Renal Tubular Secretion of Varenicline by Multidrug and Toxin Extrusion (MATE) Transporters

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Summary: Multidrug and toxin extrusion (MATE) 1 and MATE2-K, H+/organic cation antiporters, are located at the brush-border membrane of renal proximal tubules. The present study aimed to clarify the role of MATE transporters in tubular secretion of varenicline. Varenicline at a dose of 5 mg/kg was administered to wild-type and Mate1-knockout mice via the jugular vein, and its uptake was measured by high-performance liquid chromatography. The renal secretory clearance of and systemic exposure to varenicline were significantly decreased (54.6%, p < 0.05) and increased (116%, p < 0.05) respectively, by the genetic disruption of Mate1 in mice. Uptake of varenicline and [14C]tetraethylammonium (TEA) was examined in HEK293 cells transiently expressing the human (h) MATE1, hMATE2-K, mouse (m) MATE1, and hOCT2 basolateral organic cation transporter. [14C]TEA uptake in HEK293 cells expressing MATE transporters and hOCT2 was decreased in the presence of varenicline. The calculated IC50 values for hMATE1, hMATE2-K, mMATE1, and hOCT2 were 62.2 ± 6.5, 122.3 ± 67.6, 255.0 ± 37.9, and 1,003.9 ± 135.8 (µM; mean ± S.E. for three separate experiments), respectively. Varenicline uptake was significantly increased in HEK293 cells expressing mMATE1, hMATE1, or hMATE2-K cDNA as well as hOCT2 compared to empty vector-transfected cells. In conclusion, renal MATE transporters were found to be responsible for renal tubular secretion of varenicline.

Keywords: MATE; varenicline; tubular secretion; knockout mouse

Introduction

The smoking-cessation agent varenicline is a selective partial agonist of α4β2 nicotinic acetylcholine receptors; it suppresses the symptoms of nicotine withdrawal and reduces the pharmacologic reward from cigarette smoking.1) Varenicline, which exists as a cation at physiological pH, is mainly excreted in urine as an unchanged form in humans and animals2) and the individual variation in exposure to varenicline is considered to be dependent on renal function.3) Renal clearance involves glomerular filtration, tubular reabsorption, and tubular secretion. Tubular secretion of many organic cations is mediated by the basolateral organic cation transporter (OCT) 2 (SLC22A2) along with the luminal multidrug and toxin extrusion 1 (MATE1, SLC47A1) and MATE2-K (SLC47A2) functioning as luminal H+/organic cation antiporters.4,5) Various cationic compounds have been identified as substrates of MATE transporters such as tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium, cimetidine, metformin, oxaliplatin, and cisplatin.6–12) The important role of MATE transporters in the pharmacokinetics and pharmacodynamics of metformin, a cationic hypoglycemic drug, has been especially well investigated.13–18)
Because \( ^{14}\text{C} \) varenicline is significantly transported in HEK293 cells expressing human (h) OCT2, and renal clearance of and systemic exposure to varenicline were shown to be decreased to 74.9% and increased to 129%, respectively, by the concomitant administration of cimetidine in healthy volunteers, hOCT2 has been suggested to mediate the basolateral uptake of varenicline from the circulation into the kidney. However, the molecular mechanisms involved in the tubular secretion of varenicline at the luminal membrane remain to be clarified.

In the present study, MATE transporter-mediated transport of varenicline was examined with \( \text{Mate}1 \)-knockout \( (\text{Mate}1^{-/-}) \) mice recently generated in our laboratory and in vitro expression systems, in an effort to clarify whether renal MATE transporters are one of the factors responsible for the tubular secretion of varenicline.

**Materials and Methods**

**Materials:** Varenicline tartrate [formula weight: 361.37 (as is), 211.27 (free base)] was kindly provided by Pfizer (Groton, CT). TEA (2.035 GBq/mmol) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). All other chemicals used were of the highest purity available.

**Animals:** Animal experiments were carried out in accordance with the “Guidelines for Animal Experiments of Kyoto University.” All protocols were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University. Male \( \text{Mate}1^{-/-} \) (wild-type) and \( \text{Mate}1^{-/-} \) (13 weeks of age, C57BL/6 genetic background) mice generated in our laboratory were used.

**Varenicline protein binding ratio in mouse plasma:** Mouse plasma was spiked with varenicline in saline to a final concentration of 10 \( \mu \)g/mL and incubated for 30 min at 37°C. The spiked plasma was centrifuged in a Microcon Ultracel YM-30 (Merck, Darmstadt, Germany) for 30 min at 1,200 \( \times \) g. The total varenicline concentration in the spiked plasma and ultrafiltrated plasma was analyzed by high performance liquid chromatography (HPLC) after filtration through a Cosmonice Filter W (0.45 \( \mu \)m; Merck). The percentage of bound drug was calculated as follows: 100 \( \times \) (total concentration – ultrafiltrated plasma concentration)/(total concentration).

**Pharmacokinetic experiments:** Pharmacokinetic experiments were carried out as described with some modifications. Mice were anesthetized by an intraperitoneal administration of 50 mg/kg sodium pentobarbital. A catheter was inserted into the right jugular vein with polyethylene tubing (Intramedic PE-10; BD Biosciences, San Jose, CA) for drug administration. Urine was collected from the urinary bladder catheterized with SP-31 tubing (Natsume Seisakusho, Tokyo, Japan). Thereafter, 5 mg/kg varenicline and 146 mg/kg mannitol were administered as a bolus via the jugular vein. Then, 1% mannitol was administered to maintain a sufficient and constant urine flow rate by continuous infusion at 0.35 mL/h using an automatic infusion pump (Harvard Apparatus, Inc., Holliston, MA). Blood samples were collected from both femoral veins at 1, 5, 15, and 30 min and from the abdominal aorta at 60 min after drug administration. Urine was collected for 60 min after drug administration. At the end of the experiment, the kidney and liver were removed to determine the tissue concentration of varenicline. The concentrations of varenicline in plasma, urine, the renal homogenate, and the hepatic homogenate were determined by HPLC. The plasma samples were obtained by centrifugation of blood. Plasma or urine (25 \( \mu \)L) was deproteinized by adding 50 \( \mu \)L of methanol. The samples were centrifuged and supernatants from plasma or urine were diluted 4- or 200-fold with saline, respectively. The supernatants were filtered through a Cosmonice Filter W (0.45 \( \mu \)m) and varenicline concentrations were determined by HPLC. The excised tissues were gently washed, weighed, and homogenized in 9 volumes of saline. Homogenates (150 \( \mu \)L) were deproteinized by adding 300 \( \mu \)L of methanol, and the samples were centrifuged. The supernatants were filtered through a Cosmonice Filter W (0.45 \( \mu \)m) and varenicline concentrations were determined by HPLC. The levels of creatinine in plasma and urine at 60 min were determined by the Jaffé reaction using an assay kit from Wako Pure Chemical Industries.

**Determination of pharmacokinetic parameters:** The area under the blood concentration-time curve from time 0 to 60 min \( \text{AUC}_{0\text{–}60} \) was determined by the trapezoidal rule. Renal clearance \( \text{Cl}_{\text{ren}} \) of varenicline was obtained by dividing the amounts of varenicline eliminated into urine during 60 min by the \( \text{AUC}_{0\text{–}60} \). The renal secretory clearance \( \text{Cl}_{\text{sec}} \) of varenicline was calculated by subtracting creatinine clearance \( \text{Ccr} \) from \( \text{Cl}_{\text{ren}} \), taking into consideration the protein binding ratio of varenicline in mouse plasma. The non-renal clearance \( \text{Cl}_{\text{nr}} \) of varenicline was calculated as follows: \( \text{[injected dose (\( \mu \)g)/AUC}_{0\text{–}60} \) (\( \mu \)g/min/mL – renal clearance (mL/min)/mouse body weight (kg)). The kidney-to-plasma concentration ratio \( (K_{\text{a}, \text{kidney}}) \) and liver-to-plasma concentration ratio \( (K_{\text{a}, \text{liver}}) \) were calculated by dividing the tissue concentration by the plasma concentration at 60 min.

**Cell culture and transfection:** HEK293 cells (ATCC CRL-1573; American Type Culture Collection, Manassas, VA) were cultured in complete medium consisting of Dulbecco’s modified Eagle’s medium (DMEM) (Wako Pure Chemical Industries, Osaka, Japan) with 10% fetal bovine serum (Life Technologies Corporation, Carlsbad, CA) in an atmosphere of 5% CO\(_2/95% \) air at 37°C. For transient expression, HEK293 cells were seeded on 24-well (for the cis-inhibition study, 2 \( \times \) 10\(^5\) cells/well) or 12-well (for the hMATE2-K, mMATE1, and hOCT2 varenicline uptake study, 4 \( \times \) 10\(^5\) cells/well) poly-d-lysine-coated plates (BD Biocat, Franklin Lakes, NJ) and transfected with hMATE1, hMATE2-K, mouse (m) MATE1, and hOCT2 cDNA containing plasmid vectors using the LipofectAMINE 2000.
Reagent (Life Technologies Corporation), according to the manufacturer's instructions. The cells were used for uptake experiments at 48 h after transfection. The vectors used for transfection were pcDNA3.1/Hygro(+) (Life Technologies Corporation) for hMATE1 and hMATE2-K,2 pFLAG-CMV-6 (Sigma-Aldrich Co., St. Louis, MO) for mMATE1,20 and pCMV6-XL4 (OriGene Technologies, Rockville, MD) for hOCT2.21 Previously established stably expressing hMATE1 and empty vector cells were used for the hMATE1 varenicline uptake study.12 Stably expressing cells were cultured in the complete medium described above with 0.2 mg/mL hygromycin B (Life Technologies Corporation) (hMATE1) or 0.5 mg/mL G418 (Nacalai Tesque, Kyoto, Japan) (vector) in an atmosphere of 5% CO2/95% air at 37°C and seeded on 6-well poly-D-lysine-coated plates (BD Biocoat) (8 x 10⁶ cells/well) for uptake study. The cell monolayers on day 3 of culture were used for uptake experiments.

**Uptake experiments using HEK293 cells:** The uptake experiments using transiently transfected HEK293 cells were carried out as described previously.12 In cis-inhibition experiments, the cells were preincubated with 0.2 mL of incubation medium containing ammonium chloride (30 mM, 20 min at 37°C, pH 7.4) to induce intracellular acidification for MATE transporters or incubation medium (10 min at 37°C, pH 7.4) for OCT2. The medium was then removed, and 0.2 mL of incubation medium containing [14C]TEA and various concentrations of varenicline was added. The medium was aspirated off at the end of the incubation, and the cells were washed rapidly two times with 1 mL of ice-cold incubation medium. The cells were solubilized in 0.5 mL of 0.5 N NaOH, and then the radioactivity in 200 µL was determined in 3 mL of ACS II (GE Healthcare, Buckinghamshire, UK) by liquid scintillation counting. The IC50 values were calculated from the inhibition plots using the equation, V = V0/[1 + ([I]/IC50)n], by a nonlinear least square regression analysis with Kaleidagraph Version 4.00 (Synergy Software, Reading, PA). V and V0 were the uptake amounts of [14C]TEA in the presence and absence of inhibitor, respectively. [I] is the concentration of inhibitor, and n is the Hill coefficient.

In the hMATE2-K, mMATE1, and hOCT2 varenicline uptake experiment, the medium was then removed after preincubation, and 0.5 mL of incubation medium containing 100 µM varenicline was added. The medium was aspirated off at the end of the incubation, and the cells were washed rapidly two times with 2 mL of ice-cold incubation medium. Thereafter, the cells were scraped from the bottom of the well with 0.4 mL of 10 mM sodium phosphate buffer (pH 5.0) containing 1 mM SDS. Next, 0.4 mL of methanol was added and the mixture was left at room temperature for 1 h. The extraction solution was centrifuged at 15,900 g for 15 min and the supernatant was evaporated at 37°C. Then, 1,500 µL of 1 N NaOH was added to the pellet for determination of protein content.

In the hMATE1 varenicline uptake experiment, the medium was then removed after preincubation, and 0.8 mL of incubation medium containing 6 µM varenicline was added. The medium was aspirated off at the end of the incubation, and the cells were washed rapidly two times with 2 mL of ice-cold incubation medium. Thereafter, the cells were scraped from the bottom of the well with 0.4 mL of 10 mM sodium phosphate buffer (pH 5.0) containing 1 mM SDS. Next, 0.4 mL of methanol was added and the mixture was left at room temperature for 1 h. The extraction solution was centrifuged at 15,900 × g for 15 min and the supernatant was filtered through a Cosmonice Filter W (0.45 µm) and analyzed by HPLC. Then, 500 µL of 1 N NaOH was added to the pellet for determination of protein content.

In the hMATE1 varenicline uptake experiment, the medium was then removed after preincubation, and 0.8 mL of incubation medium containing 6 µM varenicline was added. The medium was aspirated off at the end of the incubation, and the cells were washed rapidly two times with 2 mL of ice-cold incubation medium. Thereafter, the cells were scraped from the bottom of the well with 0.4 mL of 10 mM sodium phosphate buffer (pH 5.0) containing 1 mM SDS. Next, 0.4 mL of methanol was added and the mixture was left at room temperature for 1 h. The extraction solution was centrifuged at 15,900 × g for 15 min and the supernatant was evaporated at 37°C. Then, 1,500 µL of 1 N NaOH was added to the pellet for determination of protein content. After being dissolved in 200 µL of solution [10 mM sodium phosphate buffer (pH 5.0) containing 1 mM SDS:methanol = 1:1], samples were filtered through a Cosmonice Filter W (0.45 µm) and analyzed by HPLC.

The protein content of each NaOH solution was determined with a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) using bovine γ-globulin as a standard.

**HPLC analysis for varenicline detection:** The concentration of varenicline was analyzed using HPLC (model LC-10A; Shimadzu, Kyoto, Japan). The conditions for the measurement of varenicline were as follows: column, TSK gel ODS-80TS (particle size, 5 µm; internal diameter, 250 mm x 4.6 mm; Tosoh, Tokyo, Japan); mobile phase, 10 mM sodium phosphate buffer pH 5.0 containing 1 mM SDS in methanol at 45:55; flow rate, 1.0 mL/min; wave length, 320 nm; injection volume, 50 µL; column temperature, 40°C. Detection limits of varenicline in HEK293 cells, plasma, urine, kidney, and liver were 0.04 µg/mL, 0.5 µg/mL, 0.05 µg/mL, 0.5 µg/mL, and 0.1 µg/mL, respectively.

**Statistical analysis:** All data are expressed as the mean ± S.E. Data were analyzed statistically using the unpaired t test (in cases where variances were not significantly different) or unpaired t test with Welch's correction (when variances were significantly different).

**Results**

**Pharmacokinetics of varenicline in Mate1+/+ and Mate1−/− mice:** Pharmacokinetic profiles of varenicline in Mate1+/+ and Mate1−/− mice were examined. The plasma concentration of varenicline was significantly higher in Mate1−/− mice than in Mate1+/+ mice at 15 min after its administration via the jugular vein (p < 0.001, Fig. 1). The pharmacokinetic parameters are summarized in **Table 1**. The AUC∞ of varenicline in Mate1−/− mice was significantly increased (116%) as compared to that in Mate1+/+ mice (p < 0.05, Table 1). Accordingly, the CLren and CLsec of varenicline in Mate1−/− mice were significantly decreased to 62.3% and 54.6% of the values in Mate1+/+ mice (p < 0.05, Table 1). On the other hand, the value of CLnr was significantly decreased to 116% of the value in Mate1+/+ mice (p < 0.05, Table 1).
Fig. 1. Plasma concentration profile of varenicline in Mate1+/+ (open circle) mice and Mate1−/− (closed circle) mice
Blood samples were collected at the time points indicated. Varenicline levels in the blood samples were determined by HPLC. Each point represents the mean ± S.E. of data from six Mate1+/+ mice and nine Mate1−/− mice. ***p < 0.001, significantly different from Mate1+/+ mice (unpaired t test).

Fig. 2. Urinary excretion of varenicline in Mate1+/+ (open column) mice and Mate1−/− (closed column) mice
Urine was collected for 60 min after varenicline administration. Each column represents the mean ± S.E. of data from six Mate1+/+ mice and nine Mate1−/− mice. *p < 0.05, significantly different from Mate1+/+ mice (unpaired t test).

Fig. 3. Tissue distribution of varenicline in Mate1+/+ (open column) mice and Mate1−/− (closed column) mice
The kidneys (a) and liver (b) were removed to determine the tissue concentration of varenicline at 60 min after varenicline administration. Each column represents the mean ± S.E. of data from six Mate1+/+ mice and nine Mate1−/− mice. *p < 0.05, significantly different from Mate1+/+ mice (unpaired t test with Welch’s correction for a, unpaired t test for b).

Table 1. Pharmacokinetic parameters of varenicline and Ccr in Mate1+/+ mice and Mate1−/− mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mate1+/+</th>
<th>Mate1−/−</th>
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<tbody>
<tr>
<td>AUC0−240 (μg min/mL)</td>
<td>72.6 ± 4.0</td>
<td>84.3 ± 3.0*</td>
</tr>
<tr>
<td>CLtotal (mL/min/kg)</td>
<td>26.0 ± 2.7</td>
<td>16.2 ± 2.2*</td>
</tr>
<tr>
<td>CLur (mL/min/kg)</td>
<td>16.3 ± 1.5</td>
<td>8.9 ± 1.8*</td>
</tr>
<tr>
<td>CLur (mL/min/kg)</td>
<td>43.8 ± 1.5</td>
<td>43.7 ± 1.9</td>
</tr>
<tr>
<td>Km (mL/g liver)</td>
<td>13.3 ± 1.7</td>
<td>14.6 ± 1.2</td>
</tr>
<tr>
<td>Kd (mL/g liver)</td>
<td>7.6 ± 0.4</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>Ccr (mL/min/kg)</td>
<td>10.9 ± 1.5</td>
<td>8.6 ± 0.7</td>
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The CLur of varenicline was calculated by subtracting Ccr from CLtotal, taking into consideration the protein binding ratio of varenicline in mouse plasma, which was 10.5%. Each value represents the mean ± S.E. of data from six Mate1+/+ mice and nine Mate1−/− mice. *p < 0.05, significantly different from Mate1+/+ mice (unpaired t test).

Similar between Mate1+/+ and Mate1−/− mice and larger than CLur (Table 1). Urinary excretion of varenicline was 36.9% and 26.7% of the administered dose in Mate1+/+ and Mate1−/− mice, respectively (p < 0.05, Fig. 2). The renal and hepatic concentrations of varenicline in Mate1−/− mice were significantly higher than those in Mate1+/+ mice (p < 0.05, Fig. 3).

Inhibitory effect of varenicline on [14C]TEA uptake by hMATE1, hMATE2-K, mMATE1, and hOCT2: We examined the interaction between varenicline and four organic cation transporters. [14C]TEA uptake by hMATE1, hMATE2-K, mMATE1, and hOCT2 were decreased in a concentration-dependent manner in the presence of varenicline (Fig. 4). The apparent IC50 values calculated from the inhibition plot in Figure 4 by nonlinear regression analysis were 62.2 ± 6.5, 122.3 ± 67.6, 255.0 ± 37.9, and 1,003.9 ± 135.8 (μM, mean ± S.E. for three separate experiments) for hMATE1, hMATE2-K, mMATE1, and hOCT2, respectively. The inhibitory effect of varenicline on [14C]TEA uptake by hMATE1 was 16.1-fold stronger than its effect on hOCT2.

Varenicline uptake by hMATE1, hMATE2-K, mMATE1, and hOCT2: Next, varenicline uptake in HEK293 cells transiently expressing hMATE1, hMATE2-K, mMATE1, and hOCT2 was examined. Significant uptake of varenicline by hMATE1 (p < 0.001, Fig. 5a), hMATE2-K (p < 0.01, Fig. 5b), and mMATE1 (p < 0.001, Fig. 5c) was observed. In addition, varenicline uptake by hOCT2-expressing cells was almost twice that of the cells transfected with an empty vector (p < 0.001, Fig. 5d).
Discussion

The principal secretion of cationic drugs in the renal brush-border membrane is mediated by the MATE transporters, which act as the luminal H⁺/organic cation antiporter. In the kidney, P-glycoprotein (Pgp) and carnitine/organic cation transporter (OCTN) 1 and OCTN2 have been examined as potential apical organic cation transporters (as well as MATE transporters) using digoxin and carnitine as a typical substrate, respectively. Among them, MATE transporters showed functional properties comparable with those of the classical H⁺/organic cation antiport system, based on their substrate specificities, comparable inhibitor potencies, and the driving force and sensitivity of the histidine residue-modifying reagent diethylpyrocarbonate and the sulfhydryl reagent p-chloromercuribenzenesulfonate. It was reported that varenicline is neither a substrate nor an inhibitor of Pgp, hOCTN1, or hOCTN2. Therefore, the membrane transporters involved in the luminal efflux of varenicline had remained unclear, although renal excretion was revealed to be the predominant pathway for varenicline’s elimination. In the present study, mMATE1 has been identified to mediate renal tubule secretion of varenicline at the luminal membrane of the kidney using Mate1 null mice. Based on the in vitro transport experiments, hMATE1, hMATE2-K, and hOCT2 as well as mMATE1 mediated the membrane transport of varenicline, and the IC₅₀ value of varenicline against the luminal hMATE1-mediated transport of [³¹C]TEA was 16.1-fold lower compared to basolateral hOCT2. Similarly, cimetidine was identified as another high affinity inhibitor of hMATE1 and hMATE2-K, as compared to hOCT2, although many cationic compounds show an opposite affinity to OCT2 as compared to MATE transporters. Therefore, MATE transporters rather than OCT2 were suggested to be involved in the drug interaction between organic cations and varenicline or cimetidine within the range of clinical dosages of both drugs.

Although only mMATE1 has been identified as an H⁺/organic cation antiporter in the mouse kidney, both hMATE1 and hMATE2-K play predominant roles in the tubular secretion of organic cations in the human kidney. mRNA expression levels of MATE1 and MATE2-K were almost the same in the human kidney. Although substrate specificity of MATE1 and MATE2-K was similar, some
specific substrates were reported. For instance, the zwitterionic drugs cephalin, cephradine, fexofenadine and the oxazolidine antibiotics PNU-288034 were MATE1 specific substrates. On the other hand, oxalplatin was a MATE2-K specific substrate. In the present study, hMATE1 and hMATE2-K as well as mMATE1 mediated the transport of varenicline in the in vitro expression systems (Figs. 5a, 5b, and 5c). The amount of varenicline taken up in hOCT2-expressing cells was almost twice that in cells transfected with empty vector, consistent with a previous report (Fig. 5d). Similarly, the amount taken up by mMATE1, hMATE1, and hMATE2-K expressing cells was almost twice that by empty vector-transfected cells.

The half-life of varenicline in mice is about 1.4 h, and 90% of an administered dose is excreted into urine within 24 h of oral administration. In the present study, the pharmacokinetic experiments were designed to use 5 mg/kg of unlabeled varenicline with sampling performed 60 min after the administration, because radiolabeled varenicline is not available. As a result, the excretion of unchanged varenicline in urine was below 40% of the administered dose during 60 min after the intravenous administration and CL\text{u}, with the lower limit of detection in plasma by HPLC being 0.5 µg/mL, preventing death from the administration of a large amount of drug (Fig. 2, Table 1). A similar event was observed in teicoplanin pharmacokinetic experiments. In a human study, co-administration of cimetidine, a potent inhibitor of hOCT2 and hMATE2-K, caused a 25.1% decrease in the renal clearance of varenicline, suggesting a contribution of these transporters to the active tubular secretion of varenicline. In this study, Matel deficiency caused a 37.7% decrease in the renal clearance of varenicline, and 45.4% decrease in the secretory clearance of varenicline in mice (Table 1). Although other mechanisms remain unclear, at least half of the tubular secretion of varenicline is due to Matel in mice.

In conclusion, based on the data from experiments in vivo and in vitro, this study provides the first evidence that renal mouse MATE1 mediates the tubular secretion of varenicline, and human MATE1 and MATE2-K can mediate the tubular secretion of varenicline.

References


