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Original Article

Increased D-[*S*-methyl-^{14}C]-methionine after Co-loading of L-tyrosine ethyl ester in C6 Glioma Cells

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Abstract

Introduction: We investigated whether trans-stimulation by amino acid ethyl ester co-loading increases D-[*S*-methyl-^{14}C]-methionine (D-[^{14}C]-Met) uptake into C6 glioma cells.

Methods: Effective amino acid ethyl esters for enhancing D/L-[^{14}C]-Met transport were determined by incubating cells at 37°C for 1 h in Na^+-containing medium together with 18.5 kBq of non-carrier-added D/L-[^{14}C]-Met and 1.0 mM of the ethyl ester chloride salt of Gly, L-Arg, L-Ser, L-Leu, L-Met, L-Tyr, L-Val, L-Lys or L-Pro. Similarly, for the time course study of D/L-[^{14}C]-Met uptake with L-Tyr-OEt, the cells were incubated for 5, 10, 30, 60 and 90 min. In addition, the effects of MeAIB (a system A-specific inhibitor) and BCH (a system L-specific inhibitor) at 1.0 mM were determined. Radioactivity was measured using a liquid scintillation counter.

Results: L-Tyr-OEt most effectively enhanced the uptake of both D- and L-[^{14}C]-Met: 4.7-fold for D-[^{14}C]-Met, and 2.5-fold for L-[^{14}C]-Met. The highest uptake value of D-[^{14}C]-Met with L-Tyr-OEt was observed 90 min or more after the start of incubation. System L was primarily responsible for mediating D/L-[^{14}C]-Met transport, and System A, which is not an amino acid exchanger, contributed to D-[^{14}C]-Met transport.

Conclusions: Trans-stimulation by L-Tyr-OEt co-loading increased D-[^{14}C]-Met uptake into C6 glioma cells in vitro.

Key words: D-[S-methyl-^{14}C]-methionine, L-tyrosine ethyl ester, trans-stimulation, system L, C6 glioma cell

1. Introduction

Neutral amino acids are transported by three main systems, systems L, A, and ASC, that have overlapping substrate specificities.[1,2)] System L is defined by being Na^+-independent and by its inhibition by 2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH). System A is defined by its Na^+ dependence and inhibition by 2-(methylamino)isobutyric acid (MeAIB), and system ASC is Na^+-dependent and intolerant of N-methylated substrates. Systems L and A have specific inhibitors, whereas system ASC does not.[1,2)] Kanai et al. previously identified a system L amino acid exchanger, LAT1 (L-type amino acid transporter 1: system L transporter isoform), in C6 rat glioma cells and demonstrated that LAT1 requires 4F2 heavy chain (4F2hc) for its functional expression.[3)]

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Expression of LAT1 in fetal rat liver and tumors suggests that LAT1 plays a critical role in cell growth and proliferation. The degree of LAT1 and ATA1 (amino acid transporter A1: system A transporter isoform) expression reportedly indicates the extent of tumor malignancy.[4,5] Most transporters can only transport compounds of similar structure, whereas LAT1 can transport essential amino acids and many functionally designed non-natural amino acids.[6-8] Four isoforms of system L transporter, LAT1 to 4, are known.[9] Of these, LAT1 and LAT2 are system L1 transporters and are amino acid exchangers. *Trans*-stimulation by administration can occur in LAT1 and LAT2, because they are amino acid exchangers.[9] *Trans*-stimulation by administration cannot occur in LAT3 and LAT4, which are system L2 transporters, because they are not amino acid exchangers.[9] The system L and ASC amino acid transporters LAT1 and ASCT-2 play roles in counter transport[10] and are very important for processes that use large amounts of amino acids, such as tumor growth and proliferation.[10] In contrast, system A transporters do not play roles in counter transport, because they are not amino acid exchangers.

To address the question "How can tumor uptake of amino acids be manipulated and improved?", Jager *et al*. commented that the main point is to find a tracer with high specific uptake in tumor tissue (first approach) and to look for methods to improve tumor uptake or improve contrast with the background (second approach).[11] The second approach comprises two tactics: manipulating the transport system (influx and especially efflux mechanisms), or manipulating the kinetics of amino acid metabolism. Lahoutte *et al*. found that tumors with pre-loaded natural amino acids increased $^{125/123}$I-3-iodo-alpha-methyl-L-tyrosine (IMT) tumor uptake and increased contrast in an *in vivo* animal imaging study.[12] These findings led us to investigate methods for enhancing the accumulation of drugs via LAT1 for improving diagnosis and treatment, and thus we attempted to enhance the function of LAT1 for the *trans*-stimulation of neutral amino acid exchange using an amino acid ethyl ester as a prodrug.[13,14]

We previously observed that L-tyrosine ethyl ester stimulated the uptake of IMT by CHO-K1 cells,[13] which are not tumor cells. However, it remained unclear whether trans-stimulation using L-tyrosine ethyl ester affects LAT1-mediated uptake of the D-isomer of [^{14}C]-methionine into tumor cells, such as C6 glioma cells. There are no published data describing an *in vitro* method for observing the amino acid exchange function for especially D-isomer amino acids. For example, there is no current method for enhancing D-[*S*-methyl-^{14}C]-methionine (D-[^{14}C]-Met) uptake or its trans-stimulation in C6 glioma cells in combination with an amino acid ester.

In this study, we investigated the effectiveness of *trans*-stimulation by co-loading of an amino acid ester on D-isomer amino acid uptake, and we determined the most effective amino acid ester for enhancing the accumulation of D-[^{14}C]-Met and L-[^{14}C]-Met in C6 glioma cells. Because D/L-[^{11}C]-Met are clinically most popular labeled essential amino acids in positron emission computed tomography (PET) study today, and it is easy to radiolabel. The results of this study could be useful for developing molecular imaging and cancer treatment agents based on D-amino acid.

2. Materials and methods

2.1. Materials

D-[^{14}C]-Met (2.22 GBq/mmol) and L-[^{14}C]-Met (2.22 GBq/mmol) were purchased from American Radiolabeled Chemicals Co. (St. Louis, MO, USA). C6 (rat glial cell tumor) cells were purchased from JCRB (Osaka, Japan). Plastic tissue culture dishes (60 mm diameter) and plastic culture flasks (surface area 25 cm^2) were purchased from Nalge Nunc International (Rochester, NY, USA). 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-amino acids, and reagent grade chemicals were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

2.2. Cell culture

C6 cells (10^5 cells/dish) were incubated in 60-mm dishes with 5 ml of Dulbecco's modified Eagle's medium (DMEM: Cat. No. D5796; Sigma Chemical Co., St. Louis,

MO, USA) containing 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere (pH 7.4). The cells were sub-cultured every 5 days using 0.02% ethylenediamine tetraacetic acid (EDTA) and 0.05% trypsin. Cells were used for experiments on the 3rd or 4th day after sub-culturing. A cell proliferation curve was prepared to determine the duration of the logarithmic growth phase.

2.3. Radioactivity measurements for screening amino acid esters

When the C6 cells reached approximately 80% confluence (3–4 days after sub-culturing), the medium was replaced with 5 ml of phosphate-buffered saline (PBS) containing Na^+ (37°C, pH 7.4) and the cells were incubated at 37°C for 10 min. Effective amino acid esters, their optimal concentration, and the duration of incubation required for enhanced D/L-[^{14}C]-Met transport were determined as follows. First, the cells were incubated at 37°C for 1 h in PBS with 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) containing 18.5 kBq of non-carrier-added D/L-[^{14}C]-Met and 1.0 mM of the ethyl ester chloride salt of Gly, L-Arg, L-Ser, L-Leu, L-Met, L-Tyr, L-Val, L-Lys or L-Pro. Ethyl esters of system L transport substrates and several additional amino acid esters were chosen for screening effective compounds. Next, the medium was removed, the cells were washed twice with 5.0 ml of cold PBS, and then the cells were solubilized using 2.0 ml of NaOH (2 N). The radioactivity of each aliquot (200µl) was measured using a liquid scintillation counter (Perkin Elmer, Waltham, MA, USA, Tri-Carb 2910TR). Data are reported as the mean of the results from four dishes for each set of experimental conditions.

We used PBS uptake solution containing sodium because it does not contain nutrients and other factors found in culture medium. Consequently, if we incubate cells in PBS for more than 90 min, the uptake data should reflect the influence of cell starvation, possibly resulting in autophagy. Furthermore, L-[^{14}C]-Met has low metabolic stability and thus the radioactivity values will include the effects of radioactive metabolites. Uptake experiments should be conducted in culture medium to maintain the cells in good condition for an extended time but neutral amino acids contained as nutrients in the uptake solution affect D/L-[^{14}C]-Met uptake. Consequently, we used PBS containing sodium as the uptake solution in this study, consistent with the sodium found in animals used in *in vivo* experiments.

2.4. Time course of D/L-[^{14}C]-Met uptake with L-Tyr-OEt

To determine the time course of D/L-[^{14}C]-Met uptake with L-Tyr-OEt, after removing the culture medium, cells at approximately 80% confluence in 5 ml of PBS and incubated for 10 min were transferred to 2.0 ml of Na^+ uptake solution containing 18.5 kBq of D/L-[^{14}C]-Met and 1.0 mM of L-Tyr ethyl ester chloride salt. The cells were then incubated for 5, 10, 30, 60 and 90 min using the same conditions as used for screening effective amino acid esters, and the radioactivity of each aliquot (200 µl) was determined by liquid scintillation counting as described above. Practical diagnostic imaging of a disease state with radiopharmaceuticals is based on net radiopharmaceutical accumulation resulting from the influx and efflux of the radiopharmaceutical into and out of organs, tissues, and cells. We did not measure the uptake rate (*e.g.*; mmol/min) of the labeled amino acid because this parameter is not typically used in the diagnosis of cancer. In an *in vitro* cell study, the cell accumulation level of the labeled amino acid following an adequate incubation time is more important than measurement of the uptake rate at the start of incubation. Although an effect of ester co-loading was observed 5 to 15 min after the start of incubation, we chose an incubation time of 60 min because this provided the enough effect of ester co-loading.

2.5. Inhibition of D/L-[^{14}C]-Met uptake by MeAIB and BCH

Uptake inhibition studies were conducted using MeAIB (a system A-specific inhibitor) and BCH (a system L-specific inhibitor) at a concentration of 1.0 mM. MeAIB was dissolved in Na^+ uptake solution, pH 7.4, and BCH was dissolved in sodium-free uptake solution (containing choline-Cl and K_2HPO_4 instead of the NaCl and Na_2HPO_4 in the Na^+ uptake solution). After removing the culture medium, each dish was washed once with 5 ml of PBS for

10 min at 37°C. The cells were then incubated with 2 ml of incubation medium containing 18.5 kBq D/L-[^{14}C]-Met for 10 min at 37°C, after which the radioactivity of each aliquot (200 μl) was determined as described above.

2.6. Statistical analysis

Data were collated as mean ± standard deviation calculated from three to five measurements, and each experiment was performed in duplicate. The results were analyzed using Student's *t* test. P-values of less than 0.0001 were taken to indicate statistical significance.

3. Results

3.1. Cell culture and proliferation curve

As shown in Fig. 1, the C6 cells showed continuous logarithmic growth after seeding, and confluent growth was seen on day 5. The cells were used in experiments on day 3 or day 4 after seeding.

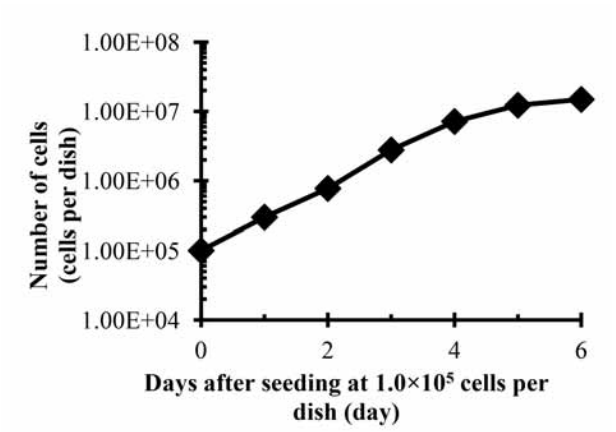

Fig. 1 Cell growth curve of C6 cells

3.2. Screening effective amino acid esters

Figure 2A shows the uptake of D-[^{14}C]-Met with various amino acid esters; the effective esters were L-Tyr-OEt, L-Lys-OEt and L-Pro-OEt ($p < 0.001$ vs. control). The results for L-[^{14}C]-Met are shown in Fig. 2B and the effective esters were L-Arg-OEt and L-Tyr-OEt ($p < 0.001$ vs. control). The uptake of L-[^{14}C]-Met was inhibited by L-Val-OEt, L-Met-OEt and L-Leu-OEt ($p < 0.001$ vs. control). L-Tyr-OEt was the most effective amino acid ester for enhancing the uptake of both D- and L-[^{14}C]-Met (2.73 ± 0.22 and 1.66 ± 0.15).

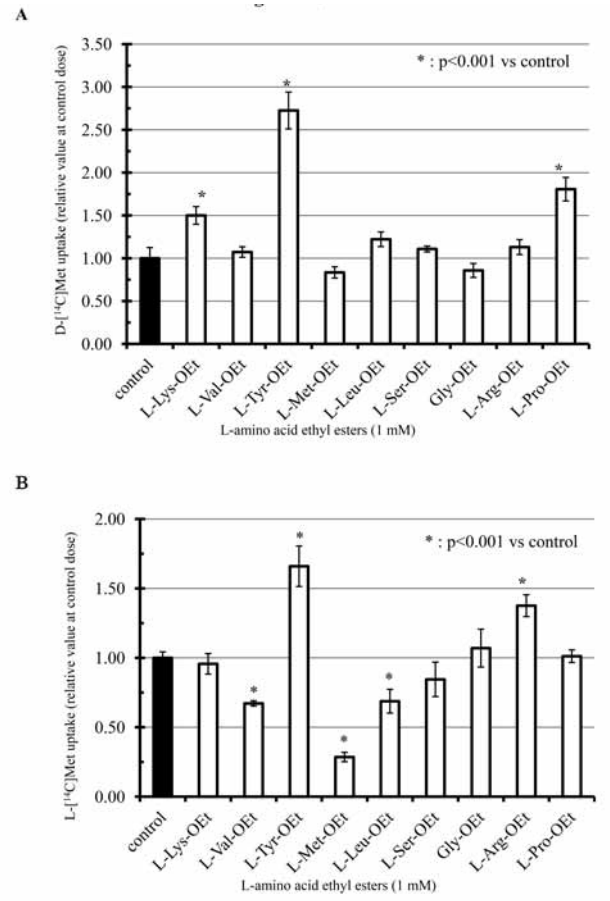

Fig. 2 Screening for effective amino acid esters

3.3. Time course of D/L-[^{14}C]-Met uptake with L-Tyr-OEt

Figure 3A shows the time course of D-[^{14}C]-Met uptake with/without L-Tyr-OEt. The highest uptake value of D-[^{14}C]-Met without L-Tyr-OEt was observed 10 min after starting the incubation, whereas the highest uptake value of D-[^{14}C]-Met with L-Tyr-OEt was observed after 90 min.

Figure 3B shows the results of the L-[^{14}C]-Met uptake time course with/without L-Tyr-OEt. The highest uptake points were 5 min for L-[^{14}C]-Met without L-Tyr-OEt and 90 min for L-[^{14}C]-Met with L-Tyr-OEt.

The use of L-Tyr-OEt to stimulate the uptake of D-[^{14}C]-Met and L-[^{14}C]-Met resulted in up to 4.7-fold higher uptake than that obtained with D-[^{14}C]-Met alone, and 2.5-fold higher uptake than that observed with L-[^{14}C]-Met alone in a time course study (Fig. 3). These *trans*-stimulation effects were observed 5 to 15 min after the start of incubation with L-Tyr-OEt, and the effects increased with time. Furthermore, some amino

acid esters stimulated the transport of D-[14C]-Met (Fig. 2). Incubation for 90 min or more may result in increased D/L-[14C]-Met uptake, but uptake saturated within several hours and then decreased. Observation with an optical microscope showed no morphological changes in the cells during the 90 min experiment.

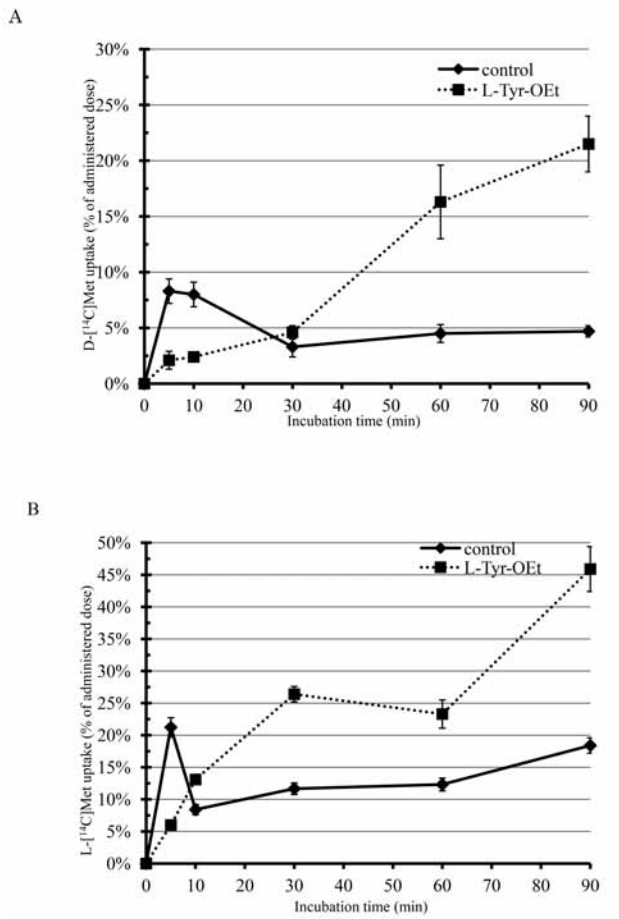

Fig. 3 Time course of D-[14C]-Met (A) and L-[14C]-Met (B) uptake

3.4. Inhibition of D/L-[14C]-Met uptake by MeAIB and BCH

Figures 4A and B show the results of the analysis of the transport mechanism of D/L-[14C]-Met. D-[14C]-Met uptake was inhibited by the addition of MeAIB or BCH. L-[14C]-Met uptake was inhibited by the addition of BCH. These data suggest that system L played a major role in mediating the transport of both D-[14C]-Met (by about 59.6%) and L-[14C]-Met (by about 71.4%), and that sodium-dependent transport played a minor role in D-[14C]-Met transport. MeAIB enhanced L-[14C]-Met uptake by about 19.6% in Na^+ uptake solution, but this increase was not statistically significant.

The present results verify that the uptake of both D-[14C]-Met and L-[14C]-Met by C6 cells is mediated primarily by the system L transporter. Furthermore, we identified for the first time that D-[14C]-Met uptake may be mediated by system A, as shown in Figure 4A.

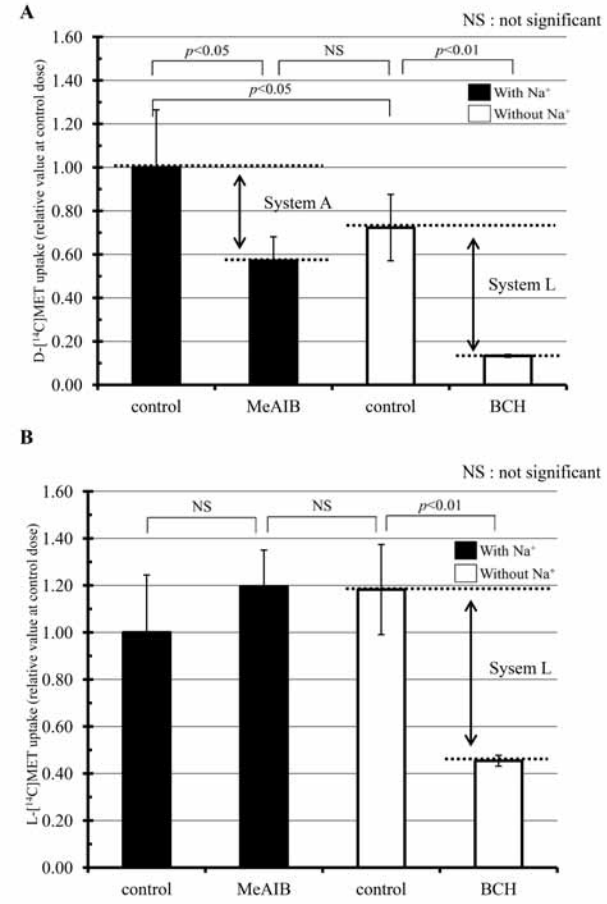

Fig. 4 Uptake inhibition study of D-[14C]-Met (A) and L-[14C]-Met (B)

4. Discussion

It is noteworthy that neutral amino acid transport in cultured glioma cells is dominated by LAT1,[15] and C6 glioma cells express LAT1 but not LAT2 in the presence of 4F2hc.[16] Four isoformes of system L transporter are known, LAT1 to 4.[9] LAT1 and LAT3 are tumor-cell-type transporters. LAT3 is expressed in specific minor tumor cells in, for example, hepatocarcinoma and prostate cancer.[9] LAT2 and LAT4 are normal-cell-type transporters[9] and there is no report suggesting LAT4 expression in C6 glioma cells. Trans-stimulation of LAT1 and LAT2 can occur following the administration of L-Tyr-OEt because LAT1 and LAT2 are amino acid

exchangers. In contrast, trans-stimulation of LAT3 and LAT4 by L-Tyr-OEt cannot occur because those isoforms cannot exchange amino acids. System A transporters are not trans-stimulation sensitive, because they are not amino acid exchangers. Therefore, LAT1 is likely the main trans-stimulation sensitive transporter mediating D/L-[^{14}C]-Met transport in C6 cells. We are currently planning to test the effect of knockout of LAT1 RNA using siRNA on trans-stimulation or inhibition using a LAT1-specific compound.

Figure 2 shows that L-Tyr-OEt was the most effective amino acid ester for enhancing the uptake of both D- and L-[^{14}C]-Met in C6 cells, although the factors underlying this observation are currently unclear. If the pool of L-Tyr amino acid is smaller than the amount of generated L-Tyr inside C6 glioma cells from L-Tyr-OEt, L-Tyr efflux should increase due to the exchanger and D/L-[^{14}C]-Met influx should also increase due to the exchanger. The normal concentration range for each amino acid pool and the Km value for the efflux of each substrate amino acid via the exchanger may be very important factors underlying why L-Tyr-OEt was the most effective amino acid ester for enhancing the uptake of both D- and L-[^{14}C]-Met in C6 cells.

As shown in Fig. 3, effects of ester co-loading were observed 5 to 15 min after the start of incubation with L-Tyr-OEt, suggesting that 5 to 15 min may be required to synthesize the amino acid inside the cell following passage of the co-loaded ester across the cell membrane and enzymatic hydrolysis inside the cell. Use of the opposite enantiomer, D-Tyr-OEt, which is not a substrate for hydrolysis enzymes, resulted in no enhanced labeled tyrosine uptake in CHO-K1 cells.[13] Consequently, we believe there is also a large increase caused by enzymatic hydrolysis in the amount of L-Tyr inside the C6 cells that becomes available due to an amino acid exchanger, most likely LAT1.

Trans-stimulation using L-tyrosine ethyl ester enhanced the uptake of the D-isomer of [^{14}C]-methionine by C6 glioma cells, possibly via LAT1. In this paper we propose an i*n vitro* amino acid ester (prodrug of an amino acid that is a substrate for efflux by an amino acid exchanger) co-loading method that might prevent competitive inhibition of influx. Competitive inhibition could be caused by preloading the amino acid which could not be removed before uptake of D- and L-[^{14}C]-Met, and would minimize the enhancement of labeled amino acid influx.

In this study, we selected L-Tyr combined with ethyl alcohol, but ethyl alcohol may not be the best alcohol for *in vivo* use because it does not specifically distribute to target tumors *in vivo*. The expression levels of the amino acid exchangers ASCT2 and LAT1 are coordinately elevated in a wide spectrum of primary human cancers.[17] Therefore, our next study will investigate the effect of other D-amino acids and the substrates for ASCT2 (D-[^{14}C]-Ala and D-[^{14}C]-Ser) and LAT1 (D-[^{14}C]-Met, D-[^{14}C]-Phe and D-[^{14}C]-Tyr),[18-20] together with the amino acid esters studied in the current investigation and other amino acid esters. Our aim will be to identify promising amino acid esters for targeting tumor tissue in mouse or rat and to address concentration dependence. Investigations on the effects of other D-amino acids with other amino acid esters are also anticipated in the near future.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1) Christensen HN. Role of amino acid transport and countertransport in nutrition and metabolism. Physiol Rev 1990;70:43-77.

2) Shotwell MA, Jaymes DW, Kilberg MS, Oxender DL. Neutral amino acid transport
system in Chinese hamster ovary cells. J Biol Chem 1981;256:5422-7.

3) 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 the heavy chain of 4F2 antigen (CD98). J Biol Chem 1998;273:23629-32.

4) Kobayashi K, Ohnishi A, Promsuk J, Shimizu S, Kanai Y, Shiokawa Y, Nagane M. Enhanced tumor growth elicited by l-type amino acid transporter 1 in human malignant glioma cells. Neurosurgery 2008;62:493-503

5) Kondoh N, Imazeki N, Arai M, Hada A, Hatsuse K, Matsuo H, Matsubara O, Ohkura S, Yamamoto M. 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.

6) Shikano N, Ogura M, Okudaira H, Nakajima S, Kotani T, Kobayashi M, Nakazawa S, Baba T, Yamaguchi N, Kubota N, Iwamura Y, Kawai K. Uptake of 3-[^{125}I] iodo-alpha-methyl-L-tyrosine into colon cancer DLD-1 cells: characterization and inhibitory effect of natural amino acids and amino acid-like drugs. Nucl Med Biol 2010;37:197-204.

7) Sato E, Yamamoto T, Shikano N, Ogura M, Nakai K, Yoshida F, Uemae Y, Takada T, Isobe T, Matsumura A. Intracellular boron accumulation in CHO-K1 cells using amino acid transport control. Appl Radiat Isot 2014;88:99-103.

8) Kanai Y, Fukasawa Y, Cha SH, Segawa H, Chairoungdua A, Kim DK, Matsuo H, Kim JY, Miyamoto K, Takeda E, Endou H. Transport properties of a system y^+L neutral and basic amino acid transporter. Insights into the mechanisms of substrate recognition. J Biol Chem 2000;275:20787-93.

9) Kanai Y. Expression of amino acid transporters in cancers and their application to cancer diagnosis and therapeutics. Membrane 2008; 33: 108-17.

10) Souba WW, Pacitti AJ. How amino acids get into cells: mechanisms, models, menus and mediators. J Parenter Enteral Nutr 1992;16:569-78.

11) Jager PL. Improving amino acid imaging: hungry or stuffed? J Nucl Med 2002;43:1207-9.

12) Lahoutte T, Caveliers V, Franken PR, Bossuyt A, Mertens J, Everaert H. Increased tumor uptake of 3-^{123}I-iodo-L-α-methyltyrosine after preloading with amino acids: an in vivo animal imaging study. J Nucl Med 2002;43:1201-6.

13) Shikano N, Ogura M, Sagara J, Nakajima S, Kobayashi M, Baba T, Yamaguchi N, Iwamura Y, Kubota N, Kawai K. Stimulation of ^{125}I-3-iodo-alpha-methyl-L-tyrosine uptake in Chinese hamster ovary (CHO-K1) cells by tyrosine esters. Nucl Med Biol 2010;37:189-96.

14) Tsukada H, Sato K, Fukumoto D, Nishiyama S, Harada N, Kakiuchi T. Evaluation of D-isomers of *O*-^{11}C-methyl tyrosine and *O*-^{18}F-fluoromethyl tyrosine as tumor-imaging agents in tumor-bearing mice : comparison with L- and D-^{11}C methionine. J Nucl Med 2006;47:679-88.

15) Nawashiro H, Otani N, Shinomiya N, Fukui S, Ooigawa H, Shima K, Matsuo H, Kanai Y, Endou H. L-type amino acid transporter 1 as a potential molecular target in human astrocytic tumors. Int J Cancer. 2006; 119: 484-92.

16) Kim DK, Kim IJ, Hwang S, Kook JH, Lee MC, Shin BA, Bae CS, Yoon JH, Ahn SG, Kim SA, Kanai Y, Endou H, Kim JK. System L-amino acid transporters are differently expressed in rat astrocyte and C6 glioma cells. Neurosci Res. 2004;50: 437-46.

17) Fuchs BC, Bode BP. Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? Semin Cancer Biol 2005;15:254-66.

18) Shikano N, Nakajima S, Kotani T, Ogura M, Sagara J, Iwamura Y, Yoshimoto M, Kubota N, Ishikawa N, Kawai K. Transport of D-[1-^{14}C]-amino acids into Chinese hamster ovary (CHO-K1) cells: implications

for use of labeled d-amino acids as molecular imaging agents. Nucl Med Biol 2007;34:659-65.
19) Utsunomiya-Tate N, Endou H, Kanai Y. Cloning and functional characterization of a system ASC-like Na^+-dependent neutral amino acid transporter. J Biol Chem 1996;271:14883-90.
20) Zander CB, Albers T, Grewer C. Voltage-dependent processes in the electroneutral amino acid exchanger ASCT2. J Gen Physiol 2013;141:659-72.

和文抄録

アミノ酸エチルエステル負荷によるトランス刺激が D-[S-methyl-^{14}C]-methionine (D-[^{14}C]-Met) の C6 神経膠腫細胞への取り込みを増加させるかどうかを検討した。どのアミノ酸エチルエステルが D/L-[^{14}C]-Met 輸送を増強するかの検討では，細胞を 1.0 mM の塩化エチルエステル塩 Gly，L-Arg，L-Ser，L-Leu，L-Met，L-Tyr，L-Val，L-Lys，L-Pro または L-Arg を添加した 18.5kBq の無担体 D/L-[^{14}C]-Met とともに，Na^+ 含有培地中，37℃で 1 時間インキュベートし集積を計測した。また，L-Tyr-OEt を添加し D/L-[^{14}C]-Met の取り込みの経時的測定を行った実験では，細胞を最大 90 分間までインキュベートして集積の計測を行った。さらに，MeAIB（A 系特異的阻害剤）および BCH（L 系特異的阻害剤）の 1.0mM における効果を測定した。L-Tyr-OEt は D- および L-[^{14}C]-Met の取り込みを最も効果的に増強したが，D-[^{14}C]-Met では 4.7 倍，L-[^{14}C]-Met では 2.5 倍であった。L-Tyr-OEt を用いた D-[^{14}C]-Met の最も高い取り込み値は，インキュベーション開始後 90 分かそれ以上の時間で観測された。システム L は主に D/L-[^{14}C]-Met 輸送の媒介に関与し，システム A は D-[^{14}C]-Met 輸送に寄与した。L-Tyr-OEt 同時負荷によるトランス刺激は，インビトロで C6 グリオーマ細胞への D-[^{14}C]-Met 取り込みを増加させた。

キーワード：D-[S-methyl-^{14}C]-methionine, L- チロシンエチルエステル , トランス刺激 , L 系 , C6 グリオーマ細胞