Involvement of Organic Cation Transporter 1 in Hepatic and Intestinal Distribution of Metformin

DE-SHENG WANG, JOHAN W. JONKER, YUKIO KATO, HIROYUKI KUSUHARA, ALFRED H. SCHINKEL, and YUICHI SUGIYAMA
Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan (D.-S.W., Y.K., H.K., Y.S.); Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Tokyo, Japan (Y.K., H.K., Y.S.); and Division of Experimental Therapy, The Netherlands Cancer Institute, Amsterdam, The Netherlands (J.W.J., A.H.S.)

Received February 11, 2002; accepted March 27, 2002

ABSTRACT
Metformin, a biguanide, is widely used as an oral hypoglycemic agent for the treatment of type 2 diabetes mellitus. The purpose of the present study was to investigate the role of organic cation transporter 1 (Oct1) in the disposition of metformin. Transfection of rat Oct1 cDNA results in the time-dependent and saturable uptake of metformin by the Chinese hamster ovary cell line with $K_m$ and $V_{max}$ values of 377 μM and 1386 pmol/min/mg of protein, respectively. Buformin and phenformin, two other biguanides, were also transported by rOct1 with a higher affinity than metformin: their $K_m$ values were 49 and 16 μM, respectively. To investigate the role of Oct1 in the disposition of metformin, the tissue distribution of metformin was determined in Oct1 gene-knockout mice after i.v. administration. Distribution of metformin to the liver in Oct1(−/−) mice was more than 30 times lower than that in Oct1(+/+) mice, and can be accounted for by the extracellular space. Distribution to the small intestine was also decreased in Oct1(−/−) mice, whereas that to the kidney as well as the urinary excretion profile showed only minimal differences. In conclusion, the present findings suggest that Oct1 is responsible for the hepatic uptake as well as playing a role in the intestinal uptake of metformin, whereas the renal distribution and excretion are mainly governed by other transport mechanism(s).

Metformin, a biguanide, has been used for the treatment of hyperglycemia in patients with type 2 diabetes mellitus. It was developed during the late 1950s, first marketed in Europe in 1959 and available in the United States in 1995. Metformin seems to ameliorate hyperglycemia by improving peripheral sensitivity to insulin, reducing gastrointestinal glucose absorption and hepatic glucose production (Caspar and Creutzfeldt, 1971; Hundal et al., 2000; Borst and Snellen, 2001), although the exact mechanism for its pharmacological action has not yet been fully determined.

Metformin is extensively eliminated from the kidney via glomerular filtration and tubular secretion, approximately 79 to 86% of an intravenous dose being recovered in urine in humans (Sirtori et al., 1978; Tucker et al., 1981). However, despite its long clinical usage, the mechanism underlying its systemic elimination is still unknown. Inhibition of the renal tubular secretion of metformin occurs during its coadministration with cimetidine, resulting in increased systemic exposure of metformin (Somogyi and Gugler, 1987). Cimetidine, a cationic compound, is also known to be cleared via tubular secretion (Somogyi and Gugler, 1983). Grundemann et al. (1999) reported that cimetidine is a substrate of rat organic cation transporters (rOct1 and rOct2), leading to the possibility of the involvement of organic cation-specific transporters in the urinary excretion of metformin.

Oc1s are a family of polyspecific organic cation transporters responsible, at least in part, for the uptake of organic cations, including xenobiotics and endogenous compounds from the systemic circulation, maintaining body fluid homeostasis, and acting as a defense system against toxic agents. Because rOct1 (Slc22a1) was first cloned from the kidney by Grundemann et al. (1994), at least two other members of the OCT

ABBREVIATIONS: Oct, organic cation transporter; rOct, rat organic cation transporter; CHO, Chinese hamster ovary; HPLC, high-performance liquid chromatography; TEA, tetraethylammonium; CL_total, systemic clearance; CL_renal, renal clearance; AUC, area under the concentration-time curve.
family, Oct2/OCT2 (Slc22a2/SLC22A2) and Oct3/OCT3 (Slc22a3/SLC22A3), have been identified in rats, mice, and humans (Koepeell et al., 1999; Inui et al., 2000). rOct1 is expressed in the liver and kidney, whereas rOct2 is expressed mainly in the kidney. Based on these findings, in the present study, we attempted to investigate the possible involvement of Oct1 in the disposition of metformin. Transfection of rOct1 cDNA results in the saturable uptake of metformin as well as two other biguanides, buformin and phenformin, in a Chinese hamster ovary (CHO) cell line, suggesting that these biguanides are substrates of rOct1. Because the Oct1 gene-knockout mouse has recently been developed by Jonker et al. (2001), further investigations with this mouse were also performed to determine the contribution of Oct1 to the tissue distribution of metformin.

**Experimental Procedures**

**Chemicals and Materials.** Metformin, phenformin hydrochloride, and cimetidine were purchased from Sigma-Aldrich (St. Louis, MO). Buformin hydrochloride, acetoneitrile of high-performance liquid chromatography (HPLC) grade, and diethyl ether were purchased from Wako Pure Chemicals (Osaka, Japan). Saline used for infusion was purchased from Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan) and pentobarbital was from Dainippon Pharmaceutical (Osaka, Japan). Deionized and distilled water, successively purified by the Milli-Q system (Millipore, Bedford, MA), was used for reagent preparation. All other chemicals were analytical grade and commercially available.

**Cell Culture and Transfection.** The parent CHO-K1 cells (JCRB9018) purchased from Japanese Health Science Research Sources Bank (Osaka, Japan) were cultured in Dulbecco’s modified Eagle’s medium/F-12 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37°C with 5% CO₂ and 95% humidity. The cDNA fragment was amplified by reverse transcription-polymerase chain reaction with the total RNA of rat liver using an RNA-PCR kit (Takara Shuzo, Osaka, Japan). For polymerase chain reaction, the primers were prepared based on the sequence reported previously (Grundemann et al., 1994). The sequences of the forward and reverse primers were 5'-TTGGGAGTGGTCAAGGAGTGC-3' and 5'-TGAAGGCGCGGGAATTCCA-3', respectively. The amplified fragment was used for library screening. For cDNA library screening, the cDNA libraries were constructed from Sprague-Dawley rat liver using the SuperScript Choice system (Invitrogen). Briefly, poly(A)⁺ RNA was fractionated by sucrose density gradient and fractions containing RNA at approximately 2 kilobase pairs were used as a template for cDNA construction using reverse transcriptase and oligo(dT) primer. After two rounds of screening, single positive colonies were isolated. After coinfection with the M13 helper phage (ExAssist; Stratagene, La Jolla, CA), the cDNA was excised in a pBluescript II SK(−) plasmid from Wako Pure Chemicals (Osaka, Japan). For polymerase chain reaction, the primers were designed as described above and evaporated to dryness. Pellets were dissolved in 200 µl of water for HPLC analysis. In the other groups of mice, urine was collected up to 1 h at 20-min intervals. The systemic clearance (CL<sub>total</sub>) and renal clearance (CL<sub>renal</sub>) were estimated, respectively, from the following equations:

\[
\text{CL}_{\text{total}} = \frac{\text{Dose}/\text{AUC}_{0-\infty}}{} \tag{2}
\]

\[
\text{CL}_{\text{renal}} = \frac{X_{\text{urine}}(0-60)/\text{AUC}_{0-60}}{} \tag{3}
\]

where AUC<sub>0-60</sub> and AUC<sub>0-60</sub> are the area under plasma concentration-time curve up to infinity and 60 min, respectively, and these values were calculated by integration of the biexponential equation, which was obtained by fitting to the plasma concentration profile. X<sub>urine(0-60)</sub> is the cumulative urinary excretion up to 60 min.

**Infusion of Metformin with Cimetidine.** Male Sprague-Dawley rats (8 weeks old, 250–280 g of body weight; Charles River Japan, Kanagawa, Japan) were anesthetized with diethyl ether and both the femoral vein and bladder were catheterized with polyethylene tubing for infusion and urine collection, respectively. Infusion was performed by a basic syringe pump (Harvard Apparatus, Holliston, MA). Metformin dissolved in saline was administered at a loading dose of 0.5 mg/kg of body weight, followed by constant infusion at 0.9 mg/kg/h for 4 h. Cimetidine was administered at the
loading dose of 24 mg/kg and an infusion rate of 50 mg/kg/h. The administered volume of saline was 8.0 ml/kg/h. Blood samples were collected from the cervical vein at 60, 120, 180, and 240 min. Urine was collected by washing the bladder with 0.5 ml of saline at 1-h intervals. Blood samples, deproteinized with 4 volumes of acetonitrile, and urine were subjected to HPLC.

**HPLC Analysis.** The HPLC system involved a model L-7100 pump and a model L-7400 UV monitor (Hitachi, Tokyo, Japan) with 300- × 3.9-mm i.d. C18 µBondapak (10 µm) purchased from Waters (Milford, MA). The mobile phase consisted of 0.01 M phosphate buffer (pH 6.5)/acetonitrile (30:70) at the flow rate of 1 ml/min. The wavelength of the UV detection was at 236 nm. The volume used for HPLC was 50 µl.

**Statistical Analysis.** Statistical analysis was performed by Student’s t test to identify significant differences between various treatment groups.

**Results**

**Uptake of Biguanides in rOct1-Transfected CHO Cells.** The time profile for the uptake of metformin (100 µM), buformin (10 µM), and phenformin (1.0 µM) by rOct1- and vector-transfected CHO cells is shown in Fig. 1, A, C, and E, respectively. Uptake was much higher in rOct1-transfected cells than in vector-transfected cells (Fig. 1, A, C, and E). Because the uptake of each compound was linear up to 5 min (Fig. 1, A, C, and E), the initial uptake was assessed as the uptake for 5 min at various substrate concentrations (Fig. 1, B, D, and F). Saturation of uptake was observed in rOct1-transfected cells, and an Eadie-Hofstee plot revealed a single saturable component for each compound (Fig. 1, B, D, and F). The kinetic parameters for the uptake of metformin, buformin, and phenformin are shown in Table 1. Phenformin has a much higher affinity, but a lower capacity, for rOct1 than metformin, and the intrinsic clearance (V_{max}/K_m) of phenformin was ~2 to 3 times higher than that of metformin (Table 1). TEA, used in the present study as a positive control, exhibited K_m and V_{max} values of 84 µM and 320 pmol/min/mg of protein, respectively. This K_m was not very different from that found in previous reports (47–100 µM; Urakami et al., 1998; Dresser et al., 2000).

**Plasma Concentration and Tissue Distribution of Metformin in Oct1(−/−) and Oct1(+/+) Mice.** The time profiles of the plasma concentration and tissue distribution of metformin at 10 min in Oct1(−/−) and Oct1(+/+) mice are shown in Fig. 2. The plasma concentration of metformin at 5 and 10 min was almost comparable for Oct1(−/−) and Oct1(+/+) mice, whereas a small (less than 2-fold) but significant difference was observed at 1 min (Fig. 2A). The difference at these earlier time points suggests that there is a smaller distribution volume in Oct1(−/−) mice than in Oct1(+/+) mice. The liver concentration of metformin was approximately 30 times higher in Oct1(−/−) mice than in Oct1(+/+) mice. The liver concentration of metformin was also observed in the duodenum, jejunum, and ileum (Fig. 2B). On the other hand, the distribution of metformin to the kidney was almost identical for the two types of mice and the difference in the distribution to the colon was minimal (Fig. 2B). The amount of metformin associated with the kidney at 10 min was 24.0 ± 2.4 and 0.690 ± 0.065% of dose in Oct1(+/+) and Oct1(−/−) mice, respectively. A 3- to 7-fold higher distribution in Oct1(+/+) than Oct(−/−) mice was also observed in the duodenum, jejunum, and ileum (Fig. 2B). On the other hand, the distribution of metformin to the kidney was almost identical for the two types of mice and the difference in the distribution to the colon was minimal (Fig. 2B). The amount of metformin associated with the kidney was 34.2 ± 6.7 and 32.1 ± 7.0% of dose in Oct1(+/+) and Oct1(−/−) mice, respectively.

**Urinary Excretion of Metformin in Oct1(−/−) and Oct1(+/+) Mice.** The urinary excretion of metformin was chased in Oct1(−/−) and Oct1(+/+) mice (Table 2). In all, 55 to 70% of the dose was recovered in the urine up to 60 min after i.v. administration. There was only a minimal difference in both the CL_{renal} and CL_{total} between Oct1(−/−) and Oct1(+/+) mice (Table 2).

**Confinement of Metformin with Cimetidine.** To demonstrate the cation-specific renal excretion of metformin in rodents, the effect of coadministration of cimetidine on the urinary excretion of metformin was investigated in rats (Fig. 3). The plasma concentration of metformin was much higher in the presence of cimetidine than in its absence (Fig. 3A). On
the other hand, the urinary excretion of metformin was significantly reduced in the presence of cimetidine (Fig. 3B).

**Discussion**

Despite the widespread use of the biguanide metformin in the treatment of hyperglycemia, the mechanism(s) underlying its disposition has not yet been clarified. Renal excretion is the major elimination pathway for metformin in humans and is much higher than the glomerular filtration rate (Pennikainen et al., 1979), suggesting involvement of tubular secretion systems, although the detailed mechanism has not yet been determined. Metformin exerts its antidiabetic effects, at least partly, via a direct and/or indirect inhibitory effect on complex 1 of the mitochondrial respiratory chain in hepatocytes (El-Mir et al., 2000). Considering that biguanide compounds are positively charged at physiological pH, there may be membrane transport system(s) for these compounds in the liver. Thus, it is important to clarify their disposition mechanism(s) to understand the factors that may affect the pharmacokinetics and pharmacodynamics of biguanides.

Gene knockout mice for Oct1, which accepts a variety of types of organic cations as substrates, have recently been established (Jonker et al., 2001). Considering that biguanides are also cationic compounds, the involvement of Oct1 in the disposition of biguanides was investigated in the present study using rOct1-transfected cells and knockout mice as the first step to clarify the pharmacokinetic mechanism(s). The increase in the saturable uptake of the three biguanides by rOct1 transfection suggests that these compounds are substrates of rOct1 (Fig. 1; Table 1). The distribution of metformin to the liver and three segments of the small intestine is much lower in Oct1(-/-) mice, compared with Oct1(+/+) mice, suggesting that Oct1 may be involved in the distribution to these tissues (Fig. 2). In the liver and duodenum of Oct1(-/-) mice, the $K_m$ values of metformin were 0.13 and 0.14, respectively, these values being comparable with the extracellular volume of these tissues (Tsuji et al., 1983). Because the plasma concentration at this time point (5–10 µg/ml corresponding to 30–60 µM) is much lower than the $K_m$ of metformin for Oct1, Oct1-mediated transport may not
be saturated. Thus, the distribution of metformin to these tissues seems to be mainly governed by Oct1 at least under linear conditions. These results are compatible with previous observations (Grundemann et al., 1994; Schweifer and Barlow, 1996; Jonker et al., 2001) and suggest that Oct1 localization in the basolateral membrane in the liver may explain its possible function as an uptake mechanism for organic cations (Meyer-Wentrup et al., 1998; Urakami et al., 1998; Karbach et al., 2000). Although the localization of Oct1 in the small intestine has not been clarified yet, the present findings suggest that it has a possible role in the basolateral uptake of biguanides, although further studies are needed to demonstrate this hypothesis.

On the other hand, neither the distribution of metformin to the kidney nor its renal excretion showed any clear difference between Oct1 (−/−) and Oct1 (+/+) mice (Fig. 2B; Table 2), suggesting that Oct1 is not the major transporter involved in the renal uptake of metformin. Because the intrinsic transport activity (Vmax/Km) of metformin by rOct1 is comparable with that of TEA, and the renal uptake of TEA is very similar to the blood flow rate, the renal uptake of metformin may also be limited by the blood flow, suggesting that the change in intrinsic renal uptake due to the lack of Oct1 may result only in a minimal difference in renal uptake in vivo. Therefore, the result obtained in vivo does not fully exclude the possible role of Oct1 in the renal uptake of metformin. Nevertheless, cimetidine inhibits the urinary excretion of metformin and increases its systemic exposure in rats (Fig. 3). A similar drug-drug interaction has also been reported in humans (Somogyi and Gugler, 1987), suggesting that a cation-specific mechanism(s) is involved in the urinary excretion of metformin, irrespective of the species. However, the difference in the dose of cimetidine and metformin was more than 50-fold in rats (Fig. 3), whereas the difference in humans was less than 2-fold (Somogyi and Gugler, 1987). Therefore, the mechanism underlying this drug interaction both in humans and rats should be discussed once additional studies have been performed. rOct2 and rOct3 are also expressed in kidney (Sugawara-Yokoo et al., 2000; Wu et al., 2000) and may be involved in the uptake and/or secretion of cationic compounds. rOct2 is also expressed in the basolateral side of the kidney (Karbach et al., 2000; Sugawara-Yokoo et al., 2000), although the substrate recognition of rOct2 has not yet been clearly distinguished from that of rOct1 (Fig. 3; Table 2), and the renal uptake of biguanides, although further studies are needed to demonstrate this hypothesis.

The present findings highlight the importance of Oct1 as the mechanism for the hepatic distribution of metformin. Its pharmacological actions include a reduction in glucose production and inhibition of the mitochondrial respiratory chain in hepatocytes (El-Mir et al., 2000; Hundal et al., 2000; Owen et al., 2000). The existence of the uptake system for metformin may be compatible with the previous finding that a lower metformin concentration is required to inhibit the respiratory chain in isolated hepatocytes than in isolated mitochondria (Owen et al., 2000). However, the metformin concentration (1–5 mM) that inhibits oxygen consumption in rat hepatocytes is higher than the Km for rOct1 (Table 2), suggesting that rOct1 might be, at least partly, saturated at such an effective concentration. Therefore, further studies are required to clarify the functional relationship between the transport of biguanides by rOct1 and their pharmacological action in hepatocytes.

Acknowledgments

We thank Setsuo Kinoshita for work in the preparation of the gene library and Naomi Morita for help in subcloning and cellular uptake studies.

References


Tsujii A, Yoshikawa T, Nishide K, Minami H, Kimura N, Nakashima E, Terakasai T, Miyamoto E, Nightingale CH, and Yamana T (1988) Physiologically based phar-

Address correspondence to: Dr. Yuichi Sugiyama, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: sugiyama@mol.f.u-tokyo.ac.jp