis; (3) 0.001 to 10 mM L-Tyr ethyl and methyl esters for concentration dependence analysis; and (4) preloading the L-Tyr esters so that esters are present 10 or 30 min prior to the start of incubation and not during the incubation for time-course analysis for 5, 10, 15, 30, 45 or 60 min. For all experiments, ^{125}I radioactivity was determined as below.

2.5. Determination of radioactivity

Following the conclusion of incubations, the medium was aspirated and the cells were rinsed twice in rapid succession with 5 ml of ice-cold incubation medium. Cells were solubilized in 1.5 ml of 1 N NaOH, and the radioactivity of each aliquot was counted. The ^{125}I radioactivity of the solubilized cells was determined using an ARC-1000M well-type scintillation counter (Alokam, Tokyo, Japan). Cell numbers were counted simultaneously on a subcultured dish using a Coulter counter (Coulter Counter Electronics, Inc., London, England).

2.6. Confirmation of de-esterification of L- and D-Tyr ethyl and methyl esters in the cell homogenates

All subsequent steps were conducted at 4°C. To prepare the cell homogenates, cells in the 60-mm dishes were scraped out with a rubber policeman, and the cells were homogenized with five volumes of ice-cold 3-mM Tris buffer (pH 7.4) supplemented with 0.25 M sucrose and 0.1 mM EDTA. The homogenates were centrifuged for 10 min at 9,000×*g*, and the pellets were discarded. The supernatants were centrifuged at 105,000×*g* for 60 min, and microsomal pellets were discarded. The resulting cytosolic fractions were stored at −20°C and used in the de-esterification study [26].

We studied de-esterification of L- and D-Tyr ethyl and methyl esters of the cells in the cell homogenates at 37°C and on ice in the Tris buffer (see above). De-esterification was studied using cell homogenates at 37°C in a time-course study for 0, 30 or 60 min. Concentrations of L- and D-Tyr in the homogenates were analyzed by HPLC with the following conditions: Nova-Pak C18; UV detection at 280 nm; eluent, water:ethanol:acetic acid (7:3:1); flow rate, 0.5 ml/min. The retention times were as follows: L- and D-Tyr, 4.0–4.3 min; L- and D-Tyr ethyl esters, 5–6 min; L- and D-Tyr methyl esters, 4.5–4.75 min.

2.7. Statistical analysis

Data were collated as mean±standard deviation of five measurements, and each experiment was performed in duplicate. Results were analyzed using Student's *t* test, and $P<.01$ was considered to be statistically significant.

3. Results

The contribution of individual transport systems to total uptake of IMT is shown by the differences in IMT uptake (Fig. 1). Low temperature inhibited IMT uptake, suggesting that uptake is carrier mediated. The Na^+-independent transport system was the main contributor to IMT uptake. These carriers could be energy independent because neither DNP nor NaN_3 inhibited IMT uptake. Moreover, neither PAH nor TEA inhibited IMT uptake, suggesting that organic anion and organic cation transport systems were not involved. However, BCH significantly inhibited uptake, suggesting that IMT is a substrate of system L. IMT was not a substrate of system A because inhibition of IMT uptake with MeAIB was not significant. IMT appears to be a substrate of system ASC as well as system L, but not to a significant degree.

As shown in Fig. 2, IMT uptake was inhibited by both L- and D-neutral amino acids (Ala, Ser, Thr, Cys, Leu, Ile, Phe, Met, Tyr, His, Trp Val and Asn) in CHO-K1 cells. In

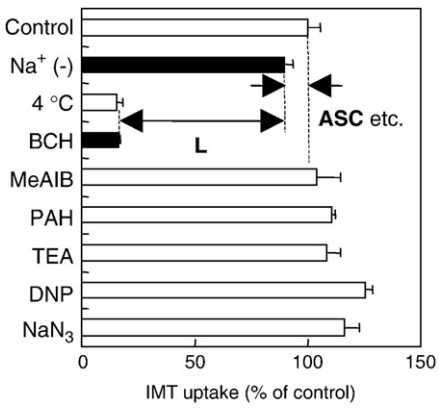

Fig. 1. Effects of various inhibitors and conditions on non-carrier-added IMT transport into CHO-K1 cells. Contributions of individual transport systems to total uptake of non-carrier-added IMT. Open and filled columns represent uptake of non-carrier-added IMT into CHO-K1 cells in Na^+-containing and Na^+-free uptake medium, respectively. *$P<.005$, **$P<.0001$ versus control.

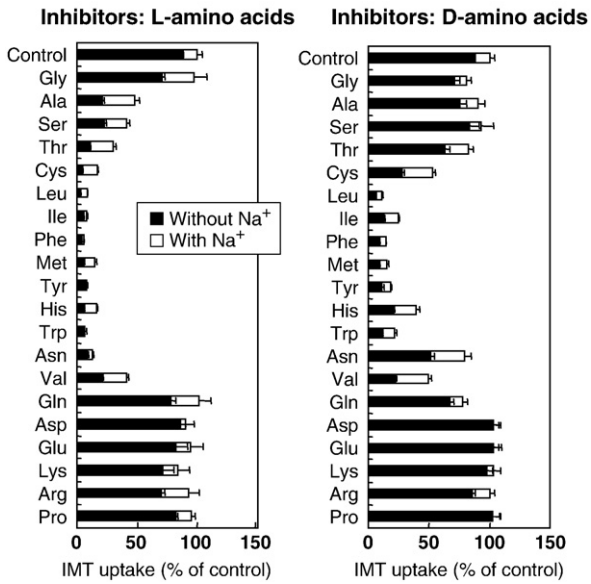

Fig. 2. Effects of natural L- and D-amino acids (1 mM) on non-carrier-added IMT transport into CHO-K1 cells. Open and filled columns represent uptake of non-carrier-added IMT into CHO-K1 cells in Na^+-containing and Na^+-free uptake medium, respectively.

contrast, the D-amino acids Ala, Ser and Thr had weaker or statistically insignificant inhibitory effects on IMT uptake. The L-isomers demonstrated higher inhibitory effects than the D-isomers.

Of the tested ethyl/methyl ester chloride salts co-administered with IMT, IMT uptake was significantly increased ($P<.001$) by the ethyl esters of L-Tyr, L-Phe and L-Ser (Fig. 3A) and the methyl esters of L-Tyr, L-Ser and L-Lys (Fig. 3B). For the L- and D-Tyr esters, the L-isomers enhanced uptake to a greater degree than the D-isomers (Fig. 3C).

In the time-course study with the ethyl and methyl esters of L-Tyr, both L-Tyr esters demonstrated significantly greater uptake after 15 to 180 min (Fig. 4A). On the other hand, the ethyl and methyl esters of L-Met did not increase IMT uptake. Co-administration of 1 mM of the L-Tyr ethyl or methyl ester with IMT induced the greatest enhancement (Fig. 4B). Preloading of the L-Tyr esters caused earlier uptake enhancement (Fig. 4C) as compared with simultaneous loading (Fig. 4A). Longer preloading interval produced a longer and enhanced steady state.

De-esterification kinetics of D-Tyr ethyl and methyl esters were very slow under the same conditions compared to L-Tyr

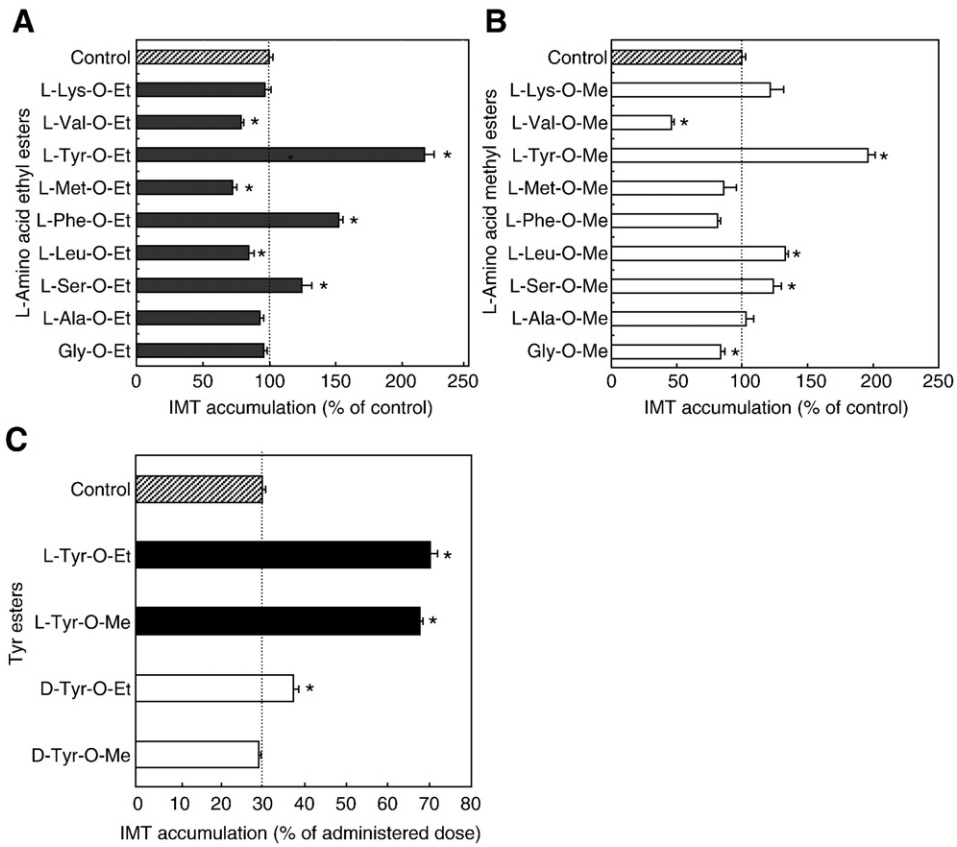

Fig. 3. Influence of amino acid esters co-administered with IMT. One millimolar of amino acid ethyl esters (A), methyl esters (B) and Tyr esters (C) on uptake of non-carrier-added IMT into CHO-K1 cells. *$P<.01$ versus uptake when not incubated in amino-acid-free medium, **not significant versus uptake when not incubated in amino-acid-free medium.

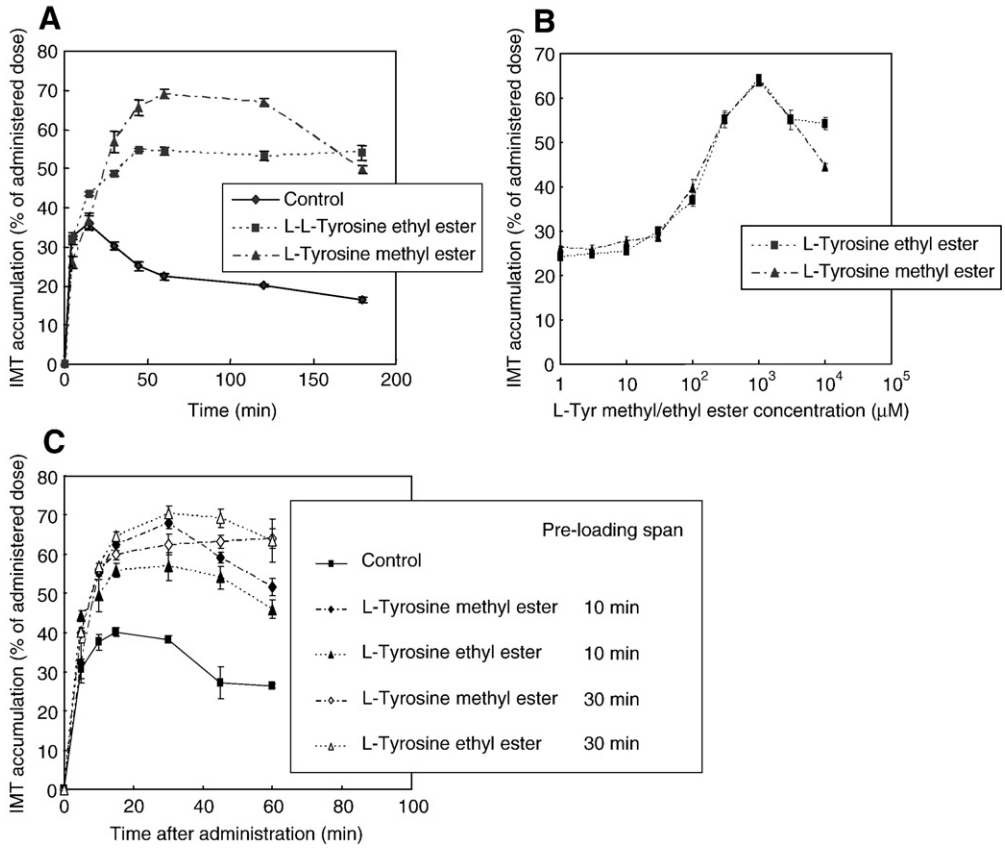

Fig. 4. Time course of uptake into CHO-K1 cells of non-carrier-added IMT co-administered with 1 mM L-Tyr ethyl ester or L-Tyr methyl ester (A), concentration dependence (B) and preloading of the esters: that is, esters present in preloading and not in incubation conditions (C).

ethyl and methyl esters (Fig. 5A and B). De-esterification kinetics of L-Tyr ethyl and methyl esters were very fast in the homogenates at 37°C (pH 7.4) and slow in ice-cold homogenates and the phosphate buffer (pH 7.4; Fig. 5C and D). Thus, the reaction was stereo selective and temperature dependent.

4. Discussion

There is no published data describing an in vitro method to observe amino acid exchanger function by enhancement of amino acid tracer uptake in tissue or cell samples—for example, by using IMT in combination with an amino acid ester. We believe that the advantage of such a method would be "function enhancement reflecting the malignancy of the tumor" [27,28] compared to methods such as immunohistochemical quantification of transporter protein using LAT1 antibody. The development of such a method is our next goal and, to that end, the focus of this preliminary study was the interventional control of IMT uptake in vitro in a cell line in order to enhance IMT uptake by stimulation of the amino acid exchanger function.

The magnitude of system L and A amino acid transporter expression reportedly indicates tumor malignancy [27,28]. We selected a system L amino acid exchanger as the target transporter and attempted to enhance its function with trans-stimulation of neutral amino acid exchange by an amino acid methyl or ethyl ester. As shown in Fig. 6, we proposed to use one of the methyl or ethyl esters of Tyr with IMT, on the basis that a methyl or ethyl ester of Tyr might act as a prodrug for the trans-stimulation of neutral amino acid exchange by the system L amino acid exchanger. Accordingly, we hoped that these lipophilic esters would diffuse into cells, allowing the ester to act as a prodrug, which could be metabolized into an amino acid by intracellular hydrolysis enzyme(s). We expected that, compared to amino acid preloading, amino acid ester loading would cause very little competitive inhibition of IMT influx via the amino acid transporter. Esterification of amino acids could mask the competitive inhibition of IMT uptake (influx transport). LAT1, a system L transporter, uses an amino acid gradient to activate exchange of intracellular amino acids generated from the prodrug for extracellular IMT. We also anticipated that the prodrug would have a durable effect within the time span of hydrolysis. The findings shown in Fig. 5 suggest that de-esterification in the cells is caused by stereo-selective, temperature-dependent enzymatic attack.

Membrane transport of IMT has already been relatively well studied with other cell lines — cultured human 86HG-

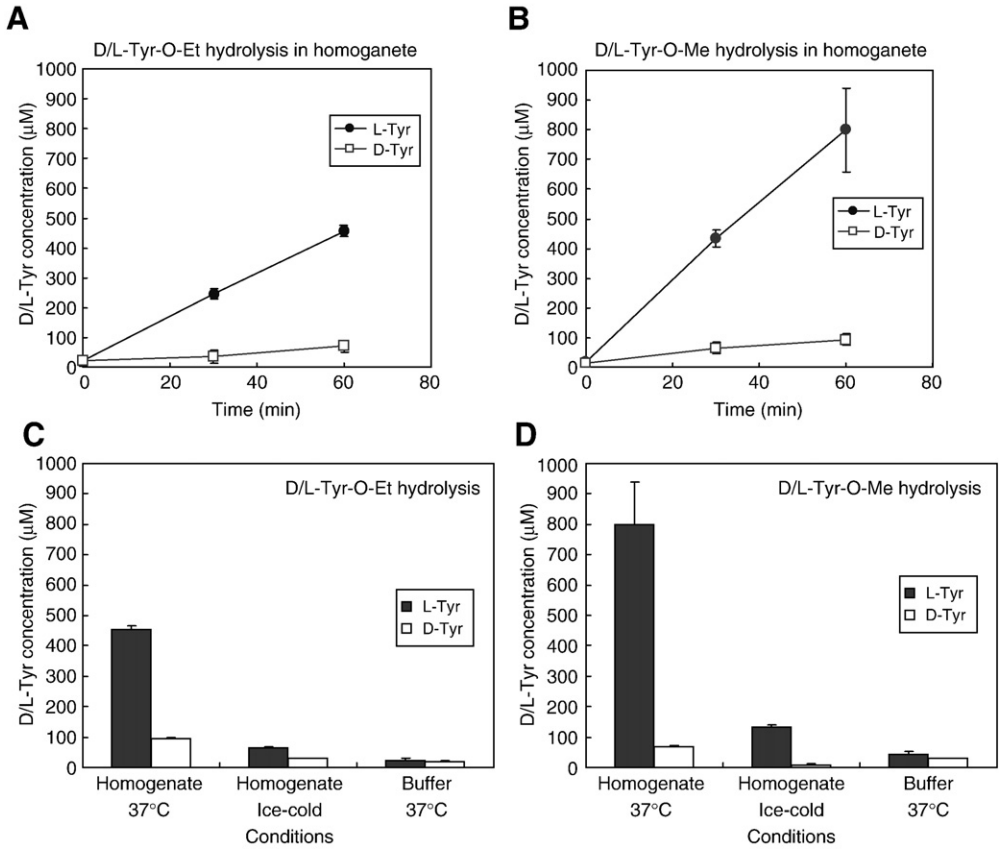

Fig. 5. De-esterification of L- and D-Tyr ethyl and methyl esters in the cell homogenates. Time-course study on de-esterification of 1 mM D/L-Tyr ethyl ester (A) or L-Tyr methyl ester (B) in the cell homogenates at 37°C. The de-esterification of L- and D-Tyr ethyl (C) and methyl (D) esters in the cells in cell homogenates at 37°C or at ice-cold conditions and in a 3-mM Tris buffer (pH 7.4).

39 glioma cells [16], rat C6 glioma cells [17], human GOS3 glioma cells [18], human Ewing's sarcoma cells [19] and human U266 myeloma cells [20] — but has not been characterized with CHO-K1. Although IMT is reportedly primarily mediated by system L in most tissues [4], it is important to determine what kind of membrane transport mediates IMT transport; therefore, we sought to verify the mechanisms of IMT uptake into CHO-K1 cells. The present results with CHO-K1 demonstrate that IMT uptake is predominantly (>70%) mediated by a temperature-dependent and energy-independent carrier, system L. Na^+-dependent systems are responsible for a relatively minor contribution to transport (<20%); IMT appears to be a substrate of system ASC but not to a significant extent. Neither system A nor organic anion and cation transport systems contribute to IMT uptake into CHO-K1 cells. As indicated by the inhibition profile shown by neutral L- and D-amino acids under Na^+-free conditions, the system L transport of IMT in CHO-K1 cells most likely involves LAT1 [29,30].

IMT uptake was significantly increased by the ethyl esters of L-Tyr, L-Phe and L-Ser and by the methyl esters of L-Tyr, L-Ser and L-Lys in the tested candidate amino acid esters. The most effective amino acid esters were the methyl and ethyl esters of L-Tyr. Generally, the esters of the aromatic amino acids were more effective than those of other amino acids such as L-Ser or L-Lys, successfully demonstrating a significant increase in IMT uptake after 15 to 180 min. The accumulation of IMT was effectively enhanced by the L-Tyr esters but not by the corresponding D-isomers, indicating that enzymes may be involved in the hydrolysis of L-Tyr ethyl/methyl esters. It is unclear why the L-Met ethyl and methyl esters did not induce activated IMT uptake; however, one possible reason is the velocity of hydrolysis of the L-Met ethyl and methyl esters in the cells and the affinity of the generated L-Met with the counter transport system. As shown in Fig. 4B, the effective interventional ester levels on IMT uptake in vitro were estimated to range from 0.1 to 1 mM for the L-tyrosine methyl/ethyl esters. However, IMT uptake decreased at excess ester concentration (over 1 mM). In Fig. 4A, the control shows a curve typical for depletion of the cells during the experiment, as LAT1 influx and efflux is reversible, showing a steady state. This can be due to the pre-incubation followed by incubation in absence of necessary amino acids.

As shown in Fig. 5, de-esterification kinetics of L-Tyr methyl ester were 1.8-fold faster than that of ethyl ester. De-esterification in cells may be approximately six times faster

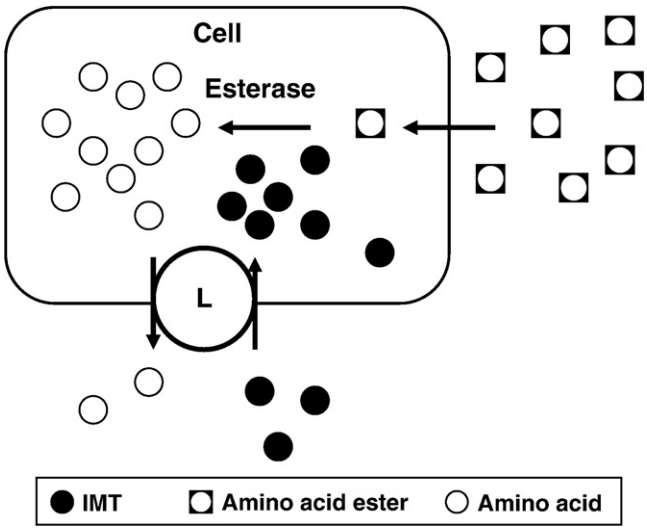

Fig. 6. Proposed mechanism of IMT uptake activation into cells by system L amino acid exchanger: ◘, amino acid esters penetrate the cell membrane as a result of the membrane's lipophilicity; ○, amino acids are metabolized by esterase within the cells; ●, IMT (system L substrates). System L amino acid exchanger function is stimulated by amino acids due to amino acid methyl or ethyl ester. The lipophilic amino acid esters could diffuse into cells, allowing the esters to act as prodrugs, which would be metabolized into amino acids by intracellular hydrolysis enzymes. A system L transporter uses an amino acid gradient for obligatory exchange of intracellular amino acids generated from the prodrugs for extracellular IMT.

than that in homogenates diluted to 1/6 cell cytosolic concentration by 3-mM Tris buffer. Because ethanol is less toxic than methanol, L-Tyr ethyl ester is a better candidate.

As shown in Fig. 4A and C, the timing of reaching the steady state of IMT accumulation may depend on when and how long the ester is loaded. Magnitude of the enhancement of preloading was similar to that of simultaneous loading. Longer preloading interval produced a longer and enhanced steady state. Preloading of L-Tyr esters caused earlier uptake enhancement as compared with simultaneous loading.

We think that the results of this study give us very important suggestions, not only for diagnosis methods but also for potential therapies with ^{131}I-labeled amino acids. However, in the in vivo situation, amino acid esters may be difficult to use because the amino acid esters would be distributed and hydrolyzed in the cells of most tissues as well as tumors, and then some kind of transport system on the cell surface would most likely carry the de-esterified amino acids out of the cell. As a result, a portion of the amino acids from the interventional amino acid esters might enter the circulation. There is also the possibility of an inhibitory effect of de-esterified amino acids on IMT uptake by in vivo tumors. The inhibitory effect of intrinsic amino acids on the intervention in the vitro study may be less than that in an vivo study.

In the clinical setting, patient preparation and interventions may improve amino acid imaging as flowing. Increased tumor uptake, by threefold, of ^{11}C-5-hydroxytryptophan by neuroendocrine tumors after administration of carbidopa was observed by a group from Uppsala [31]. This was presumably caused by inhibition of peripheral metabolism (decarboxylation), which leads to prolonged and higher exposure of the tracer to the tumor. Prior amino acid administration increases IMT tumor accumulation and image contrast. This effect can be explained by the increased antiporter activity of the amino acid transport system L in preloaded conditions [32]. Recently, we examined the fundamental changes in the pharmacokinetics of IMT after probenecid loading in mice implanted with colon cancer DLD-1 cells using IMT. We found that preloading with probenecid yields better tumor images with good tumor/normal tissue radioactivity ratios by blocking IMT excretion from the kidney [33].

In conclusion, to propose a method to quantify amino acid uptake function via amino acid exchanger LAT1, which indicates tumor malignancy, we conducted a preliminary study on the effect of amino acid esters in combination with IMT on accumulation in CHO-K1 cells. LAT1 was selected as the target transporter to enhance the amino acid exchanger function with trans-stimulation by amino acid esters because it is the main contributor to IMT uptake. IMT uptake was significantly increased by the ethyl esters of L-Tyr, L-Phe and L-Ser and by the methyl esters of L-Tyr, L-Ser and L-Lys (in fact, uptake was increased by two- to threefold with the L-Tyr methyl/ethyl esters). Thus, the L-Tyr methyl/ethyl esters were the most effective compounds for enhancing IMT uptake. We are planning to use L-Tyr ethyl ester and other related compounds (e.g., dipeptides) co-administered with IMT to enhance amino acid uptake for in vitro cell examination of tumor tissue.

Acknowledgments

We wish to thank Akihiro Imura and Shinya Nakazawa (Ibaraki Prefectural University) for their excellent technical assistance. 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 for the Encouragement for Young Scientists 2001, 2002, 2004, 2005, 2006 and 2008; and Japan Atherosclerosis Research Foundation Grant 2008.

References

[1] Biersack HJ, Coenen HH, Stoecklin G, Reichmann K, Bockische A, Oehr P, et al. Imaging of brain tumors with L-3-[I-123]iodo-α-methyl tyrosine and SPECT. J Nucl Med 1989;30:110–2.

[2] Kuwert T, Woesler B, Morgenroth C, Lerch H, Schafers M, Palkovic S, et al. Diagnosis of recurrent glioma with SPECT and iodine-123-α-methyl tyrosine. J Nucl Med 1998;39:23–7.

[3] Jager PL, Franssen EJF, Kool W, Szabo BG, Hoeckstra HJ, Groen HJM, et al. Feasibility of tumor imaging using L-3-[iodine-123]-iodo-alpha-methyl-tyrosine in extracranial tumors. J Nucl Med 1998;39: 1736–43.
[4] Langen KJ, Pauleit D, Coenen HH. 3-[^{123}I]Iodo-α-methyl-L-tyrosine: uptake mechanisms and clinical applications. Nucl Med Biol 2002;29: 625–31.
[5] Langen KJ, Muhlensiepen H, Holschibach M, Hautzel H, Jansen P, Coenen HH. Transport mechanisms of 3-[^{123}I]iodo-α-methyl-L-tyrosine in a human glioma cell line: comparison with [^{3}H-methyl]-L-methionine. J Nucl Med 2004;41:1250–5.
[6] Kawai K, Fujibayashi Y, Saji H, Yonekura Y, Konishi J, Kubodera A, et al. A strategy for study of cerebral amino acid transport using iodine-123-labeled amino acid radiopharmaceutical: 3-iodo-alpha-methyl-L-tyrosine. J Nucl Med 1991;32:819–24.
[7] Kawai K, Fujibayashi Y, Yonekura Y, Konishi J, Saji H, Kubodera A, et al. An artificial amino acid radiopharmaceutical for single photon emission computed tomographic study of pancreatic amino acid transports ^{123}I-3-iodo-alpha-methyl-L-tyrosine. Ann Nucl Med 1992;6: 169–75.
[8] Kawai K, Fujibayashi Y, Yonekura Y, Tanaka K, Saji H, Konishi J, et al. Canine SPECT studies for cerebral amino acid transport by means of ^{123}I-3-iodo-α-methyl-L-tyrosine and preliminary kinetic analysis. Ann Nucl Med 1995;9:47–50.
[9] Christensen HN. Role of amino acid transport and counter-transport in nutrition and metabolism. Physiol Rev 1990;70:43–77.
[10] Palacin M, Kanai Y. The ancillary proteins of HATs: SLC3 family of amino acid transporters. Pflugers Arch 2004;447:490–4.
[11] Kanai Y, Hediger MA. The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. Pflugers Arch 2004;447:469–79.
[12] Verrey F, Closs EI, Wagner CA, Palacin M, Endou H, Kanai Y. CATs and HATs: the SLC7 family of amino acid transporters. Pflugers Arch 2004;447:532–42.
[13] Weissbach L, Handlogten ME, Christensen HN, Kilberg MS. Evidence of two Na-independent neutral amino acid transport systems in primary cultures of rat hepatocytes. J Biol Chem 1982;256:12006–11.
[14] Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H. Expression cloning and characterization of a transporter for large neutral amino acids activated by heavy chain of 4F2 antigen (CD98). J Biol Chem 1998;273:23629–32.
[15] Yanagida O, Kanai Y, Chairoungdua A, Kim DK, Segawa H, Nii T. Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. Biochim Biophys Acta 2001;1514:291–302.
[16] Reimann B, Stoegbauer F, Kopka K, Halffer H, Lasic M, Schirmachr A, et al. Kinetics of 3-[^{123}I]iodo-L-α-methyl tyrosine transport in rat C6 glioma cells. Eur J Nucl Med 1999;26:1274–8.
[17] Riemann B, Kopka K, Stogbauer F, Halfter H, Ketterler S, Vu Phan TQ, et al. Kinetic parameters of 3-[^{123}I]iodo-L-α-methyl tyrosine ([^{123}I]IMT) transport in human GOS3 glioma cells. Nucl Med Biol 2001;28:293–7.
[18] Franzius C, Kopka K, Valen F, Eckervogt V, Riemann B, Sciuk J. Characterization of 3-[^{123}I]iodo-L-α-methyl tyrosine transport ([^{123}I] IMT) transport into human Ewing's sarcoma cells in vitro. Nucl Med Biol 2001;28:123–8.
[19] Lahoutte T, Caveliers V, Dierickx L, Vekeman M, Everaert H, Mertens J, et al. In vitro characterization of the influx of 3-[^{125}I]iodo-L-α-methyl tyrosine and 2-[^{125}I]iodo-L-tyrosine into U266 human myeloma cells: evidence for system T transport. Nucl Med Biol 2001;28:129–34.
[20] Prante O, Deichen JT, Hocke C, Kuwert T. Characterization of uptake of 3-[^{131}I]iodo-alpha-methyl-L-tyrosine in human monocyte-macrophages. Nucl Med Biol 2004;3:365–72.
[21] Shotwell MA, Jayme DW, Kilberg MS, Oxender DL. Neutral amino acid transport systems in Chinese hamster ovary cells. J Biol Chem 1981;256:5422–7.
[22] Hayer M, Bonisch H, Bruss M. Molecular cloning, functional characterization and genomic organization of four alternatively spliced isoforms of the human organic cation transporter 1 (hOCT1/ SLC22A1). Ann Hum Genet 1999;63:473–82.
[23] Fukumoto M, Kurohara A, Yoshimura N, Yoshida D, Akagi N, Yoshida S. Relationship between ATP synthesis and ^{201}Tl uptake in transformed and non-transformed cell lines. Nucl Med Commun 1998; 19:1169–75.
[24] Trinchieri G, De Marchi M. Antibody-dependent cell-mediated cytotoxicity in humans. II. Energy requirement. J Immunol 1975; 115:256–60.
[25] Shikano N, Kawai K, Nakajima S, Nishii R, Flores II LG, Kubodera A, et al. Renal accumulation and excretion of radioiodinated 3-iodo-α-methyl-L-tyrosine. Ann Nucl Med 2004;18:225–32.
[26] Sagara J, Sugita Y. Characterization of cytosolic glutathione *S*-transferase in cultured astrocytes. Brain Res 2002;902:190–7.
[27] Kobayashi K, Ohnishi A, Promsuk J, Shimizu S, Kanai Y, Shiokawa Y, et al. Enhanced tumor growth elicited by L-type amino acid transporter 1 in human malignant glioma cells. Neurosurgery 2008;62: 493–503.
[28] Kondoh N, Imazeki N, Arai M, Hada A, Hatsuse K, Matsuo H, et al. Activation of a system A amino acid transporter, ATA1/SLC38A1, in human hepatocellular carcinoma and preneoplastic liver tissues. Int J Oncol 2007;31:81–7.
[29] Shikano N, Kanai Y, Kawai K, Ishikawa N, Endou H. Characterization of 3-[^{125}I]iodo-α-methyl-L-tyrosine transport via human L-type amino acid transporter 1. Nucl Med Biol 2003;30:31–7.
[30] Shikano N, Kanai Y, Kawai K, Inatomi J, Kim do K, Ishikawa N, et al. Isoform selectivity of 3-^{125}I-iodo-alpha-methyl-L-tyrosine membrane transport in human L-type amino acid transporters. J Nucl Med 2003; 44:244–6.
[31] Eriksson B, Bergstrom M, Orlefors H, Sundin A, Langstrom B. Use of PET in neuroendocrine tumors: in vivo applications and in vitro studies. Q J Nucl Med 2000;44:68–76.
[32] Lahoutte T, Caveliers V, Franken PR, Bossuyt A, Mertens J, Everaert H. Increased tumor uptake of 3-^{123}I-iodo-L-alpha-methyltyrosine after preloading with amino acids: an in vivo animal imaging study. J Nucl Med 2002;43:1201–6.
[33] Nakajima S, Shikano N, Kotani T, Ogura M, Nishii R, Yoshimoto M, et al. Pharmacokinetics of 3-[^{125}I]iodo-α-methyl-L-tyrosine, a tumor imaging agent, after probenecid loading in mice implanted with colon cancer DLD-1 cells. Nucl Med Biol 2007;34:1003–8.