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Metformin is a Superior Substrate for Renal Organic Cation Transporter OCT2 rather than Hepatic OCT1

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Summary: Although metformin, a cationic agent for type II diabetes, shows its pharmacological effect in the liver, the drug is mainly eliminated into urine. The tissue selectivity based on the function of drug transporters is unclear. In the present study, the transport of metformin was examined using HEK293 cells transiently transfected with five human renal organic ion transporter cDNAs. Human OCT1 and OCT2, but not OAT1, OAT3 or OCT2-A, stimulated the uptake. A kinetic analysis of metformin transport demonstrated that the amount of plasmid cDNA for transfection was also important parameter to the quantitative elucidation of functional characteristics of transporters, and both human and rat OCT2 had about a 10- and 100-fold greater capacity to transport metformin than did OCT1, respectively. In male rats, the mRNA expression level of rOCT2 in the whole kidneys was 8-fold greater than that of rOCT1 in the whole liver. The in vivo distribution of metformin in rats revealed that the expression level of renal OCT2 was a key factor in the control of the concentrative accumulation of metformin in the kidney. These findings suggest that metformin is a superior substrate for renal OCT2 rather than hepatic OCT1, and renal OCT2 plays a dominant role for metformin pharmacokinetics.

Key words: organic cation transporter; kidney; liver; diabetes; biguanide; TEA

Introduction

Tissue-specific organic cation transporters contribute to the hepatic- or renal-selective distribution of cationic compounds including endogenous substrates, drugs, and their metabolites. Gründemann et al. 1) isolated a rat organic cation transporter, rOCT1 (slc22a1), which was preferentially expressed in the liver and kidney. We isolated rOCT2 (slc22a2) which was homologous to rOCT1, and found that its mRNA was solely expressed in the kidney. 2) In humans, OCT1 (SLC22A1) is primarily expressed in the liver, 3) and the human (h)OCT1 could not be detected in the human kidney. 4) In addition, hOCT2 (SLC22A2) was found to be the most abundant organic cation transporter in the basolateral membranes of human kidney. 4) Because OCT1 and OCT2 were similar in substrate specificity, it had been difficult to explain the difference in the tissue distribution of cationic drugs between liver and kidney. 5)

One of the biguanide agents, metformin, is extensively excreted into urine, mostly via the tubular secretion. 6) However, it has been considered that the pharmacological target organ is the liver, and the lactic acidosis is one of the severe side effects of the drug. Wang et al. 7) reported that the OCT1 mediated the intestinal and hepatic distribution of metformin in rats and mice. The relation between the OCT1 expression and lactic acidosis was reported by use of OCT1 null mice. 8) Using the electrophysiological technique in Xenopus oocytes, Dresser et al. 9) suggested that metformin and phenformin interacted with hOCT1 and hOCT2. Recently, we demonstrated that metformin was a substrate for the renal OCT2. 10) However, there is no...
information about the contribution of OCT1 and OCT2 on the in vivo pharmacokinetics of metformin. The quantitative characteristics of metformin transport by OCT1 or OCT2 are needed to understand the hepatic or renal selectivity of metformin.

In the present study, the quantitative analyses for metformin transport activity, using the limited amount of transfected cDNA, have been performed to clarify the difference in the transport capacity as well as the substrate affinity between OCT1 and OCT2 using both the human and rat clones. The results clearly revealed that metformin was a superior substrate for the renal OCT2 rather than hepatic OCT1.

Methods

Materials: [Biguanidine-14C]metformin hydrochloride (26 mCi/mmoll) was purchased from Moravek Biochemicals, Inc. (Brea, CA). Metformin and 1-methyl-4-phenylpyridinium iodide were obtained from Sigma-Aldrich Co. (St. Louis, MO). All other compounds used were of the highest purity available.

Cell culture and transfection: HEK 293 cells (ATCC CRL-1573, American Type Culture Collection, Manassas, VA) were cultured in complete medium consisting of Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum in an atmosphere of 5% CO2/95% air at 37°C, and used as host cells. pCMV6-XL4 plasmid vector (OriGene Technologies, Rockville, MD) DNA (800 ng) containing hOCT1, hOCT2, and hOCT2-A cDNA, and pBK-CMV vector (Stratagene, La Jolla, CA) DNA (800 ng) containing hOAT1, hOAT3, rOCT1, and rOCT2 cDNA were used to conduct the transient expression analysis, as described.10 The transfectant stably expressing hOCT1 was established as described previously.10,12 The cell monolayers were used at day 3 of culture for uptake experiments.

Uptake experiments: Cellular uptake of cationic compounds was measured with monolayer cultures of HEK293 cells grown on poly-D-lysine-coated 24-well plates.10,12 The incubation medium for uptake experiments contained: 145 mM NaCl, 3 mM KCl, 1 mM CaCl2, 0.5 mM MgCl2, 5 mM D-glucose, and 5 mM HEPES (pH 7.4). The pH of the medium was adjusted with NaOH or HCl. The cells were preincubated with 0.2 mL of incubation medium for 10 min at 37°C. The medium was then removed, and 0.2 mL of incubation medium containing [14C]metformin or [ethyl-1-14C]tetraethylammonium (TEA) bromide was added. The medium was aspirated off at the end of the incubation, and the monolayers were rapidly rinsed twice with 1 mL of ice-cold incubation medium. The cells were solubilized in 0.5 mL of 0.5N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. The protein content of the solubilized cells was determined by the method of Bradford,13 using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) with bovine γ-globulin as a standard.

mRNA expression of organic cation transporters: The expression levels of hOCT1, hOCT2, rOCT1, and rOCT2 in HEK293 transfectants were quantified as described previously with some modifications.6 Briefly, total cellular RNA was isolated from specimens using a MagNA Pure LC RNA isolation Kit II (Roche Diagnostic GmbH, Mannheim, Germany) and was reverse-transcribed to cDNA. Real-time polymerase chain reaction (PCR) was performed using the ABI prism 7700 sequence detector (Applied Biosystems, Foster, CA). Glyceraldehyde-3-phosphate dehydrogenase mRNA was also measured as an internal control with glyceraldehyde-3-phosphate dehydrogenase Control Reagent (Applied Biosystems).

For quantification of the organic cation transporter mRNAs in the whole liver or kidneys in rats, we harvested each tissue from Wistar male rats weighing between 300 g and 330 g (12 weeks old, n = 4), and the isolated whole liver or kidneys were minced and incubated in the RNA Later® (QIAGEN GmbH, Hilden, Germany) for 10 hours at 4°C. After re-mincing the tissues, a part of the mixed tissue samples were subjected to total RNA extraction. The total RNA extraction, reverse-transcription and real-time PCR were performed as described above.

In Vivo intravenous administration study: The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University. Male and Female Wistar/ST rats weighing 210–240 g were anesthetized with sodium pentobarbital and the femoral artery and vein were cannulated with polyethylene tubing. Tracer amounts of metformin (1 mg/kg) dissolved in saline were administered as a bolus via the catheterized right femoral vein. Blood samples were collected at 0.5, 1, 1.5, 2, 2.5, and 3 min from the left femoral artery. Three minutes after the injection, the kidneys and livers were collected immediately after sacrifice. The excised tissues were gently washed, weighed, and homogenized in 3 volumes of saline.14 Aliquots (100 μL) of blood and tissue homogenates were deproteinized with methanol (200 μL) and then subjected to HPLC.

HPLC analysis: A high-performance liquid chromatograph LC-10AD (Shimadzu Co., Kyoto, Japan) was equipped with an UV spectrophotometric detector (SPD-100A; Shimadzu Co.) adjusted to 236 nm for metformin and integrator (Chromatopac C-R8A;

The accession numbers of cDNAs used in the present study were as follows: hOCT1, X98332; hOCT2, X98333; hOCT2-A, AB075951; hOAT1, AB009698; hOAT3, AF097491; rOCT1, X78855; rOCT2, D83044.
Fig. 1. Transport activity for [14C]Metformin by HEK293 cells transiently expressing human organic ion transporters.

(A) HEK293 cells transfected with pCMV6-XL4 vector (open circle), hOCT1 (open triangle), hOCT2 (closed circle), or hOCT2-A (closed triangle) were incubated for the specified periods at 37°C with 0.2 mM (9.26 kBq/mL) [14C]metformin. (B) HEK293 cells transfected with pBK-CMV vector (open circle), hOAT1 (open triangle), or hOAT3 (closed circle) were incubated for the specified periods at 37°C with 0.2 mM (9.26 kBq/mL) [14C]metformin. Each point represents the mean ± S.E. of three monolayers. (C-E) HEK293 cells transfected with pCMV6-XL4 vector (open column), hOCT1 (closed column) (C), hOCT2 (closed column) (D), or hOCT2-A (closed column) (E) were incubated at 37°C for 1 min with 0.2 mM of 5 μM (10.36 kBq/mL) [ethyl-1-14C]tetraethylammonium (TEA) bromide. (F) HEK293 cells transfected with pBK-CMV vector (open column), and hOAT1 (closed column) were incubated at 37°C for 1 min with 0.2 mM of 5 μM (9.25 kBq/mL) p-[glycyl-14C]aminohippuric acid (PAH). (G) HEK293 cells transfected with pBK-CMV vector (open column), and hOAT3 (closed column) were incubated at 37°C for 1 min with 0.2 mM of 20 nM (37 kBq/mL) [6,7-3H(N)]estrone sulfate (ES) ammonium salt. Each column represents the mean ± S.E. of three monolayers.

Selective Metformin Transport Via OCT2

Shimadzu Co.). The stationary phase was a Cosmosil 5C18-MS-II column (4.6-mm inside diameter × 150 mm, Nacalai Tesque, Kyoto Japan). The flow rate was 1 mL/min, and the column temperature was maintained at 40°C. The mobile phase consisted of 60% phosphate buffer (10 mM, pH 6.5) and 40% methanol.

Analytical methods: The plasma concentration at 0 min was extrapolated assuming that the concentration data could be fitted to the two-compartment model. The area under the plasma concentration-time curve until 3 min (AUC0–3min) was calculated by the trapezoidal rule. The tissue uptake clearance of metformin was calculated as dividing the tissue accumulation at 3 min by the AUC0–3min.14)

Statistical analysis: Data are expressed as the mean ± S.E. Data were analyzed statistically using a one-way analysis of variance (ANOVA) followed by Fisher’s t test. Significance was set at P < 0.05.

Results

[14C]Metformin by organic ion transporters: First, we examined the [14C]metformin uptake by HEK293 cells transfected with hOCT1, hOCT2, hOCT2-A, hOAT1, and hOAT3 cDNAs. The uptake was markedly stimulated in the OCT2-transfected cells. On the other hand, much less [14C]metformin was taken up by OCT1-transfected cells than OCT2-transfected cells. Other transfectants did not show an increase in the uptake of metformin (Figs. 1A and 1B). The functional expression of each transporter was confirmed using typical substrates (Figs. 1C–1G).

Difference in metformin transport capacity between OCT1 and OCT2: Next, we assessed whether the difference in the uptake of [14C]metformin via hOCT1 and hOCT2 was dependent on the expression level in the transfectants. After transfection with several amounts of hOCT1 or hOCT2 cDNA, each mRNA level (Fig. 2A) and metformin uptake (Fig. 2B) was evaluated. The metformin uptake by each hOCT1- or hOCT2-cDNA transfected HEK293 cells was saturated at the high mRNA level range. Figure 2C shows that the uptake of metformin by hOCT2 was much greater than that by hOCT1 at various mRNA levels. We recently reported that the apparent Km and Vmax values for the transport of metformin by HEK293 cells stably expressing hOCT2 were 1.38 ± 0.21 mM and 11.9 ± 1.5 nmol/mg protein/min, respectively.10) In the current study, we also established HEK293 cells stably expressing hOCT1. The apparent Km and Vmax values for the uptake of metformin by cells stably expressing hOCT1 were 4.95 ± 1.12 mM and 4.34 ± 0.59 nmol/mg protein/min, respectively. Because the hOCT1 and hOCT2
metformin transport.

porters. were suggested to be derived by the transfected trans-

the empty vector (data not shown), the present data

mRNA were not detected in the cells transfected with

DNA solution to give the final volume (800 ng

well) before transfec-

monolayers. (B) HEK293 cells transfected with several amounts of hOCT1 cDNA (open

closed circle and closed triangle), and 750 ng/well of pCMV6-XL4 empty vector were incubated for 2

mRNA in the absence (circle) or presence (triangle) of 5 mM MPP (pH 7.4).

Open and closed triangles are overlapping. Unlabeled metformin was

Fig. 2. Effects of mRNA expression levels of hOCT1 and hOCT2 on [14C]metformin transport.

(A) HEK293 cells cultured in 24-well plate were transfected into a

well with 2, 10, 50, 200, and 800 ng of plasmid cDNA coding hOCT1

or hOCT2. The pCMV6-XL4 empty vector DNA was added to the

DNA solution to give the final volume (800 ng/well) before transfection. Total cellular RNA was extracted from HEK293 cells transfected with several amounts of hOCT1 cDNA or hOCT2 cDNA. The mRNA

levels of hOCT1 (open circle) and hOCT2 (closed circle) were deter-
minal by real-time PCR. Each point represents the mean ± S.E. of three

monolayers. (B) HEK293 cells transfected with several amounts

of hOCT1 cDNA (open circle) or hOCT2 (closed circle) were incubated for 2 min at 37°C with

0.2 mL of 10 μM (9.26 kBq/mL) [14C]metformin. Each point represents the mean ± S.E. of three

monolayers. (C) An illustration of the correlation between the mRNA

level of hOCT1 or hOCT2 (A), and the accumulation of [14C]metformin (B).

(D) HEK293 cells transfected with 50 ng/well of hOCT1 cDNA (open circle

and open triangle) or hOCT2 (closed circle and closed triangle), and

750 ng/well of pCMV6-XL4 empty vector were incubated for 2

min at 37°C with 0.2 mL of 10 μM (9.26 kBq/mL) [14C]metformin in the absence (circle) or presence (triangle) of 5 mM MPP (pH 7.4).

Open and closed triangles are overlapping. Unlabeled metformin was

added to [14C]metformin to give the final concentrations indicated.

Each point represents the mean ± S.E. of three monolayers.

mRNA were not detected in the cells transfected with the empty vector (data not shown), the present data were suggested to be derived by the transfected trans-

Kinetic evaluation of metformin transport by OCTs:

To establish more of a quantitative difference between hOCT1 and hOCT2 in the transport of metformin, we examined the concentration-dependence of [14C]met-

formin uptake without saturating the mRNA expression levels using cells transfected with each OCT-cDNA (50 ng/well) and vector-cDNA (750 ng/well) (Fig. 2D).

Table 1 shows the apparent Km and Vmax values for the uptake of metformin by hOCT1 and hOCT2. The clearance of metformin, Vmax/Km, was much higher in hOCT2-transfectants. Concerning the expression level of each transporter mRNA, the intrinsic clearance of the hOCT2-mediated uptake of metformin was about 10-fold that for the hOCT1-mediated uptake (Table 1).

Prior to examining the tissue distribution of metformin in rats, we ascertained the correspondence between hOCTs and rOCTs. As shown in Fig. 3, the expression-

level dependent manners of metformin transport by rOCT1 and rOCT2 was similar in comparison with those of human orthologue transporters. The apparent intrinsic clearance of rOCT2-mediated uptake of metformin was about 100-fold greater than rOCT1 (Table 1). To confirm the transport activity of these OCTs, we also examined the similar experiments using TEA as a reference substrate (Fig. 4). The expression-

level dependent manners of TEA uptakes by OCTs were observed. Although hOCT2-mediated uptake of TEA was tended to be similar with hOCT1 (Fig. 4A), rOCT2-

mediated TEA uptake was much greater than rOCT1 (Fig. 4B).

To estimate the tissue intrinsic clearance of metformin in the liver or kidney focusing on the organic cation transporters, we quantified the mRNA expression amounts of rOCT1 and rOCT2 in the whole liver (12.3 ± 0.3 g/rat, mean ± S.E. of four rats) and kidney (2.1 ± 0.03 g/rat, mean ± S.E. of four rats) of rats. As shown in the Fig. 5, the mRNA expression level of rOCT2 in the kidneys was 10.3 and 6.5 fold higher in comparison with the renal rOCT1 and the hepatic rOCT1, respectively. Next, the tissue intrinsic clearance of rOCT-

mediated uptake of metformin in each tissue per rat was estimated in combination with the data in Table 1. The renal intrinsic clearance of rOCT2-mediated uptake of

Table 1. Apparent Km values of [13C]metformin uptake by human or rat OCT1 and OCT2.

<table>
<thead>
<tr>
<th>OCT</th>
<th>Km (mM)</th>
<th>Vmax (pmol/mg protein/min)</th>
<th>Intrinsic clearance* (mL/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOCT1</td>
<td>1.47 ± 0.19</td>
<td>396 ± 42</td>
<td>5.09 ± 0.52</td>
</tr>
<tr>
<td>hOCT2</td>
<td>0.99 ± 0.03</td>
<td>1444 ± 81</td>
<td>54.49 ± 4.64</td>
</tr>
<tr>
<td>rOCT1</td>
<td>3.73 ± 0.15</td>
<td>145 ± 6</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>rOCT2</td>
<td>0.63 ± 0.09</td>
<td>1446 ± 55</td>
<td>36.98 ± 4.39</td>
</tr>
</tbody>
</table>

*Values of intrinsic clearance were calculated as follows: Vmax (pmol/mg protein)/Km (mM)/mRNA expression level (amol/mg protein). Experimental conditions are in the legends of Fig. 2D and Fig. 3D.
Fig. 3. Effects of expression levels of rOCT1 and rOCT2 on $[^{14}C]$metformin transport.

The experimental conditions were correspondingly conducted to those of Fig. 2. HEK293 cells were transfected with the several amounts of plasmid cDNA coding rOCT1 or rOCT2, and pBK-CMV empty vector DNA instead of hOCT1 or hOCT2, and pCMV6-XL4 empty vector, respectively.

Fig. 4. Effects of expression levels of organic cation transporters on $[^{14}C]$TEA transport.

The experimental conditions were correspondingly conducted as described for Fig. 2B. HEK293 cells were transfected with the several amounts of plasmid cDNA coding hOCT1 (open circle, A), hOCT2 (closed circle, A), pCMV6-XL4 empty vector DNA (A), rOCT1 (open circle, B) or rOCT2 (closed circle, B), and pBK-CMV empty vector DNA (B).

Fig. 5. mRNA expression levels of rOCT1 and rOCT2 in whole liver and kidney.

After harvesting the whole liver and kidneys from the male rats (12 weeks, 300–330 weighing), total RNA was extracted as described in the method section. The mRNA levels of rOCT1 and rOCT2 in each tissue were determined by real-time PCR. The wet weights of liver and kidneys were 12.3 ± 0.3 and 2.1 ± 0.03 g/rat (mean ± S.E. of four rats), respectively. Each point represents the mean ± S.E. of four rats. N.D., not detected.

Fig. 6. Plasma concentration curves (A) and tissue uptake clearance (B) of metformin in rats.

Metformin (1 mg/kg) was administered as a bolus via the right femoral vein of male (open circle) or female (closed circle) rats. (A) Blood samples were collected from the left femoral artery at 0.5, 1, 1.5, 2, 2.5, and 3 min after the injection. Each point represents the mean ± S.E. of five rats. (B) Three minutes after the administration of metformin, the tissues were harvested. The concentration of metformin in each tissue was measured. The tissue uptake clearance of metformin was calculated as dividing the tissue accumulation at 3 min by the AUC$_{0–3min}$ from each rat. The wet weights of liver and kidneys in male rats were 8.5 ± 0.3 and 1.94 ± 0.05 g/rat, and those in female rats were 7.9 ± 0.3 and 1.5 ± 0.04 g/rat, respectively (mean ± S.E. of five rats). Each column represents the mean ± S.E. for five rats. *$p<0.05$, **$p<0.01$, significant differences.
metformin was negligible compared to rOCT2.

In vivo pharmacokinetic role of rOCT1 and rOCT2 on metformin tissue distribution: Furthermore, we examined the tissue distribution of metformin in vivo focusing on rOCT1 and rOCT2. We previously reported that the levels of rOCT2 mRNA and protein in the kidney were much higher in males than females, but there was no gender-based difference in the mRNA expression of renal rOCT1. Considering these findings, we examined the contribution of rOCT2 to the tissue distribution of metformin using male and female rats. The plasma concentrations of metformin until 3 min after the intravenous administration and the tissue uptake clearances of metformin were determined (Fig. 6). The tissue uptake clearance of metformin in the male kidney was much higher than that in male liver, female liver, and female kidney.

Discussion

In the present study, we have quantitatively elucidated the substrate specificity of OCTs. hOCT2 was identified as a superior transporter mediating the uptake of metformin among several human organic ion transporters examined (Fig. 1), and two transporters, OCT1 and OCT2, had distinct substrate affinity for metformin (Figs. 2 and 3). Although the expression-level dependent profile of TEA uptake by hOCT1 and hOCT2 was similar, the TEA uptake activity of rOCT2 was much greater in comparison with rOCT1 (Fig. 4). By analysing the relation between the expression level and transport activity, some differences on the transport characteristics between rOCT1 and rOCT2 would be clarified. It had been considered that OCT1 and OCT2 possess similar multispecificities for various compounds. However, recent reports suggest that some chemical compounds such as guanidine and creatinine could be used to determine the molecular selectivity between OCT1 and OCT2. In addition, hOCT2-A, a splicing variant of hOCT2, did not transport metformin (Fig. 1A). Therefore, metformin may be an useful probe substrate for clarifying the structural determinant(s) to distinguish the substrate specificities between hOCT2 and hOCT2-A. By use of the transfectants with a limit amount of cDNA, we have simultaneously demonstrated that metformin, which is a derivative of guanidine, was dominantly transported by OCT2 compared to OCT1. To our knowledge, this is the first report demonstrating the quantitative difference in the transport activities of metformin between OCT1 and OCT2. Information about the molecular determinants of substrate binding has emerged gradually. The use of guanidine derivatives including metformin, will clarify the chemical structures required for specific transport by OCT2.

In rat kidney, rOCT2 expression levels were higher in male than female rats, and the uptake of TEA in the renal slices was greater in the male rats. In the present study, the renal uptake clearance of metformin was also higher in male than female rats, comparable with previous findings. In the male rats, the mRNA expression level of rOCT2 in the kidneys was markedly higher than the hepatic rOCT1 (Fig. 5), and the estimated renal intrinsic clearance of metformin by rOCT2 was about 600 times larger than that of the liver by rOCT1. The in vivo tissue uptake clearance of metformin in the male kidney was about 6 times larger than that in the male liver (Fig. 6), and therefore, the plasma flow rate in the kidney might be a limiting factor for metformin renal distribution. Considering the expression levels and the intrinsic clearance of transporter-mediated metformin uptake, there is little contribution of rOCT1 on renal uptake of metformin. Because the hOCT1 was not detected in the human kidney, the hOCT2 was considered as a primary organic cation transporter determining the renal distribution of cationic drugs. These results indicate that rat is still useful animal for pharmacokinetic studies of metformin focusing on the hepatic rOCT1 and renal rOCT2. This is the first report to estimate the expression amounts of organic cation transporter isoforms in the whole liver and kidneys, and these data will be useful to quantitative evaluation of the tissue selectivity of cationic drugs in rodents.

In the present study, the accumulation of metformin in the liver was much lower than that in the kidney (Fig. 6). Because the plasma flow rate could be a limited step, the difference between metformin accumulation into the kidneys and livers was small compared to the in vitro estimation (Table 1, Figs. 5 and 6). However, the expressional dominancy of the rOCT2 in male kidney reflected the concentrated accumulation of metformin in comparison with that in the female rats. Therefore, the expressional amount as well as substrate specificity of transporters in each tissue was suggested to help understanding the tissue selectivity of cationic drugs. In the humans, the kidney function as well as the age was postulated as the predictors for pharmacokinetics of metformin. Although the OCT1 protein is expressed in the kidney and liver in the rat, the expression of OCT1 in the human kidney is negligible. Therefore, considering the present in vitro and in vivo results, it was suggested that renal OCT2 should be more important for the pharmacokinetics of metformin in comparison with hepatic OCT1.

The mechanisms behind the pharmacological actions of metformin have been described, including decreased hepatic glucose production and increased glycogenesis
and lactate production. The liver had been generally considered to be the primary gluconeogenic organ, except in acidic conditions. But several studies have provided considerable evidence that mammalian kidney can make glucose and release it under various conditions.\textsuperscript{21} Since 1938, it has been said that animals’ kidney is also a producer of glucose.\textsuperscript{19,22} Stumvoll \textit{et al.}\textsuperscript{20} suggested an important role for the human kidney in glucose homeostasis, using the combined isotopic-net renal balance approach. Furthermore, the role of the kidney in gluconeogenesis during diabetes has been studied.\textsuperscript{21}

In the present study, the concentrative accumulation of glucose and release it under various conditions.\textsuperscript{21} Since 1938, it has been said that animals’ kidney is also a producer of glucose.\textsuperscript{19,22} Stumvoll \textit{et al.}\textsuperscript{20} suggested an important role for the human kidney in glucose homeostasis, using the combined isotopic-net renal balance approach. Furthermore, the role of the kidney in gluconeogenesis during diabetes has been studied.\textsuperscript{21}

In the present study, the concentrative accumulation of metformin in the kidney by OCT2 has been revealed. These backgrounds, including the present results, suggest that the pharmacological effects of metformin in the kidney as well as liver may be important. Clinical studies of biguanides in diabetic patients should be performed to clarify the pharmacodynamic as well as pharmacokinetic significance of renal OCT2 for the control of blood glucose levels.

In conclusion, the quantitative difference in the metformin transport between OCT1 and OCT2 has been firstly clarified using the limited amount of transfected cDNA, and it has been suggested that the renal OCT2 plays a dominant role for metformin pharmacokinetics.

\textbf{References}


