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Detection of maleate-induced Fanconi syndrome by decreasing accumulation of ^{125}I-3-iodo-α-methyl-L-tyrosine in the proximal tubule segment-1 region of renal cortex in mice: a trial of separate evaluation of reabsorption

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Objective: Fanconi syndrome is a renal dysfunction characterized by various combinations of renal tubular transport dysfunction involving amino acids, glucose, protein and other substances. Most reabsorption of amino acids occurs in proximal renal tubule segment 1 (S1). The present study evaluated the possibility of early detection of drug-induced Fanconi syndrome, based on decreased renal accumulation of ^{125}I-3-iodo-α-methyl-L-tyrosine (^{125}I-IMT), an amino acid transport marker, in the S1 region of renal cortex. The present experimental model used maleate (MAL)-induced Fanconi syndrome in mice. Results were compared between ^{125}I-IMT and 3 other clinical renal radiopharmaceuticals: ^{99m}Tc-2,3-dimercaptosuccinic acid (^{99m}Tc-DMSA); ^{99m}Tc-mercaptoacetylglycylglycylglycine (^{99m}Tc-MAG$_3$); and ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA). ***Methods:*** Male ddY mice (age, 6 weeks; body weight, 25 g) were used to create a Fanconi model of renal dysfunction. A single dose of maleate disodium salt was administered by intraperitoneal injection (6 mmol/kg). Hematoxylin and eosin (HE) staining of the renal cortex, renal autoradiography and measurement of renal radioactivity of labeled compounds were performed at 30, 60, 90 and 120 min after MAL injection. At 5 min after injection of labeled compounds (18.5 kBq for accumulation experiment, 670 kBq for autoradiography), animals were sacrificed by ether overdose and kidneys were removed. For the accumulation experiment, radioactivity was measured using a well-type scintillation counter. For autoradiography, 20-μm sections of frozen kidney were used with Bio-Imaging Analyzer. ***Results:*** At 30 min after MAL injection, HE staining showed pyknosis in some proximal tubule cells. At that time, accumulations of ^{125}I-IMT and ^{99m}Tc-DMSA in the S1 region were approximately 67% and 55% of control levels ($p < 0.005$). MAL increased accumulation of ^{99m}Tc-DTPA in the S1 region, but had no effect on accumulation of ^{99m}Tc-MAG$_3$ in the S1 region. ***Conclusions:*** Decreased accumulation of ^{123}I-IMT in the S1 region appears to represent a useful marker for detection of MAL-induced Fanconi syndrome. In future, we plan to assess the efficacy of using ^{125}I-IMT to monitor renal dysfunction induced by nephrotoxic clinical drugs.

Key words: reabsorption, 3-iodo-α-methyl-L-tyrosine, maleate-induced Fanconi syndrome, renal cortex, proximal tubule segment 1

INTRODUCTION

FANCONI SYNDROME is a renal dysfunction associated with a variety of metabolic disorders, including tyrosinemia, cystinosis, Wilson's disease, glycogen storage disease,[1] galactosemia and oculocerebrorenal syndrome of Lowe.[2,3]

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This syndrome is characterized by various combinations of proteinuria, aminoaciduria, glucosuria, phosphaturia, bicarbonaturia, increased electrolyte excretion, uricosuria and proximal renal tubular acidosis.[2] Fanconi syndrome is often observed in patients with drug-induced acute renal failure after nephrotoxic treatment. Cases have been observed after exposure to tetracycline,[4] streptozotocin,[2,3] gentamycin,[5] valproate,[4,6] 4-pentenoate,[7] cystine,[8,9] ifosfamide,[10–13] maleate,[14,15] heavy metals[16] and succinylacetone.[17]

Clinically, ^{99m}Tc-2,3-dimercaptosuccinic acid (^{99m}Tc-DMSA) has been used not only to visualize the kidneys, but also to assess individual kidney function.[18] Fanconi syndrome is associated with low renal uptake of ^{99m}Tc-DMSA, despite a relatively normal glomerular filtration rate (GFR).[19] However, the mechanisms of ^{99m}Tc-DMSA accumulation remain unclear.[18]

Dysfunction of amino acid resorption is characteristic of Fanconi syndrome, and begins in the early phase of drug-induced nephrotoxicity. In heavy metal-induced Fanconi syndrome, for example, renal amino acid handling is a highly sensitive marker of nephrotoxicity.[16] Markers of amino acid transport may thus be effectively used to monitor Fanconi syndrome.

After glomerular filtration, most amino acids undergo intensive resorption in proximal renal tubule segment 1 (S1). Accumulation of amino acids in the S1 region of the renal cortex may reflect renal handling of amino acids. We postulated that decreased accumulation of amino acids in the S1 region could be used to detect Fanconi syndrome, which is a multiple transport dysfunction of the proximal tubule. The present study evaluated use of ^{123}I-3-iodo-α-methyl-L-tyrosine (^{123}I-IMT) as a renal functional imaging agent for detection of Fanconi syndrome. Originally, ^{123}I-IMT was developed as a functional imaging agent for L-tyrosine (L-Tyr) transport mechanisms in the brain and pancreas (Fig. 1).[20–22] Use of ^{123}I-IMT has been seen clinically for single photon emission computed tomography (SPECT) of tumors, as a marker of up-regulated amino acid transport.[23–26]

The present study used an experimental model consisting of maleate (MAL)-induced Fanconi syndrome in mice,[15,17,27] as established by Dimopoulou et al.[27] This model has been successfully used in urine biochemical studies to characterize multiple transport dysfunctions in the proximal tubule of MAL-induced Fanconi syndrome in mice.[27]

Particularly for changes to renal accumulation in the S1 region of the renal cortex, the results obtained for ^{125}I-IMT were compared with results for 3 clinical renal radiopharmaceuticals: ^{99m}Tc-DMSA, ^{99m}Tc-mercaptoacetylglycylglycylglycine (^{99m}Tc-MAG$_3$), and ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA).

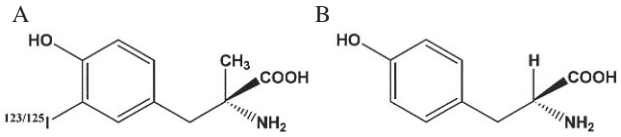


Fig. 1 Comparison of chemical structures of $^{123/125}$I-3-iodo-α-methyl-L-tyrosine ($^{123/125}$I-IMT) (A) and L-Tyr, the mother compound (B).

MATERIALS AND METHODS

Materials and preparation of labeled compounds

Reagent-grade MAL and α-methyl-L-tyrosine were acquired from Sigma-Aldrich Japan (Tokyo, Japan). Chloramine-T and other chemicals of reagent grade were purchased from Kanto Chemical (Tokyo, Japan). In the present basic study, for convenience, ^{125}I-labeled IMT (^{125}I-IMT) was used instead of ^{123}I. The radioisotope ^{125}I-NaI (8.1×10^{19} Bq/mol) was obtained from Amersham Pharmacia Biotech UK (Buckinghamshire, UK). Non-carrier-added ^{125}I-IMT was prepared using the conventional chloramine-T method, with α-methyl-L-tyrosine as the precursor, as previously reported.[20–22] A Nova-Pak C18 chromatography column (3.9 × 300 mm; Waters, Milford, MA, USA) was used for separation and purification of ^{125}I-IMT. To assess labeling efficiency and radiochemical purity, a silica gel thin-layer chromatography kit (TLC, catalogue number Art. 5553; Merck, Darmstadt, Germany) was used.

^{99m}Tc-DMSA, ^{99m}Tc-MAG$_3$ and ^{99m}Tc-DTPA were all obtained from Nihon Medi-Physics (Hyogo, Japan) and Daiichi Radioisotope Laboratories (Tokyo, Japan). Animals used in all experiments were male ddY mice (body weight, 25 g; age, 6 weeks). All animal experiments were approved by the ethical committees of Ibaraki Prefectural University of Health Sciences.

Fanconi model of renal dysfunction in mice

Groups of 3 mice were administered MAL by intraperitoneal injection of a single dose of 6 mmol/kg body weight.[27] Control mice received intraperitoneal injection (i.p.) of saline. Injected mice were allowed free movement in a separate cage for each mouse. Proximal tubule cells in the renal cortex of control and MAL-treated mice were examined using hematoxylin and eosin (HE) staining and a CX41 optical microscope (Olympus, Tokyo, Japan).

Measurement of renal accumulation

For assessment of whole-kidney accumulation (Fig. 5), groups of 5 control or MAL-treated mice were administered 0.1 m*l* of ^{125}I-IMT, ^{99m}Tc-DMSA, ^{99m}Tc-MAG$_3$, or ^{99m}Tc-DTPA (18.5 kBq) by injection into the tail vein. Mice were anesthetized with ether inspiration and sacrificed by heart puncture at 30, 45, 60, 90 and 120 min after administration of the labeled compounds, and kidneys were resected. Radioactivity of samples was quantified

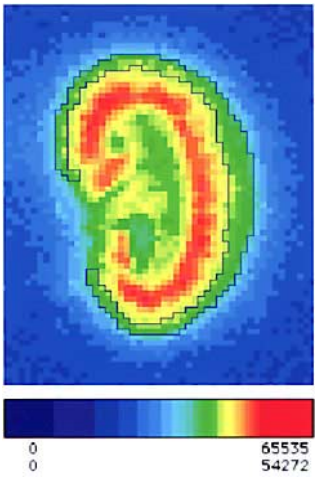


Fig. 2

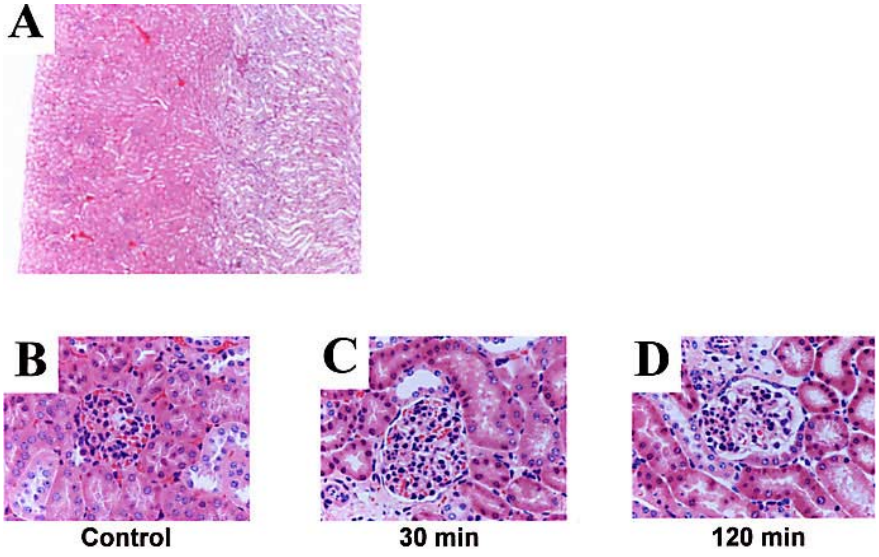
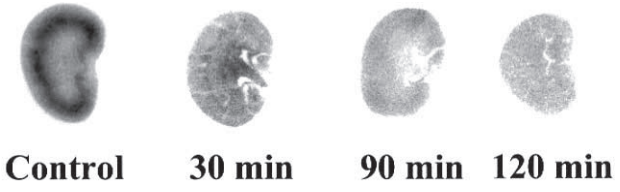


Fig. 3



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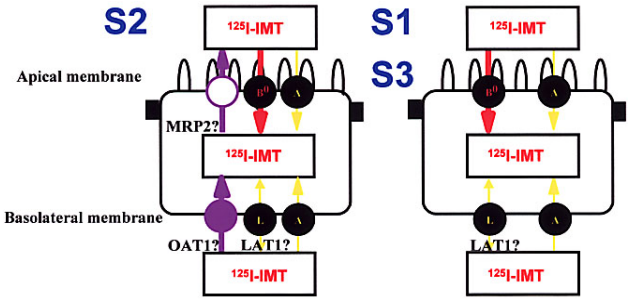

Fig. 6

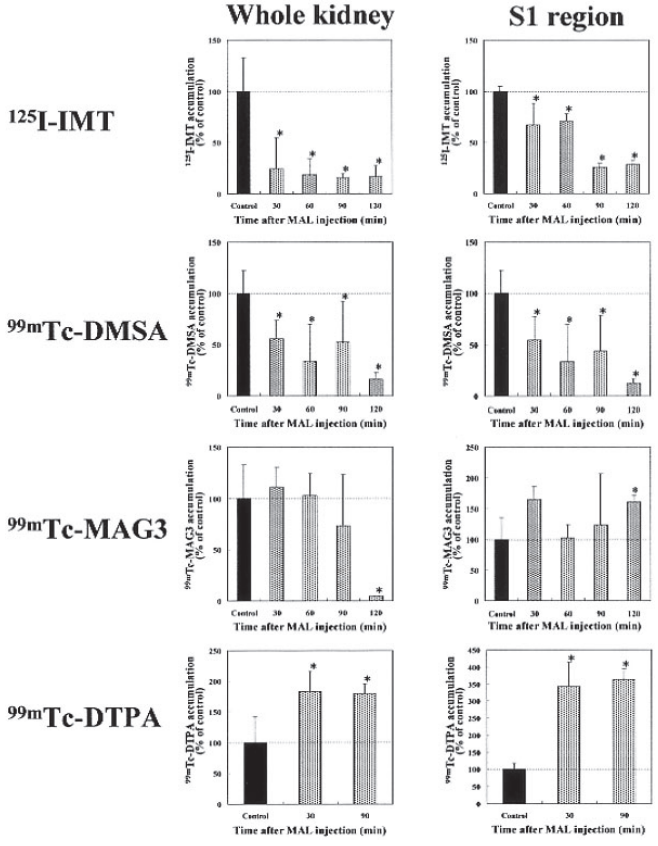

Fig. 5

Fig. 2 Accumulation of ^{125}I-IMT in control kidney (no MAL treatment). Images were processed using a Bio-Imaging Analyzer. S1 is located on the outer side of the fringe of the renal cortex (green region), while S2 is located on the inner side (red region). Open squares in the S1 region represent regions of interest (ROIs) for evaluation of amino acid transport based on ^{125}I-IMT accumulation. Numbers 0 to 65535 (16 bits/pixel) under the color bar represent the full range of gradation fitted on an exponential contrast curve that corresponds to the photo-stimulated luminescence (PSL) value of the Bio-Imaging Analyzer. The original range of 0 to 65535 was changed to 0 to 54272, so the red area indicates the S2 region.

Fig. 3 Optical microscopy of mouse renal cortex in MAL-induced Fanconi syndrome model. Control mice (A, B) and MAL-treated mice at 30 min (C) and 120 min (D) after 6 mmol/kg MAL administration. Magnification: 40× (A); 200× (B–D).

Fig. 4 Mouse kidney autoradiography in MAL-induced Fanconi syndrome model after ^{125}I-IMT administration.

Fig. 5 Accumulation of ^{125}I-IMT, ^{99m}Tc-DMSA, ^{99m}Tc-DTPA and ^{99m}Tc-MAG$_3$ in mouse kidney in a model of MAL-induced Fanconi syndrome. Left: whole kidneys (quantified using a well-type scintillation counter). Right: S1 region (quantified using a Bio-Imaging Analyzer system). *$p < 0.005$ vs. control

Fig. 6 Putative major membrane transport of ^{125}I-IMT in proximal tubule cells. Systems A, L and B^0 are neutral amino acid transport systems that mediate ^{125}I-IMT transport into proximal tubule cells. Some studies using tumor cell lines have indicated that system A does not contribute to ^{125}I-IMT transport. In S2 cells, cumulative excretion of ^{125}I-IMT via organic anion transporter 1 (OAT1) and multi-drug-resistant protein 2 (MRP2) is thought to be probenecid-sensitive.

using an ARC-380 well-type scintillation counter (Aloka, Tokyo, Japan).[28,29]

In vivo *autoradiography of kidneys*

For assessment of accumulation in the S1 region (Fig. 5), groups of 4 control or MAL-treated mice were administered 0.1 m*l* of ^{125}I-IMT (670 kBq) by injection into the tail vein. After 5 min, mice were sacrificed by inspiration of excess ether, placed in carboxymethyl cellulose embedding medium (Nacalai, Kyoto, Japan), and frozen at −15°C for at least 12 h. A CM3050 S autocryotome (Leica, Nussloch, Germany) was used to cut 20-μm coronal sections from posterior to anterior. Sections were dried at −15°C for another 24 h, then kept in contact with BAS-SR2040 imaging plates (Fuji Photo Film, Kanagawa, Japan) for 6 days. Images were processed using a BAS 5000 Bio-Imaging Analyzer (Fuji Photo Film).[28,29] Accumulation of IMT in the S1 region was measured. Figure 2 show regions of interest (ROIs) for evaluation of amino acid transport. High levels of organic anion transporter 1 (OAT1) are reportedly expressed in S2 cells.[30] OAT1 is thought to be the main contributor to the marked ^{125}I-IMT accumulation that has been observed in S2 cells.[31] The S1 region is located on the outer side of the fringe of the renal cortex (green region in Fig. 2). In the S1 region, mean count of 296 pixels in the ROIs was 14,400 counts/pixel/6 days of contact with imaging plates.

Statistical analysis

Data were collated as mean ± standard deviation of 3–5 measurements. Results were analyzed using Student's t test. Values of $p < 0.005$ were considered indicative of statistical significance.

RESULTS

Fanconi model of renal dysfunction

HE staining showed pyknosis in some proximal tubule cells 30 min after MAL injection (Fig. 3). No other obvious changes were observed in the glomerulus or proximal tubule. At 120 min after MAL injection, pyknosis was observed in distal tubules and some proximal tubule cells.

Renal accumulation

Figure 4 shows renal accumulation of ^{125}I-IMT versus time after MAL injection. Time-dependent decreases in renal accumulation of ^{125}I-IMT were apparent.

Figure 5 shows relative renal accumulation of labeled compounds (compared to control) versus time after MAL injection. Total ^{125}I-IMT radioactivity in MAL-treated kidneys decreased to 24.32% ± 25.10% of the control level ($p < 0.005$) at 30 min after MAL injection. In S1 (where most amino acid resorption occurs), ^{125}I-IMT radioactivity of MAL-treated kidneys decreased to 66.83% ± 15.63% of control level ($p < 0.005$) at 30 min after MAL injection (Fig. 5). Accumulation of ^{99m}Tc-DMSA in the S1 region was reduced to approximately 54.67% ± 22.93% of control levels ($p < 0.005$) at 30 min after MAL injection. Accumulation of ^{99m}Tc-DTPA in the S1 region was increased about 3.5-fold, compared to controls, at 30 min after MAL injection. Accumulation of ^{99m}Tc-MAG_3 had not decreased at 120 min after MAL injection.

DISCUSSION

Fanconi syndrome is an inherited or acquired disorder involving transport defects in the proximal renal tubule, including impaired resorption of amino acids, glucose, sodium, potassium, calcium, phosphate, bicarbonate, uric acid and low-molecular-weight proteins.[19]

In clinical settings, dysfunction of resorption in the proximal tubule can be assessed by testing for decreased accumulation of ^{99m}Tc-DMSA in the renal cortex. In addition, biochemical indications of multiple transport dysfunctions in the proximal tubule suggest the presence of Fanconi syndrome. Tests using ^{99m}Tc-DTPA and ^{99m}Tc-MAG_3 frequently demonstrate relatively normal GFR and proximal tubular secretion.

Despite the clinical use of ^{99m}Tc-DMSA in assessing relative renal function, the mechanisms by which ^{99m}Tc-DMSA accumulates in the kidney remain unclear.[18] Evaluation of renal handling of amino acids may allow direct detection of Fanconi syndrome. The present basic study was thus performed to evaluate the usefulness of detecting decreased accumulation of amino acid in the renal cortex as an indicator of Fanconi syndrome.

We selected $^{123/125}$I-IMT as a candidate amino acid transport marker, as $^{123/125}$I-IMT is a metabolically stable artificial amino acid that was developed as a functional imaging agent for the brain and pancreas.[20–22] Clinical use has also been made of ^{123}I-IMT for SPECT imaging of tumors, as high levels of amino acids accumulate in actively proliferating tumor cells, and this process can be monitored by assessing ^{123}I-IMT accumulation.[23–26]

In the present study, we expected to be able to assess dysfunction of amino acid transport by testing for decreased accumulation of ^{123}I-IMT in the S1 region of the renal cortex, as most resorption of amino acids occurs in S1.

The present MAL-induced mouse Fanconi syndrome model was described by Dimopoulou et al.[27] The model includes urine biochemistry changes similar to those reported for patients with Fanconi syndrome. In the study by Dimopoulou et al., urinary excretion of amino acids was significantly elevated within 30 min after injection of MAL (6 mmol/kg body weight intraperitoneal), and continued to increase with time.[27] Marked aminoaciduria in mice after MAL injection is characterized by multifold increases in levels of most amino acids present in urine of normal mice, and by significant concentrations of neutral amino acids (e.g., phenylalanine, leucine and tyrosine)

that are not present in significant quantities in normal urine.[27]

Dimopoulou et al. also reported that ^{99m}Tc-DTPA, a radiopharmaceutical freely excreted by glomerular filtration, exhibits significantly delayed pharmacokinetics as a dose-dependent effect. Although ^{99m}Tc-DTPA does not accumulate in normal renal cortex, accumulation is seen in the renal cortex of the present model of Fanconi syndrome, suggesting that the renal cortex in the present model is abnormal.[27] Dimopoulou et al. suggested that ^{99m}Tc-DTPA is an effective marker for early detection of abnormal GFR in Fanconi syndrome. Significantly disturbed ^{99m}Tc-DTPA pharmacokinetics are seen in all organs of MAL mice by 90 min after administration of a single MAL-dose of 6 mmol/kg.[27] According to the report, a mix of proximal tubule transport defects with abnormal glomerular function seems to be obtained by 90 min after MAL injection in this model.[27] The present study identified accumulation of ^{99m}Tc-DTPA by 30 min after MAL injection, suggesting some degree of abnormal GFR (Fig. 5).

Whether proximal tubular secretion remains normal is unclear, but no significant change in accumulation of ^{99m}Tc-MAG_3 was observed until 120 min after MAL injection (Fig. 5). Due to this late response to MAL administration, ^{99m}Tc-MAG_3 is not suitable for early detection of MAL-induced Fanconi syndrome. In contrast, accumulation of ^{125}I-IMT and ^{99m}Tc-DMSA significantly and rapidly decreased within 30 min after MAL injection (Fig. 5).

Accumulation of ^{125}I-IMT due to resorption in the apical membrane of S1 cells may be affected by both abnormal GFR and nephrotoxicity of filtrated MAL. This second factor appears to have a greater effect on renal handling of amino acids in the present model, as urinary excretion of natural amino acids continued to increase over time.[27]

Recent findings regarding the mechanism of Fanconi syndrome have led to the following hypothesis: administration of MAL to mice induces dysfunction of Na^+-K^+-ATPase.[9] Sodium-concentration-linked, energy-dependent amino acid transport systems such as system B^0, which is thought to be the main contributor to resorption of neutral amino acids, are functionally damaged by MAL.[27] These effects of MAL lead to Fanconi syndrome with aminoaciduria.

In a recent study, we observed marked accumulation of ^{125}I-IMT in the renal cortex,[28] whereas moderate accumulation of radiolabeled L-Tyr was observed in another study.[26] The ^{125}I-IMT used in the present study was derived from L-Tyr, which was modified by α-methylation for metabolic stability, and 3-iodination of the phenol group for radiolabeling (Fig. 1).[29] Due to structural differences between ^{125}I-IMT and natural L-Tyr, renal physiological accumulation of ^{125}I-IMT is greater than that of ^{14}C-labeled L-Tyr, particularly in S2 of the proximal tubule.[29] Greater accumulation of ^{125}I-IMT in the S2-located region may be due to α-methylation of natural L-Tyr.[29]

A recent theory concerning ^{125}I-IMT accumulation in renal proximal S1–S3 cells is consistent with the present findings (Fig. 6).[28] Clarification of these accumulation mechanisms may aid in the development of ^{125}I-IMT as a renal imaging agent. Two major types of transport pathway are responsible for ^{125}I-IMT accumulation in the renal cortex proximal tubule cells, as described below.

The first category includes pathways involving amino acid transport systems. Systems L, A and B^0, and perhaps some minor amino acid transporters, contribute to these pathways. Langen et al. reported that system A does not contribute to ^{125}I-IMT transport in certain tumor cell lines.[26] However, 2-(methylamino)isobutyric acid significantly inhibits ^{125}I-IMT transport in normal human proximal tubule cells.[28] After glomerular filtration, most tubular resorption of neutral amino acids via system B^0 occurs in the apical membranes of S1 cells. This suggests that the S1 region (green region of renal cortex in Fig. 2) is suitable for evaluation of amino acid transport using $^{123/125}$I-IMT. L-type amino acid transporter 2 (LAT2) in the basolateral membranes of S1 cells mediates transport of neutral amino acids from S1 cells into the blood. The isoform of system L that contributes to ^{125}I-IMT resorption in the basolateral membrane is thought to be LAT1, rather than LAT2. We recently reported that LAT1 mediates influx of ^{125}I-IMT, while LAT2 does not.[30] In future studies, we plan to examine efflux of IMT via LAT1 and LAT2.

The second category includes pathways involving organic anion transport systems in S2 cells. Mother L-Tyr does not show affinity for OAT1,[31,32] but ^{125}I-IMT renal accumulation and excretion via the kidneys are inhibited by probenecid and *p*-aminohippurate (PAH). This suggests that ^{125}I-IMT has affinity for organic anion transport systems. OAT1 is expressed in the basolateral membrane of the middle segment of the proximal tubule (S2 cells), and mediates cumulative secretion of PAH and other organic anions with multi-drug-resistant protein 2 (MRP2) and other proteins in the apical membrane.[33] We recently reported that MAL displays affinity for OAT1.[31] In the present study, autoradiography (ARG) at 30 min after injection of MAL (Fig. 4) showed that accumulation of ^{125}I-IMT was markedly inhibited by MAL in the S2 region (red region of renal cortex in Fig. 2). Total ^{125}I-IMT accumulation in the whole kidney was reduced 30 min after injection of MAL (Fig. 5), but this may be due to inhibition of ^{125}I-IMT accumulation by MAL in S2. Accumulation of ^{125}I-IMT in S2 cells is the result of a combination of contributions from organic anion and amino acid transport systems. This suggests that the S2 region is not suitable for evaluation of amino acid transport or resorption using ^{125}I-IMT.

Overall, most ^{125}I-IMT accumulation in the S1 and S2

regions of mouse renal cortex is due to activity of neutral amino acid transport systems (S1 cells) and organic anion transport systems (S2 cells) in the proximal tubule, respectively.

The present experimental study suggests that $^{123/125}$I-IMT is an effective marker for detecting renal tubular transport dysfunction in drug-induced Fanconi syndrome. Methods such as tumor imaging with renal imaging in the same study by ^{123}I-IMT may thus help improve the safety of treatment with nephrotoxic anticancer drugs. In future studies, we plan to assess the efficacy of using ^{125}I-IMT to monitor renal dysfunction induced by nephrotoxic clinical drugs.

CONCLUSIONS

We evaluated use of ^{123}I-IMT as a renal functional imaging agent for detection of drug-induced Fanconi syndrome. MAL-induced Fanconi syndrome was detected as decreases in accumulation of ^{125}I-IMT and ^{99m}Tc-DMSA in the S1 region of the proximal tubule of mouse renal cortex within 30 min after MAL administration by intraperitoneal injection (6 mmol/kg). The present findings suggest that decreases in ^{123}I-IMT accumulation by amino acid transporters in the S1 region offer a useful marker for detection of MAL-induced Fanconi syndrome.

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