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Transcellular transport of radioiodinated 3-iodo-α-methyl-L-tyrosine across monolayers of kidney epithelial cell line LLC-PK$_1$

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Objective: 3-[^{123}I]iodo-α-methyl-L-tyrosine ([^{123}I]IMT) is an imaging agent for amino acid transport. In order to obtain fundamental data related to tumor imaging with [^{123}I]IMT and renal physiological accumulation of [^{123}I]IMT, we investigated the transport characteristics of [^{125}I]IMT in porcine kidney epithelial cell line LLC-PK$_1$ using cell monolayers grown on microporous membrane filters. ***Methods:*** LLC-PK$_1$ monolayers were created on a collagen-coated microporous (3 μm) membrane (4.7 cm^2). To examine transcellular transport (secretion and reabsorption) and accumulation, the monolayers were incubated for up to 90 min at 37°C with 18.5 kBq [^{125}I]IMT in Dulbecco's phosphate-buffered saline (pH 7.4) as an uptake solution. After incubation, transcellular transport was assessed by quantifying the radioactivity of the solutions on each side of the monolayer. For the accumulation experiment, the cells were solubilized in NaOH solution, and the radioactivity was quantified. For the inhibition experiment, the inhibitor was added at a final concentration of 1 mM. For the pH dependence experiment, the pH of the apical-side uptake solution was varied from pH 5 to pH 8. Transport of [^{14}C]Tyr was examined for comparison. ***Results:*** Bi-directional transcellular transport of [^{125}I]IMT was observed, corresponding to secretion and reabsorption in proximal tubule. Accumulation of [^{125}I]IMT from the basolateral side (1.62 ± 0.15%) and the apical side (2.62 ± 0.35%) was observed at 90 min. 2-Amino-bicyclo[2,2,1]heptane-2-carboxylic acid (a specific inhibitor of system L), L-Tyr (mother compound of [^{125}I]IMT) and 2-aminoisobutyric acid (an inhibitor of system L and A) inhibited both directional transport ($p < 0.01$) and accumulation ($p < 0.01$). 2-(Methylamino)isobutyric acid (a specific inhibitor of system A) appeared to inhibit transport and accumulation, but the results were not significant. Decreasing apical pH significantly enhanced accumulation of [^{125}I]IMT from both sides ($p < 0.001$), whereas accumulation of mother L-Tyr was significantly suppressed. ***Conclusions:*** The inhibition experiments suggest that the main contributor to [^{125}I]IMT transport is system L, rather than Na^+-dependent transport, in both apical and basolateral membrane. [^{125}I]IMT was transported by the system that transported L-Tyr, but the observed pH dependence of transport suggests that different mechanisms are involved in accumulation of [^{125}I]IMT and [^{14}C]Tyr.

Key words: amino acid transport, system L, epithelial cell line, LLC-PK$_1$, 3-iodo-α-methyl-L-tyrosine

INTRODUCTION

3-[^{123}I]Iodo-α-methyl-L-tyrosine ([^{123}I]IMT, Fig. 1), a non-metabolizable artificial amino acid, was developed as an imaging agent for activity of L-Tyr transport.[1–3] The majority of transport studies using [^{123}I]IMT have examined transport in tumor cell lines[4]; e.g., rat C6 glioma

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cells,[5] human GOS3 glioma cells[6] and human Ewing's sarcoma cells.[7] In a recent study, we observed marked accumulation of [^{125}I]IMT in the renal cortex,[8] whereas a moderate accumulation of radiolabeled L-Tyr has been observed.[9] These findings raised questions about the mechanisms of [^{123}I]IMT accumulation and membrane transport in a kidney epithelial cell line.

LLC-PK$_1$ is an epithelial cell line from a Hampshire pig kidney. LLC-PK$_1$ cells are polarized epithelial cells with apical and basolateral membrane domains.[10–13] We presumed that transcellular transport studies with artificial amino acids would be an interesting new application of this epithelial cell line. A confluent monolayer of LLC-PK$_1$ cells forms an oriented monolayer with tight junctions, and exhibits reabsorption of electrolytes and some nutrients via the microvilli of the apical membrane.[13] LLC-PK$_1$ has a hexose and phosphate transport system with characteristics similar to those observed in the proximal tubule.[10–13] Expression of amino acid transport systems L, A and ASC in LLC-PK$_1$ cells has been observed.[13] LLC-PK$_1$ cells are stable through more than 300 serial passages, with no evidence of transformation.[13] LLC-PK$_1$ retains several of the characteristics of differentiated renal proximal tubular cells. It has been employed extensively as a model for analysis of several functions in the proximal tubules.[14]

In the present study, we attempted to clarify mechanisms of transcellular transport of [^{125}I]IMT by LLC-PK$_1$ cell monolayers grown on collagen-coated micro-porous membrane filters (Fig. 2).[10]

MATERIALS AND METHODS

Materials

Reagent-grade 2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH), 2-(methylamino)isobutyric acid (MeAIB), 2-aminoisobutyric acid (AIB), α-methyl-L-tyrosine and Chloramine-T were acquired from Sigma-Aldrich Japan K.K. (Tokyo, Japan). Natural L-amino acids and other chemicals of reagent grade were purchased from Kanto Chemical Co., Tokyo, Japan. [^{125}I]NaI (8.1×10^{19} Bq/mol) was obtained from Amersham Pharmacia Biotech UK (Buckinghamshire, UK). A Nova-Pak C18 (3.9 × 300 mm; Waters, Milford, MA, USA) was used for separation and purification. Silica gel thin-layer chromatography kit (TLC, catalogue number Art. 5553) was obtained from Merck (Darmstadt, Germany). LLC-PK$_1$ cells were obtained from Dainippon Pharmaceuticutical Co. (Osaka, Japan). Plastic tissue culture flasks (surface area, 25 cm^2) were purchased from Nalge Nunc International (Roskilde, Denmark), and Transwell cell chambers (surface area, 4.71 cm^2) were purchased from Costar (Cambridge, MA, USA).

Preparation of labeled compounds

No-carrier-added [^{125}I]IMT was prepared using the conventional chloramine-T method, as described elsewhere.[1,2] Synthesis of non-radioactive IMT was performed according to the method of Krummeich et al.[15] [^{14}C]Tyr and [^{14}C]inulin were obtained from American Radiolabeled Chemicals (USA).

Cell cultures and monolayer preparation

Cell line studies were performed using the methods described by Saitoh et al., with modifications, as follows.[14] LLC-PK$_1$ cells were maintained by serial passages in 25-

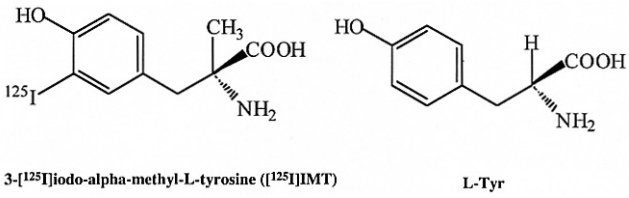

Fig. 1 Chemical structures of 3-[^{123}I]iodo-α-methyl-L-tyrosine ([^{123}I]IMT) and L-Tyr.

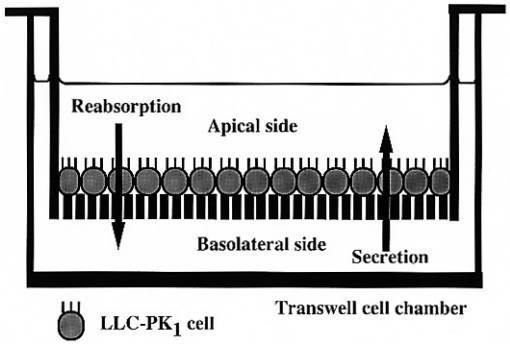

Fig. 2 LLC-PK$_1$ cell monolayer grown on a permeable support in Transwell cell chambers.

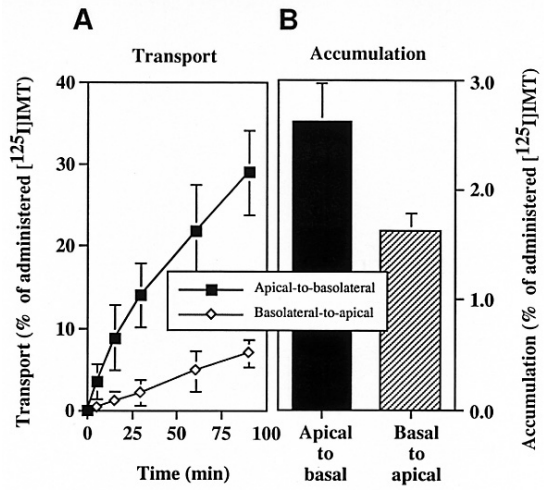

Fig. 3 Transcellular transport of [^{125}I]IMT (A) and accumulation at 90 min of incubation (B) at 6 days after inoculation. Values represent the mean ± S.D. (n = 4–5 monolayers). *p < 0.001.

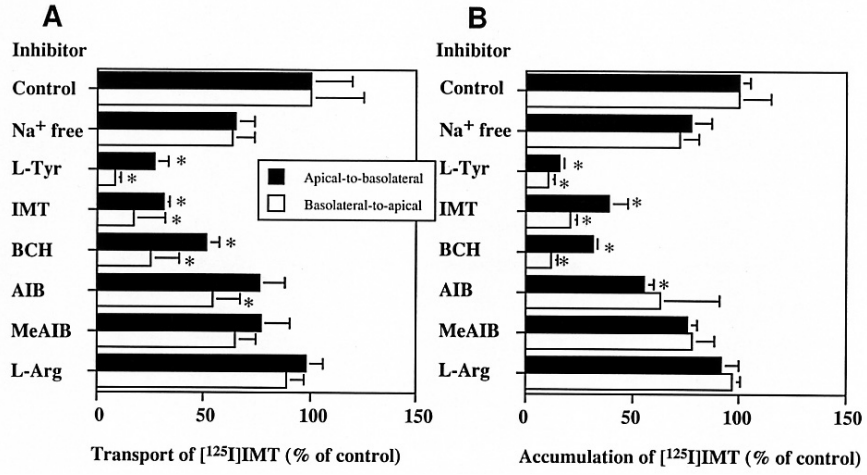

Fig. 4 Effects of inhibitors (1 mM) on transcellular transport (A) and accumulation (B) of [^{125}I]IMT. Each column represents mean ± S.D. of 3 to 5 LLC-PK$_1$ cell monolayers grown on a permeable support. *$p < 0.01$.

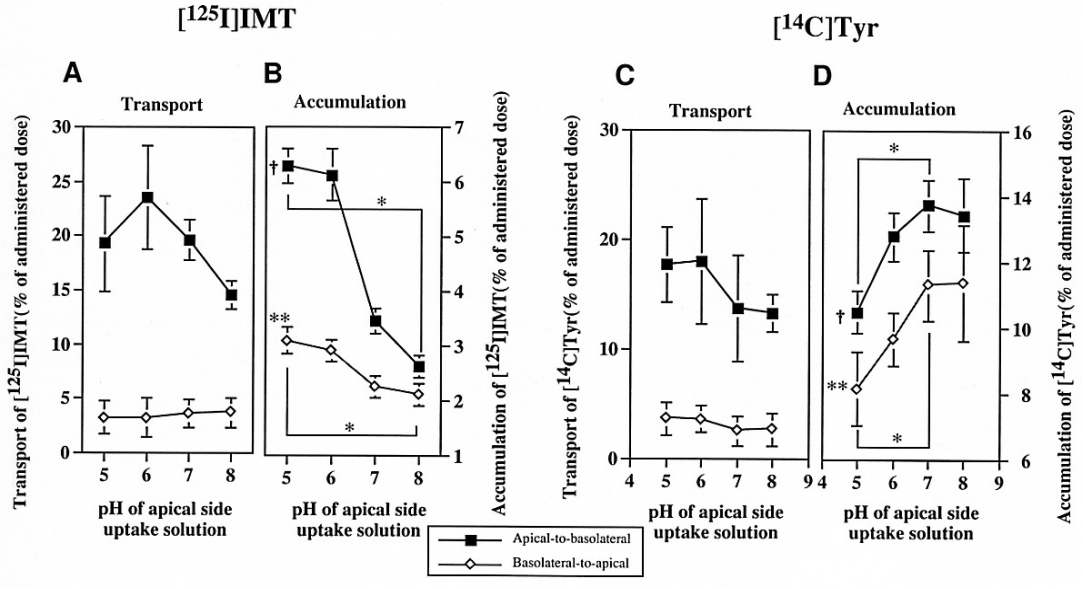

Fig. 5 Dependence of transcellular transport (A) and accumulation (B) of [^{125}I]IMT and dependence of transcellular transport (C) and accumulation (D) of [^{14}C]Tyr on apical pH. *$p < 0.001$. Accumulation of [^{14}C]Tyr (D) was significantly greater than accumulation of [^{125}I]IMT (B) ($p < 0.001$), via both apical membrane (†) and basolateral membrane (**). There was no significant difference between transcellular transport of [^{125}I]IMT (A) and that of (C) [^{14}C]Tyr.

cm^2 cell culture flasks. The cells were fed with Dulbecco's modified Eagle's medium (Sigma-Aldrich Japan K.K., Tokyo, Japan) supplemented with L-glutamine (2 mM) and 10% fetal bovine serum without antibiotics, in an atmosphere of 7.5% 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. To prepare monolayers on a micro-pores support, cells were seeded at a density of 5×10^5 cells/cm^2 on polycarbonate membrane filter (pores, 3 μm) in Transwell cell chambers. The volume of medium inside and outside the Transwell chamber was 1.5 and 2.6 m*l*, respectively. Fresh medium was replaced every 2 days. The cells were used between the 4th and 6th days.

Measurement of transepithelial transport and cellular accumulation

Using monolayer cultures grown in Transwell chambers, we measured transepithelial transport and accumulation of [^{125}I]IMT. Dulbecco's phosphate-buffered saline (PBS; pH 7.4 adjusted by adding HCl or NaOH solution) containing 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_2, 1 mM $CaCl_2$, and 0.5 mM MgCl was used as the incubation

medium. In Na^+-free medium, we replaced the NaCl and Na_2HPO_4 normally present in PBS with, respectively, choline chloride and K_2HPO_4. In general experiments, the cell monolayers were preincubated with 2 m*l* of incubation medium on each side for 10 min at 37°C, after the removal of culture medium from both sides of the monolayers. Then, we added 2 m*l* of incubation medium containing [^{125}I]IMT (18.5 kBq) either to the basolateral or apical side, and added 2 m*l* of incubation medium to the opposite side. We incubated the monolayers for a specified period of time at 37°C or 4°C. WE used [^{14}C]inulin (18.5 kBq), a compound that is not transported by the cells, to evaluate paracellular fluxes and extracellular trapping of radioactivity. We added, L-Tyr, IMT, BCH, AIB, MeAIB or L-Arg at a final concentration of 1 mM for inhibition studies. Cells were incubated for 90 min at 37°C with 18.5 kBq [^{125}I]IMT. The pH (hypothetic urine pH) of apical side incubation medium was adjusted to 5.0, 6.0, 7.0 or 8.0 by adding HCl or NaOH solution for the pH dependence experiment. We fixed the pH of the basolateral side at 7.4 (hypothetic blood pH). We incubated the cells for 90 min at 37°C with 18.5 kBq [^{125}I]IMT or [^{14}C]Tyr added to the apical or basolateral side.

For transport measurements, at the specified time, an aliquot (50 μl) of the incubation medium on the opposite side was obtained, and the radioactivity was measured. For accumulation studies, we removed the medium by suction at the end of the incubation period, and then rapidly washed the monolayers twice with 2 m*l* of ice-cold incubation medium on each side. We solubilized the cells on the filters in 0.5 m*l* of 1 N NaOH, and then measured the radioactivity of the aliquots (100 μl) of the incubation medium on each side. We used an ARC-1000M well-type scintillation counter (Aloka), and an LS6500 liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA) in Clear-sol II (Nacalai Tesque Inc., Kyoto, Japan) to determine the ^{125}I and ^{14}C radioactivity of the collected media and the solubilized cell monolayers.

Statistical analysis

The values obtained in each experiment were expressed as the mean ± S.D. Statistical comparisons between groups were performed using Student's *t* test.

RESULTS

[^{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.

For monolayer preparation, apical-to-basolateral and basolateral-to-apical leak of [^{14}C]inulin was <0.26% and < 0.07% of the total [^{14}C]inulin, respectively, up to 120 min. The amount of [^{14}C]inulin trapped in the extracellular space from both sides was <0.003% of the total [^{14}C]inulin. Because of formation of tight junctions, little of the administered dose of [^{14}C]inulin leaked to the opposite side of the LLC-PK_1 monolayer.

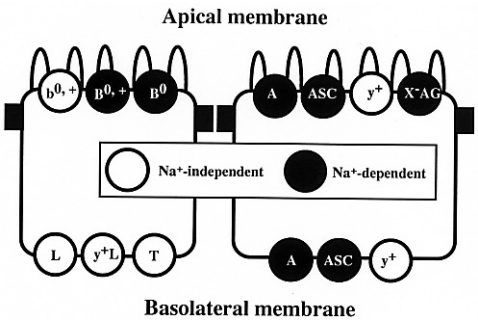

Fig. 6 Principal amino acid transport systems expressed in intact kidney epithelial cells.

As shown in Figure 3, apical-to-basolateral transport of [^{125}I]IMT (29.01 ± 5.17%) was much greater than basolateral-to-apical transport (6.95 ± 0.17%) for up to 90 min (Fig. 3A). Accumulation of [^{125}I]IMT from the basolateral side (1.62 ± 0.16%) and apical side (2.62 ± 0.35%) was observed at 90 min of incubation (Fig. 3B).

In the examination of substrate specificity of the basolateral and apical amino acid transport system in LLC-PK_1 cells, at 1 mM, the amino acids BCH, L-Tyr, AIB and IMT inhibited [^{125}I]IMT transport and accumulation from both sides ($p < 0.01$). MeAIB exhibited an inhibitory effect, but this was not significant. Na^+-dependent transport was not the main contributor to the transport from the basolateral to apical side, or the transport from the apical to basolateral side. L-Arg, a basic amino acid, did not inhibit [^{125}I]IMT transport or accumulation (Fig. 4).

We evaluated effects of apical-side pH on transcellular transport and accumulation of [^{125}I]IMT. When pH of apical incubation buffer was increased from 5.0 to 8.0 (pH of basolateral side fixed at 7.4), accumulation of [^{125}I]IMT from both basolateral and apical side significantly decreased ($p < 0.001$), whereas basolateral-to-apical and apical-to-basolateral transport were not significantly affected (Fig. 5 A, B). When pH of apical incubation buffer was increased from 5.0 to 8.0, accumulation of [^{14}C]Tyr from both basolateral and apical sides significantly increased ($p < 0.001$), whereas basolateral-to-apical and apical-to-basolateral transport of [^{14}C]Tyr was not significantly affected (Fig. 5 C, D).

DISCUSSION

Secretion and reabsorption are not, respectively, reverse reabsorption and reverse secretion, because the nature of efflux and influx via a membrane is not the same in all cases. In intact renal proximal tubule cells, basolateral membrane transport involves the L, A and ASC systems, whereas, in apical membrane, the $b^{0,+}$, $B^{0,+}$, B^0, A and ASC systems contribute to neutral amino acid transport

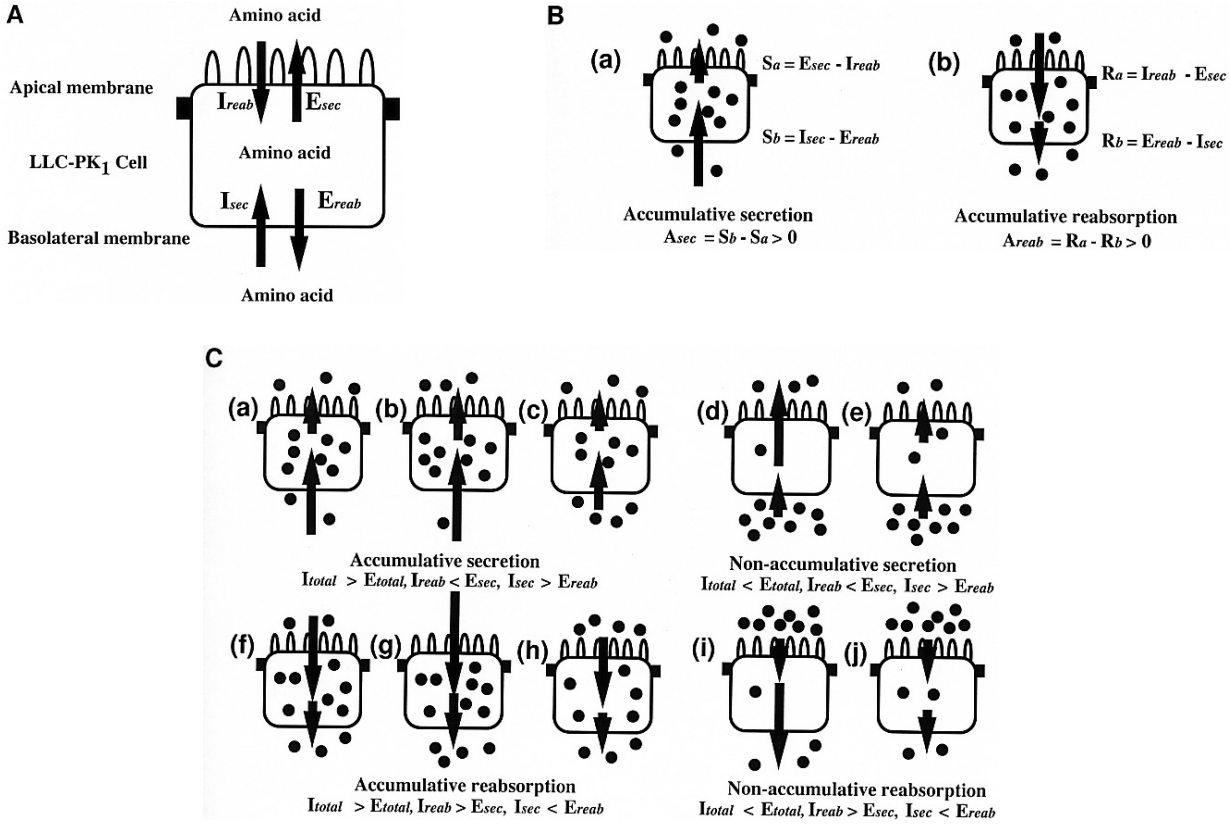

Fig. 7 Scheme showing amino acid pathway in LLC-PK$_1$ (A). Simplified model of amino acid transport via apical and basolateral membrane (B) and (C). ●: amino acid. Length of arrow corresponds to velocity of transport of amino acids.

(Fig. 6).[16] System $b^{0,+}$ is a Na^+-independent transport system involved in transport of neutral amino acids (Leu, Ile, Val, Phe, Tyr, Trp, His, Met, Gln, Asn Thr, Cys, Ser and Ala) and basic amino acids (Arg, Lys and Orn) in apical membrane.[17] System B^0 is the main neutral amino acid transport system predominantly involved in reabsorption of aromatic amino acids on the apical side of proximal tubule cells, but it has not been fully characterized at the molecular level.[18,19] Palacin et al. have suggested the presence of an Na^+-dependent transport system (system $B^{0,+}$) on the apical side of proximal tubule cells.[20] Recently, cDNA of a system T transporter designated TAT1 (T-type amino acid transporter 1) was cloned.[21] Expression of TAT1 has been detected in basolateral membrane of proximal tubule cells.

Tumor cell line studies have revealed that systems L, A, T and $B^{0,+}$ (and/or B^0) play roles in [^{123}I]IMT transport.[3] Studies of [^{123}I]IMT uptake in tumor cells have shown that the majority of transport takes place via the Na^+-independent system L (>70%), and that relatively minor uptake takes place via the Na^+-dependent system $B^{0,+}$ (<20%). Lahoutte et al. reported that system T also mediates [^{123}I]IMT transport into U266 human myeloma cells.[22] [^{123}I]IMT transport via system T accounted for up to 43.8% of all uptake into U266 cells.[22] Studies of human TAT1 show that it transports three aromatic amino acids (Tyr, Phe and Trp) in an Na^+-independent manner. It is noteworthy that α-methyl-L-tyrosine was not a substrate.[21] Tyr is a substrate of system $b^{0,+}$ transport.[17] However, it has also been shown that the Na^+-independent carrier systems $b^{0,+}$ and y^+ do not play roles in [^{123}I]IMT uptake.[3] It is unclear whether system y^+L is involved in [^{123}I]IMT uptake (Fig. 6).[3]

In the present study, [^{125}I]IMT concentration of intracellular fluid via apical membrane at 90 min in pH 7.4 was 9.5 times that of its uptake solution, and that of [^{125}I]IMT via basal membrane was 5.9 times its uptake solution. For [^{14}C]Tyr, the values were 56.3 times (via basolateral membrane) and 9.1 times (via basal membrane) that of its uptake solution, respectively. This indicates that uptake of [^{125}I]IMT and [^{14}C]Tyr is due to carrier-mediated transport against an outwardly directed amino acid concentration gradient, rather than facilitated diffusion. But the higher accumulation of [^{14}C]Tyr, relative to [^{125}I]IMT, were contrary to prediction, because renal physiological accumulation of radiolabeled [^{123}I]IMT is reportedly higher than that of radiolabeled Tyr. Recently, we also found probenecid-sensitive rapid urinary secretion and

marked accumulation of [^{125}I]IMT in an S2-like segment of mouse kidney.[8] Figure 3A does not indicate [^{125}I]IMT secretion but [^{125}I]IMT reabsorption, in net function of transcellular transport by LLC-PK$_1$ monolayer. Rabito et al. reported that LLC-PK$_1$ shows close similarity to the pars recta of the renal proximal tubule, which contains S2 and S3 cells.[10] The lack of accumulative *p*-aminohippurate secretion[11] and pathological observation of transmission electron micrograph of confluent LLC-PK$_1$ monolayer[10] suggest that LLC-PK$_1$ cell shows similarity to S3 cells. Natural amino acids are reabsorbed via S1, S2 and S3 cells, and secreted via S2 cells.

Figure 7A is a scheme showing a hypothetical amino acid pathway in transcellular transport with LLC-PK$_1$. Here, I*reab* and E*sec* were velocity of reabsorptive influx and velocity of secretive efflux in apical membrane. I*sec* and E*reab* were velocity of secretive influx and velocity of reabsorptive efflux in basolateral membrane. I*total* and E*total* are given by two equations: I*total* = I*reab* + I*sec*; and E*total* = E*sec* + E*reab*. Figure 7B and Figure 7C show some conditions of transcellular transport of substrates based on this idea. If I*total* > E*total*, transcellular transport of substrate is accumulative (Fig. 7C (a), (b), (c), (f), (g) and (h)). If I*total* < E*total*, transcellular transport of substrate is not accumulative (Fig. 7C (d), (e), (i) and (j)). On the other hand, if I*reab* < E*sec* and I*sec* > E*reab*, transcellular transport of substrate is secretive (Fig. 7C (a), (b), (c), (d) and (e)). If I*reab* > E*sec* and I*sec* < E*reab*, transcellular transport of substrate is reabsorptive (Fig. 7C (f), (g), (h), (i) and (j)). In the present study with LLC-PK$_1$ monolayer in normal uptake condition, transport of [^{125}I]IMT and [^{14}C]Tyr occurred as accumulative reabsorption (apical-to-basolateral transcellular transport).

The mechanisms of the apical-to-basolateral transcellular transport (reabsorption) of [^{125}I]IMT (Fig. 3) are explained in Figure 7B (b) and Figure 7C (f). The reabsorptive transport velocity in basolateral membrane (R*b* = E*reab* – I*sec*) is a rate-determining step velocity of reabsorption. According to the data shown in Figure 3A, R*b* = 0.322% dose/min/4.7-cm^2. The reabsorptive transport velocity in apical membrane R*a* = I*reab* < E*sec* (>0) should be larger than the velocity R*b*, because of accumulative transport (Fig. 7B (b)). For accumulation velocity in reabsorption, the following equation defines the relationship between each membrane transport velocity and A*reab*: A*reab* = R*a* – R*b* = (I*reab* – E*sec*) – (E*reab* – I*sec*) = (I*reab* + Isec) – (E*sec* + E*reab*) = I*total* – E*total* > 0. According to the data shown in Figure 3B, A*reab* = 0.029% dose/min/4.7-cm^2. Thus, R*a* = A*reab* + R*b* = 0.029 + 0.322 = 0.351% dose/min/4.7-cm^2.

An alternative explanation of the mechanisms of the basolateral-to-apical transcellular transport (secretion) of [^{125}I]IMT (Fig. 3) is shown in Figure 7B (a) and Figure 7C (a). The secretive transport velocity in apical membrane S*a* = E*sec* – I*reab* (= 0.077% dose/min/4.7-cm^2) is a rate-determining step velocity of secretion. The secretive transport velocity in basolateral membrane S*b* = I*sec* – E*reab* (>0) should be larger than the velocity S*a*, because of accumulative transport (Fig. 7B (a)). The accumulation velocity in secretion A*sec* is given by the following equation: A*sec* = S*b* – S*a* = (I*sec* – E*reab*) – (E*sec* – I*reab*) = (I*reab* + I*sec*) – (E*sec* + E*reab*) = I*total* – E*total* > 0. According to the data shown in Figure 3B, A*sec* = 0.018% dose/min/4.7-cm^2. Thus, S*b* = A*sec* + S*a* = 0.018 + 0.077 = 0.095% dose/min/4.7-cm^2.

In the present inhibition study, bi-directional transcellular transport (secretion and reabsorption) and accumulation of [^{125}I]IMT were observed in the LLC-PK$_1$ cell monolayers (Figs. 3, 4 and 5). Transport of [^{125}I]IMT was inhibited by BCH, L-Tyr and AIB, which are substrates of system L. The amino acid BCH markedly inhibited both directional accumulation and transport ($p < 0.01$) of [^{125}I]IMT. System L uptake has been defined as uptake of an amino acid in Na^+-free buffer which is subject to inhibition by an excess of BCH.[23,24] The size of the difference between uptake of [^{125}I]IMT in buffer with Na^+ and without Na^+ suggests that Na^+-dependent transport is not the main pathway. These results suggest that transport of neutral amino acids by LLC-PK$_1$ cells is dominated by Na^+-independent transport (i.e., system L), as indicated by the inhibitory effect of BCH under Na^+-free conditions. This is consistent with a previous report of system L-mediated transport of L-dopa, a neutral amino acid, in LLC-PK$_1$ cells.[25] The decreases in accumulation and transport of [^{125}I]IMT caused by the inhibitors shown in Figure 4 are explained by the models in Figure 7B (e) and (j). Inhibited influx and efflux via apical and basolateral membrane reduce transport and accumulation of [^{125}I]IMT.

Shotwell et al. studied pH dependence of activity of the neutral amino acid transport systems L, A and ASC in Chinese hamster ovary cells.[26] Lowered pH enhanced uptake of substrates via system L, and substrate uptake via system A was abolished when the pH was lowered.[26] In the present study, transcellular transport (not significant) and accumulation of [^{125}I]IMT from both directions were enhanced by lowered pH ($p < 0.001$) (Fig. 5A). These results indicate that system L transport and accumulation of [^{125}I]IMT across the apical membrane can be stimulated by acidifying the medium on the apical side.

The pH-dependent accumulation of [^{125}I]IMT and [^{14}C]Tyr shown in Figure 5B and Figure 5D suggests that pH of extra-cellular fluid on the apical side could affect transport system activity in the membrane in contact with the extra-cellular fluid (apical-to-basolateral accumulation), and opposite side of the extra-cellular fluid (basolateral-to-apical accumulation). Despite formation of tight junctions, the slight leakage of the administered dose of [^{14}C]inulin to the opposite side of the LLC-PK$_1$ monolayer suggests that hydrogen ions in apical-side uptake solution also leak to the opposite side. It is generally considered most likely that lower pH stimulates influx via system L transporter in both membranes. Stimu-

lation of the apical-side membrane by contact with the extra-cellular fluid is greater than that of the opposite side membrane. This mechanism explains the increase in [^{125}I]IMT accumulation in low pH conditions (Fig. 5B). Figure 7C (b) shows the results for secretion in low pH, and Figure 7C (g) shows the results for reabsorption in lower pH. The increasing accumulation with lower pH may be due to the difference in the magnitude of stimulation between the two membranes.

Also in the present study, accumulation of [^{14}C]Tyr from both directions was diminished by lowered pH ($p < 0.001$) (Fig. 5B). This suggests that system A contributes to accumulation of [^{14}C]Tyr. These findings suggest differences in mechanisms of accumulation between [^{125}I]IMT and [^{14}C]Tyr. It appears most likely that lower pH diminishes influx via system A transporter in both membranes. Figure 7C (c) shows results for secretion in low pH, and Figure 7C (h) shows results for reabsorption in low pH. The decrease of [^{14}C]Tyr accumulation shown in Figure 5D in lower pH conditions can be explained using this model. The decreasing accumulation with lower pH may be due to the difference in magnitude of abolishment between the two membranes.

The high functional expression of system L transport in both sides of the membranes may be a representative characteristic by which LLC-PK$_1$ can be distinguished from intact normal renal proximal cells. Incidentally, several characteristics of LLC-PK$_1$ cells are comparable to those of tumor cell lines. It has been reported that LLC-PK$_1$ cells grow rapidly in monolayer cultures. Furthermore, they are stable through more than 300 serial passages, with no evidence that the cells undergo transformation.[13] The finding that system L is conspicuously up-regulated in many kinds of tumors and transformed cell lines is consistent with the observed increase in uptake of amino acids for rapid cell growth and proliferation.[23] On the other hand, studies of transport systems in the apical membrane of LLC-PK$_1$ cells have shown the presence of system A and ASC,[10,27] but have not identified Na^+-dependent neutral amino acid transport systems ($B^{0,+}$ and/or B^0) of the type responsible for Leu reabsorption by the proximal tubule *in vivo*.[10,11,27] These properties are different from those of intact epithelial cells in proximal tubule. As a tool for studying artificial amino acid transport in epithelia, transcellular transport studies constitute an interesting new application of LLC-PK$_1$ cells. However, investigation of the mechanism of high [^{125}I]IMT accumulation with the present experimental system would be facilitated by using intact kidney proximal tubule cells (S2) from the renal cortex.

CONCLUSIONS

We investigated transcellular transport of [^{125}I]IMT in LLC-PK$_1$ (porcine kidney epithelial cell line) monolayers grown on collagen-coated microporous membrane filters. We observed bi-directional transcellular transport of [^{125}I]IMT, by LLC-PK$_1$ cell monolayers, corresponding to secretion and reabsorption in proximal tubule. This transport was shared with transport of L-Tyr, a mother compound of drug design. The main transport pathway of [^{125}I]IMT in LLC-PK$_1$ cells was system L. Na^+-dependent systems contributed a smaller amount to the total uptake. Differences in mechanisms of accumulation between [^{125}I]IMT and [^{14}C]Tyr are suggested by their respective pH dependence.

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REFERENCES

1. 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: 819–824.
2. Kawai K, Fujibayashi Y, Saji H, Yonekura Y, Konishi J, 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: 169–175.
3. 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: 31–37.
4. Langen K-J, Pauleit D, Coenen HH. 3-[^{123}I]Iodo-α-methyl-L-tyrosine: uptake mechanisms and clinical applications. *Nucl Med Biol* 2002; 29: 625–631.
5. Reimann B, Stoegbauer F, Kopka K, Halffer H, Lasic M, Schirmacher A, et al. Kinetics of 3-[^{123}I]iodo-L-α-methlyl tyrosine transport in rat C6 glioma cells. *Eur J Nucl Med* 1999; 26: 1274–1278.
6. Riemann B, Kopka K, Stogbauer F, Halfter H, Ketteler 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: 293–297.
7. Franzius C, Kopka K, Valen F, Eckervogt V, Riemann B, Sciuk J, et al. 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: 123–128.
8. Shikano N, Kawai K, Flores II LG, Nishii R, Kubota N, Ishikawa N, et al. An artificial amino acid 4-iodo-L-*meta*-tyrosine: Biodistribution and excretion via kidney. *J Nucl Med* 2003; 44: 625–631.

9. Jager PL, Vaalburg W, Pruim J, de Vries EG, Langen KJ, Piers DA. Radiolabeled amino acids: basic aspects and clinical applications in oncology. *J Nucl Med* 2001; 42: 432–445.
10. Rabito CA. Occluding junctions in a renal cell line (LLC-PK_1) with characteristics of proximal tubular cells. *Am J Physiol* 1986; 250: F734–743.
11. Rabito CA, Karish M. Polarized amino acid transport by an epithelial cell line of renal origin (LLC-PK_1). *J Biol Chem* 1983; 258: 2543–2547.
12. Sepulveda FV, Pearson JD. Cationic amino acid transport by two renal epithelial cell lines: LLC-PK_1 and MDCK cells. *J Cell Physiol* 1985; 123: 144–150.
13. Hull RN, Cherry WR, Weaver GW. The origin and characteristics of a kidney cell strain, LLC-PK_1. *In Vitro* 1976; 12: 670–677.
14. Saito H, Ohtomo T, Inui K. Na^+-dependent uptake of 1,5-anhydro-D-glucitol via the transport systems for D-glucose and D-mannose in the kidney epithelial cell line, LLC-PK_1. *Nippon Jinzo Gakkai Shi* 1996; 38: 435–440.
15. Krummeich C, Holschbach M, Stocklin G. Direct n.c.a. electrophilic radioiodination of tyrosine analogues: their *in vivo* stability and brain-uptake in mice. *Appl Radiat Isot* 1994; 45: 929–935.
16. Christensen HN. Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev* 1990; 70: 43–77.
17. Chairoungdua A, Segawa H, Kim JY, Miyamoto K, Haga J, Fukui Y, et al. Identification of an amino acid transporter associated with the cystinuria-related type II membrane glycoprotein. *J Biol Chem* 1999; 274: 28845–28848.
18. Maenz DD, Patience JF. L-Threonine transport of pig jejunal brush border membrane. *J Biol Chem* 1992; 267: 22079–22086.
19. Lynch AM, McGivan JD. Evidence for a single common Na^+-dependent transporter for alanine, glutamine, leucine and phenylalanine in brush-border membrane vesicles from bovine kidney. *Biochem Biophys Acta* 1987; 899: 176–184.
20. Palacin M, Estevenz R, Bertran E, Zorano A. Molecular biology of mammalian plasma membrane amino acid transports. *Physiol Rev* 1998; 78: 969–1054.
21. Kim DK, Kanai Y, Chairoungdua A, Matsuo H, Cha SH, Endou H. Expression cloning of a Na^+-independent aromatic amino acid transporter with structural similarity to H^+/monocarboxylate transporters. *J Biol Chem* 2001; 276: 17221–17228.
22. 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: 129–134.
23. 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: 23629–23632.
24. Segawa H, Fukasawa Y, Miyamoto K, Takeda E, Endou H, Kanai Y. Identification and functional characterization of a Na^+-independent neutral amino acid transporter with broad substrate selectivity. *J Biol Chem* 1999; 274: 19745–19751.
25. Soares-da-silva P, Serrao MP. Molecular modulation of inward and outward apical transporters of L-dopa in cells. *Am J Physiol Renal Physiol* 2000; 279: F736–746.
26. Shotwell MA, Jayme DW, Kilberg M, Oxender DL. Neutral amino acid transport system in Chinese hamster ovary cells. *J Biol Chem* 1981; 256: 5422–5427.
27. Sepulveda FV, Pearson JD. Characterization of neutral amino acid uptake by cultured epithelial cells from pig kidney. *J Cell Physiol* 1982; 112: 182–188.