茨城県立医療大学紀要　第24巻
A S V P I Volume 24

Original Article

An L-type Amino Acid Transporter-1 Specific Imaging Agent: Structure-function Relationships of Radioiodinated Tyrosine Derivatives

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Abstract

Introduction: We recently reported the high isoform selectivity of 3-^{125}I-α-methyl-L-tyrosine (^{125}I-IMT) to L-type amino acid transporter-1 (hLAT1) of the two system L1 membrane transporters. Additional tyrosine derivatives were examined herein to identify a more hLAT1-selective compound.

Methods: An uptake study using 4-^{125}I-L-*meta*-tyrosine (4-^{125}I-*m*Tyr), 6-^{125}I-L-*meta*-tyrosine (6-^{125}I-*m*Tyr) and 3-^{125}I-L-tyrosine (3-^{125}I-Tyr) was performed using transporter-expressing *Xenopus laevis* oocytes. Oocytes were injected with 17.6 ng of hLAT1 or hLAT2 complementary RNA (cRNA) and 7.4 ng of human 4F2hc cRNA in a molar ratio of 1:1. Two days after injection, uptake of the tyrosine derivatives was measured in Na^+-free uptake solution containing 18.5 kBq of noncarrier-added tyrosine derivative by determining the radioactivity in the oocytes after incubation for 30 min at room temperature.

Results: The order of higher selectivity was 4-^{125}I-*m*Tyr > ^{125}I-IMT > 3-^{125}I-Tyr > L-^{14}C(U)-Tyr ≒ 6-^{125}I-*m*Tyr.

Conclusion: hLAT exhibited the highest transport specificity towards 4-^{125}I-*m*Tyr of the five tyrosine derivatives tested.

Key words: system L1, tyrosine derivatives, human L-type amino acid transporter 1, human 4F2hc, *Xenopus laevis* oocyte

1. Introduction

Several amino acid transport systems have been described, of which one, system L, is a Na^+-independent transport system and a major route for providing cells with large neutral amino acids, including branched and aromatic amino acids.[1)] System L1 and L2 (components of system L) genes are classified into two families of solute carriers (SLCs), the SLC7 and SLC43 families, respectively, based on the standards established by the Human Gene Nomenclature Committee.[2–5)] The SLC7 family is further divided into the cationic amino acid transporter family and the L-type amino acid transporter (LAT) family.[2–5)] The two known isoforms of system L1 belong to the LAT family and are named type 1 (LAT1) and type 2 (LAT2). The LAT1 and LAT2 transporters of system L1, but not the LAT3 and LAT4 transporters of system L2, form a heterodimeric complex with an

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additional protein, the heavy chain of the 4F2 antigen (CD98; 4F2hc/SLC3A2), which is required for LAT1/2 transport function. LAT1/2 and LAT 3/4 also differ in that LAT1 and LAT2 selectively transport neutral amino acids by obligatory exchange mechanisms, whereas LAT3 and LAT 4 do not display obligatory exchange mechanisms. All these transporters show partly overlapping expression patterns and substrates, although some differences in their expression and substrates have been reported.[2–5)]

We are currently studying radioiodinated tyrosine derivatives with the intent of developing radiopharmaceuticals.[6-10)] Recently we reported the transporter selectivity of 3-$^{123/125}$I-iodo-α-methyl-l-tyrosine ($^{123/125}$I-IMT), which is derived from tyrosine. $^{123/125}$I-IMT is transported by two isoforms of system L1 human L-type amino acid transporters (LATs), designated hLAT1 and hLAT2.[11)] Human LAT1 and hLAT2 must bind with the heavy chain of human 4F2 cell-surface antigen (h4F2hc) for system L1 to be functionally expressed.[12,13)]

In this pilot test, we performed uptake studies using 4-^{125}I-L-*meta*-tyrosine (4-^{125}I-*m*Tyr), 6-^{125}I-L-*meta*-tyrosine (6-^{125}I-*m*Tyr) and 3-^{125}I-L-tyrosine (3-^{125}I-Tyr) (Fig. 1), hLAT1 or hLAT2, and h4F2hc-coexpressing *Xenopus laevis* oocytes, and compared the results obtained with those previously reported for ^{123}I-IMT and l-^{14}C(U)-Tyr.[11)]

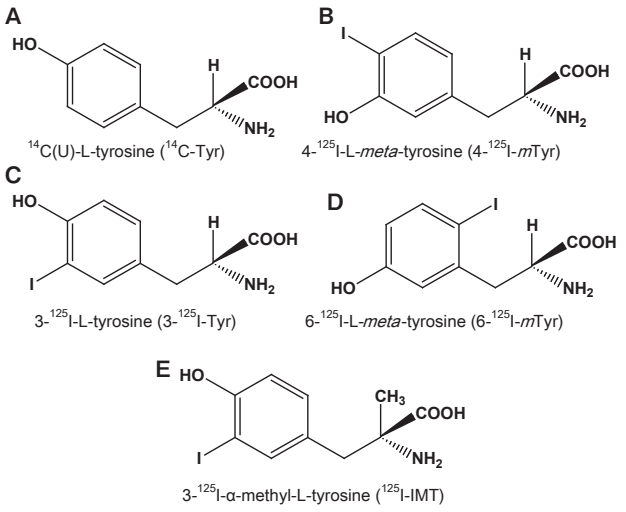

Fig. 1 Chemical structurers of l-^{14}C(U)-Tyr (A), 4-^{125}I-L-*meta*-tyrosine (4-^{125}I-*m*Tyr) (B), 3-^{125}I-L-tyrosine (3-^{125}I-Tyr) (C), 6-^{125}I-L-*meta*-tyrosine (6-^{125}I-*m*Tyr) (D) and 3-^{125}I-iodo-α-methyl-l-tyrosine (^{125}I-IMT) (E).

2. Materials and Methods

2.1. Labeled Compounds

Reagent grade chemicals (Aldrich Chemical Co., Milwaukee, WI) were used throughout. ^{125}I-NaI (8.1 × 10^{19} Bq/mol) was obtained from Amersham Pharmacia Biotech (Buckinghamshire, U.K.). Noncarrier-added 4-^{125}I-*m*Tyr, 6-^{125}I-*m*Tyr and 3-^{125}I-Tyr were prepared by the conventional chloramine-T method described previously.[6,7)]

2.2. Uptake Studies with X. laevis Oocytes

Animal experiments were approved by the ethics committees of affiliated universities. In vitro transcription was performed as described previously[11-13)] to obtain complementary RNAs (cRNAs) for hLAT1 and hLAT2 using T3 RNA polymerase in pBluescript II SK− (Stratagene, La Jolla, CA) linearized with Xho I, and T7 RNA polymerase for h4F2hc in plasmid pZL1 (Invitrogen, Carlsbad, CA) linearized with BamHI. Expression studies were conducted by injecting 17.6 ng of hLAT1 or hLAT2 cRNA and 7.4 ng of h4F2hc cRNA (molar ratio, 1:1) into *X. laevis* oocytes. The control group consisted of *X. laevis* oocytes injected with water instead of cRNA solution. Uptake of radiolabeled amino acids was measured 2 days after injection by placing the oocytes in a Na^+-free uptake solution (100 mmol/L choline chloride, 2 mmol/L KCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 10 mmol/L Hepes, 5 mmol/L Tris, pH 7.4; incubation for 30 min at room temperature) containing a 18.5 kBq/mL ^{125}I-rabeled tyrosine derivative.

3. Results

Labeling efficiency was >80%; after purification, the radiochemical purities of the ^{125}I-labeled tyrosine derivatives were >95% and their specific radioactivity was >8.1 × 10^{19} Bq/mol. Figure 2 shows the results obtained in this study for 4-^{125}I-*m*Tyr, 3-^{125}I-Tyr and 6-^{125}I-*m*Tyr, as well as the results obtained previously with ^{125}I-IMT and l-^{14}C(U)-Tyr.[11)] The uptakes of ^{125}I-IMT, 4-^{125}I-*m*Tyr and 3-^{125}I-Tyr by *X. laevis* oocytes via the

hLAT1-h4F2hc heterodimer were higher than their uptake by the hLAT2-h4F2hc heterodimer ($P < 0.005$), whereas the uptakes of 6-^{125}I-*m*Tyr and l-^{14}C(U)-Tyr were not significantly different between hLAT1-h4F2hc- and hLAT2-h4F2hc-coexpressing *X. laevis* oocytes (Fig. 2). The transport activity via hLAT1 followed the order (from high to low): 3-^{125}I-Tyr > 4-^{125}I-*m*Tyr > L-^{14}C(U)-Tyr > ^{125}I-IMT > 6-^{125}I-*m*Tyr.

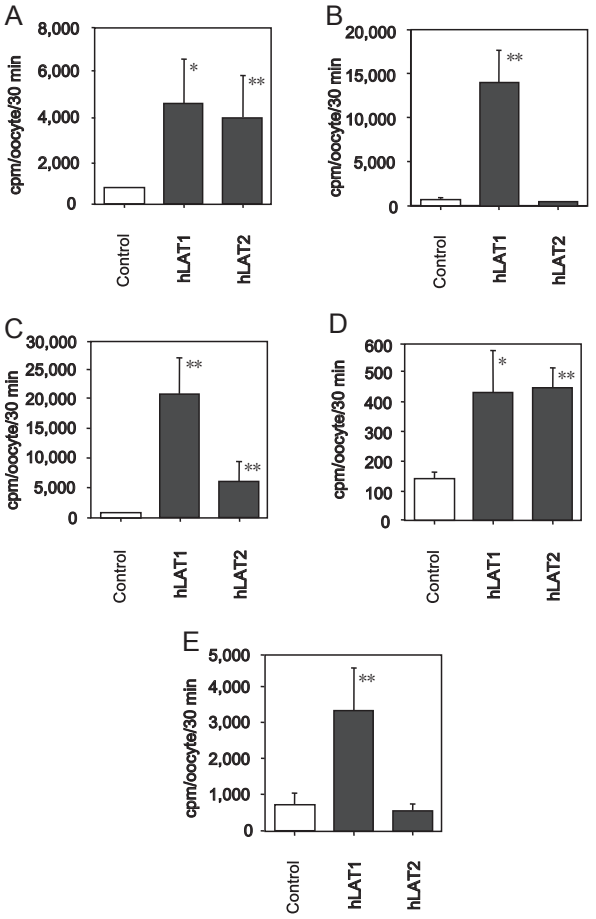

Fig. 2 Comparison of isoform selectivity of hLAT1 or hLAT2 and h4F2hc-coexpressing *X. laevis* oocytes for l-^{14}C(U)-Tyr (A), 4-^{125}I-*m*Tyr (B), 3-^{125}I-Tyr (C), 6-^{125}I-*m*Tyr (D) and ^{125}I-IMT (E). The controls were water-injected oocytes. Uptake of each radiolabeled amino acid was measured in Na^+-free uptake solution containing 18.5 kBq radiolabeled amino acid. 4-^{125}I-*m*Tyr transport showed hLAT1-h4F2hc selectivity. Data represent the average +/- s.e.m. of the uptake of each labeled amino acid. A significant difference from the control value is indicated by *P<0.05; **P<0.005. The data for ^{125}I-IMT and l-^{14}C(U)-Tyr are reprinted with permission from Ref. 11.

4. Discussion

Rat LAT1 was the first LAT1 to be identified, by Kanai et al. in 1998.[14] The relationship between LAT1 and cell proliferation, tumor malignancy, and the transport of essential amino acids has been described.[4] Many malignant tumors highly express LAT1, such as tumors resulting from colon, breast, head and neck, genital, gastric, thyroid and thymic cancers, and soft-tissue sarcoma and hepatocellular carcinoma.[15] LAT1 is highly expressed on the membranes of activated white blood cells.[16]

LAT1 is an important target for various therapy drugs, including KYT-0353,[17] Melphalan[18] and L-boronophenylalanine,[19] for the natural immunosuppressant brasilicardin A (a very high affinity specific inhibitor of the LAT1 transport of activated leukocytes),[16] and for the diagnostics ^{123}I-IMT and L-3-[^{18}F] fluoro-alpha-methyl tyrosine[20].

LAT1 and LAT2 are members of the system L1 amino acid transporters and selectively transport neutral amino acids by obligatory exchange mechanisms.[4,12] These transporters exhibit broad substrate selectivity for various neutral amino acids but LAT1 and LAT2 have different roles.[8] For example, LAT1 exhibits higher affinity (Km = 20–40 μmol/L) but lower capacity toward Leu, Ile, Phe, Met, Tyr, His, Trp, and Val, and accepts the d-isomers of Leu, Phe, and Met as substrates.[12] In contrast, the heterodimeric complex of LAT2 and 4F2hc is involved in the transcellular transport of neutral amino acids through the epithelia and blood-tissue barriers.[13] Furthermore, LAT1 prefers larger neutral amino acids with branched or aromatic side chains, whereas LAT2 exhibits lower affinity (Km = 30–300 μmol/L) but a higher capacity and remarkably broad substrate selectivity, including the small neutral amino acids Gly, Ala, Ser and Thr.[13]

We speculated that the increase in molecular weight or bulkiness caused by the introduction of an iodine atom into the phenol ring of Tyr could change the affinity of Tyr for these amino acid transporters (Fig. 1). The molecular weight of Tyr (or *m*Tyr) and alpha-methyl tyrosine is about 181 Da and 195 Da, respectively, and the atomic weight of the stable isotope of iodine is about 127 Da.

Thus, the atomic weight of iodine accounts for approximately 41% and 38% of the molecular weight of $C_9H_{11}NO_3I$ (for 3-I-Tyr, 4-I-*m*Tyr and 6-I-*m*Tyr) and $C_{10}H_{13}NO_3I$ (for IMT), respectively. The present results indicate that the amino acid transporters exhibited different specificities towards these molecularly altered amino acids (Fig. 2). Also, differences in the position of the iodine and hydroxyl groups in the phenol ring, and the presence of an alpha-methyl group, resulted in altered specificities of the system L1 amino acid transporters for these derivatives.

The selectivity by hLAT1 followed the order (from high to low): 4-^{125}I-*m*Tyr > ^{125}I-IMT > 3-^{125}I-Tyr > L-^{14}C(U)-Tyr ≒ 6-^{125}I-*m*Tyr, suggesting the importance of structure function relationships. The position of iodine in the phenol ring appears to be an important factor in the system selectivity of radioiodinated tyrosine derivatives. In the present study, hLAT1 was highly selective for 4-^{125}I-*m*Tyr and 3-^{125}I-Tyr (Figs. 2 B and C), whereas system L1 exhibited affinity for 6-^{125}I-*m*Tyr (Fig. 2 D). LAT1 was highly specific for derivatives mono-iodinated at the *meta/para* positions of the phenol ring, whereas hLAT1 and hLAT2 exhibited reduced affinity for derivatives mono-iodinated at the *ortho* position of the ring. System L1 exhibited affinity for alpha-methylated 3-^{125}I-Tyr, as indicated by the results for alpha-methylated 3-^{125}I-Tyr (IMT) (Figs. 2 C and E).

Transport by hLAT1 exhibits the highest specificity for 4-^{125}I-*m*Tyr and we are presently investigating 4-^{125}I-*m*Tyr as a potential imaging agent because of its high tumor cell uptake via hLAT1, very high hLAT1 selectivity, and rapid clearance from blood.[8)]

5. Conclusion

The heterodimeric complexes hLAT1-4F2hc and hLAT2-4F2hc are system L1 transporters. 4-^{125}I-*m*Tyr was specifically transported by hLAT1-4F2hc, whereas the parent L -^{14}C(U)-Tyr did not demonstrate isoform selectivity.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

The authors thank Tomoaki Murakami, Makoto Hira, Miya Kikuchi, Kohichi Hamazaki, Michiko Nishikido, Mayo Hakamada, and Rinko Sasaki (Ibaraki Prefectural University of Health Sciences) for their technical assistance. This work was supported by Grants-in-Aid for Scientific Research (#26461801) 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 a Japan Atherosclerosis Research Foundation Grant in 2018.

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和文抄録

我々はこれまでに, L-type amino acid transporter-1 (hLAT1) に対する 3-^{125}I- α -methyl-L-tyrosine (^{125}I-IMT) の高いアイソフォーム選択性を報告した。今回，さらに他の hLAT1 選択的化合物を探索するために 4-^{125}I-L-*meta*-tyrosine (4-^{125}I-*m*Tyr), 6-^{125}I-L-*meta*-tyrosine (6-^{125}I-*m*Tyr)，3-^{125}I-L-tyrosine (3-^{125}I-Tyr) を，hLAT1 または hLAT2 発現 *Xenopus laevis* 卵母細胞を用いて調べた。その結果，選択性の高い順は 4-^{125}I-*m*Tyr > ^{125}I-IMT > 3-^{125}I-Tyr > L-^{14}C(U)-Tyr ≒ 6-^{125}I-*m*Tyr であった。hLAT は，4-^{125}I-*m*Tyr に対して最も高い輸送特異性を示した。