Involvement of Renal Efflux Transporter MATE1 in Renal Excretion of Flecainide

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Flecainide, an anti-arrhythmic drug, undergoes renal excretion through active renal tubular secretion in addition to passive glomerular filtration. The contribution of renal uptake and efflux transporters in active renal tubular secretion of flecainide remains unclear except that flecainide is a substrate of human multidrug resistance protein 1 (MDRI). To elucidate renal efflux and uptake transporters involved with active renal tubular secretion of flecainide, we conducted in vitro interaction studies of flecainide using organic cation transporter 2 (OCT2), multidrug and toxin extrusion (MATE) 1, and MATE2-K. Uptake transporter inhibition assays using hOCT2-Chinese hamster ovary (CHO), hMATE1-CHO, and hMATE2-K-Madin Darby canine kidney strain II (MDCKII) cells revealed that flecainide (2.5 µM) inhibited hMATE1-mediated transport by 40% with an IC50 value of 6.7 µM; however, it showed no or weak inhibitory effects on hOCT2- and hMATE2-K-mediated transport. For investigating flecainide as a substrate of hMATE1, the accumulation of flecainide in hMATE1-CHO was compared with that in control cells. Uptake transporter substrate assay revealed that flecainide (1 µM) showed 1.11-fold accumulation though the hMATE1-related active transport was significantly decreased in the presence of quinidine (42.0 ± 23.9 vs. 11.8 ± 4.1 pmol/mg in transfected cells; p < 0.05). These results suggest that flecainide is a weak substrate of hMATE1, which is involved in the renal tubular secretion of cationic drugs, and hMATE1 may be less important in the pharmacokinetic drug–drug interaction for renal excretion of flecainide. However, in vivo drug–drug interaction studies of flecainide with substrates of hMATE1 may be needed because flecainide has the potential to inhibit hMATE1.

Key words flecainide; transporter; renal tubular secretion; multidrug and toxin extrusion

INTRODUCTION

Flecainide is a strong sodium channel blocker commonly used to treat various supraventricular tachyarrhythmias. 1,2 Flecainide undergoes hepatic metabolism, and around 40% of a single dose is excreted as an unchanged drug in the urine. 3,4 Since renal impairment prolongs flecainide elimination and thereby increases its serum concentrations, 5 renal excretion of flecainide is a major elimination pathway. Unchanged flecainide is excreted by active renal tubular secretion in addition to passive glomerular filtration. 6 However, the mechanism underlying active renal tubular secretion in the renal excretion of flecainide remains unknown.

Drug transporters mediate active renal tubular secretion through uptake in the basolateral membrane and efflux in the apical membrane of renal proximal tubules. 7 A previous in vitro study has reported that renal excretion of flecainide is associated with multidrug resistance protein 1 (MDRI) in the renal tubule. 8 Although organic cation transporter (OCT) 2 for basolateral uptake and multidrug and toxin extrusion (MATE) 1 and MATE2-K for apical efflux are also involved in the renal tubular secretion of cationic drugs, 9 the contribution of renal uptake and efflux transporters in active renal tubular secretion of flecainide, a cationic drug, remains unclear. Because drug–drug interactions in renal excretion of flecainide may involve the inhibition of renal uptake and efflux transporters, it is important to understand the involvement of these transporters in renal tubular secretion of flecainide. To elucidate the transporter involved with renal tubular secretion of flecainide, we conducted in vitro interaction studies of flecainide using OCT2, MATE1, and MATE2-K.

MATERIALS AND METHODS

Chemicals Flecainide was kindly supplied by Eisai Co. (Tokyo, Japan). Verapamil, quinidine, cimetidine, and pyrimethamine were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). [14C]Metformin was purchased from Moravek Biochemicals (Brea, CA, U.S.A.). All other chemicals were commercially available and were of analytical grade.

Uptake Transporter Inhibition Assay Uptake transporter inhibition assays were performed using hOCT2 and hMATE1, and hMATE2-K which were stably expressed in Chinese hamster ovary (CHO) and Madin Darby canine kidney strain II (MDCKII) cells, respectively. The transfected cell lines were developed and characterized by SOLVO Biotechnology (Szeged, Hungary). Cells were cultured in a 1:1 mixture of Dulbecco’s modified Eagle’s medium (DMEM)–Ham’s F12 (DMEM–F12; Lonza, Allendale, NJ, U.S.A.) for hOCT2 and hMATE1, and DMEM glucose (1 g/L) (Lonza) for hMATE2-K at 37°C in an atmosphere with 5% CO2. Cells were plated onto standard 96-well tissue culture plates at a density of 1 × 104 cells/well. Uptake studies were performed for 24 h for hOCT2 and hMATE2-K or 48 h for hMATE1 after seeding. Before the experiment, the medium was removed, and the cells were washed twice with 100 µL of Henseleit–Krebs buffer at pH 7.4 for hOCT2 or pH 8.0 for hMATE1 and hMATE2-K (prepared from Sigma Chemicals, Sigma-Aldrich). Uptake experiments were conducted at 37°C in 50 µL of Henseleit–Krebs buffer (pH 7.4 for hOCT2 or pH 8.0

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for hMATE1 and hMATE2-K) containing the probe substrate and flecainide or solvent. A pH value of 8.0 was chosen for uptake experiments for hMATE1 and hMATE2-K to create an outward-directed proton gradient in order to drive cellular uptake of the organic cationic substrate. \[^{14}C\]Metformin was used as the probe substrate at a concentration of 2 µM for hOCT2, 3.43 µM for hMATE1, and 10 µM for hMATE2-K. Uptake transporter inhibition of flecainide was examined at the concentration of 2.5 and 25 µM. The 2.5 µM flecainide corresponded to the concentration at the upper limit of therapeutic range for trough flecainide concentrations. 

Verapamil (100 µM), quinidine (100 µM), and pyrimethamine (10 µM) were used as the reference inhibitors of hOCT2, hMATE1, and hMATE2-K, respectively. Organic solvent concentration was equal in all wells and did not exceed 1%. After the experiment, cells were washed twice with 100 µL of Henseleit–Krebs buffer and lysed with 50 µL of 0.1 M NaOH. Radiolabelled probe substrate transport was determined by measuring an aliquot (35 µL) from each well for liquid scintillation counting. The experiments were run in triplicate.

**Uptake Transporter Substrate Assay**

Uptake transporter substrate assay was performed using hMATE1-CHO as described above. Cells were cultured at 37°C in an atmosphere with 5% CO\(_2\) and were plated on standard 24-well tissue culture plates at a density of 2 × 10\(^5\) cells/well. The uptake of flecainide was determined at a 20-min incubation time point and at the flecainide concentrations of 1 µM. In order to confirm the interaction, the transporter specific uptake of flecainide was determined in the presence of 100 µM cimetidine or quinidine, reference inhibitors. Before the experiment, the medium was removed, and the cells were washed twice with 300 µL of Henseleit–Krebs buffer (pH 8.0) (prepared from Sigma Chemicals). Cellular uptake of flecainide was measured by adding 300 µL of Henseleit–Krebs buffer containing flecainide and by incubating the cells at 37°C. Reactions were quenched by removing the Henseleit–Krebs buffer containing flecainide, and the cells were washed twice with 300 µL of Henseleit–Krebs buffer. Cells were lysed by adding 300 µL of MeOH–H\(_2\)O (2:1) and were incubated for 20 min at 4°C. The amount of flecainide in the cell lysates was determined by an Agilent 6460 Triple Quadrupole (LC/MS System equipped with an Agilent 1290 infinity LC System (Agilent Technologies, Inc., Santa Clara, CA, U.S.A.). A Poroshell 120 EC-C18 column (3.0 i.d. ×50 mm, Agilent Technologies, Inc.) was used as the analytical column and was maintained at 25°C. Mobile phase comprised 2 mM of ammonium formate and acetonitrile with 0.1% formic acid (58:42, v/v), and the flow rate was set at 0.5 mL/min. A Jetstream electrospray ionization source that operated in positive ionization mode was applied for mass spectrometric detection of flecainide. Multiple-reaction monitoring (MRM) was used for mass spectrometric detection. The optimized MRM fragmentation transition of m/z (415.1 → m/z 398.0) for flecainide was monitored at a fragmentor voltage of 120 V and a collision energy of 25 V. The flecainide quantification range was 0.2–10000 nM. The amount of protein in each well was quantified using the BCA Protein assay kit (Thermo Scientific, Rockford, IL, U.S.A.).

**Fig. 1.** Inhibition of OCT2- (A), MATE1- (B), and MATE2K- (C) Mediated Metformin Transport by Flecainide at the Concentration of 2.5 and 25 µM

Verapamil (100 µM), quinidine (100 µM), and pyrimethamine (10 µM) were used as the reference inhibitors of hOCT2, hMATE1, and hMATE2-K, respectively. * Significant difference compared to the control cells (p < 0.05).
The experiments were run in triplicate.

**Data Analysis** GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, U.S.A.) was used for curve fitting and determination of reaction parameters. IC<sub>50</sub> values were derived from a four parametric logistic equation [log (inhibitor) vs. response–variable slope]; the curve was fitted to the relative activity vs. flecainide concentration plot using non-linear regression. Top (maximal response) and bottom (maximally inhibited response) values were not constrained to constant values of 100 and 0, respectively, unless noted otherwise. In the uptake transporter inhibition assay, the test for statistical significance was a one-way ANOVA with a Dunnett’s test. In the MATE1 substrate assay, a t-test was used to determine if differences between the accumulated amount of flecainide or metformin in the control and hMATE1-transfected cells were statistically significant ($p < 0.05$).

**RESULTS**

**Uptake Transporter Inhibition Assay** For the investigation of the interaction of flecainide with hOCT2, hMATE1, and hMATE2-K, we performed an uptake transporter inhibition assay on the uptake transport of the probe substrate metformin. The hOCT2, hMATE1, and hMATE2-K cells showed high uptake activity of the probe substrate metformin (28.52-, 12.31-, and 14.14-fold accumulation, respectively). Lower concentration (2.5 µM) of flecainide inhibited hMATE1- and hMATE2-K-mediated transport by 40 and 17%, respectively, whereas it did not inhibit hOCT2-mediated metformin transport (Fig. 1). Higher concentration (25 µM) of flecainide inhibited hMATE1- and hMATE2-K-mediated transport by 40 and 17%, respectively, whereas it did not inhibit hOCT2-mediated metformin transport (Fig. 1). We further examined the inhibitory effect of flecainide on hMATE1-mediated metformin transport using eight concentrations of flecainide (0.14–300 µM). Flecainide showed a concentration-dependent inhibition of MATE1-mediated metformin transport, with a calculated IC<sub>50</sub> value of 6.7 µM (Fig. 2).

**Uptake Transporter Substrate Assay** For the investigation of flecainide as a substrate of hMATE1, we performed an uptake transporter substrate assay using hMATE1-CHO cells. The accumulation of flecainide in hMATE1-CHO cells was significantly greater than that in the control cells at 1 µM after 20 min incubation ($p < 0.05$; Fig. 3). The relative accumulation of flecainide in hMATE1-CHO and control cells was compared based on the presence and absence of the reference inhibitors cimetidine (100 µM) or quinidine (100 µM). Flecainide showed 1.11-fold accumulation, which was not changed in the presence of cimetidine or quinidine (1.11- and 1.14-fold, respectively; Fig. 3). The hMATE1-related active transport was 42.0 ± 23.9 pmol/mg in transfected cells, which was significantly decreased in the presence of quinidine (11.8 ± 4.1 pmol/mg; $p < 0.05$) but was not changed in the presence of cimetidine (37.1 ± 10.0 pmol/mg). The presence of quinidine also decreased the passive accumulation of flecainide irrespective of the cell line (410.7 ± 19.7 vs. 94.4 ± 1.0 pmol/mg in transfected cells and 368.7 ± 13.6 vs. 82.6 ± 4.0 pmol/mg in the control cells; Fig. 3). The function of hMATE1 in the applied hMATE1-CHO cells was confirmed by the positive control experiments (21.32-fold accumulation of metformin), which showed that quinidine inhibited hMATE1-mediated metformin transport (Fig. 3).

**DISCUSSION**

Renal tubular secretion is an important pathway for the renal excretion of flecainide because it has been shown that renal tubular secretion accounts for approximately 80% of the renal clearance of flecainide. We provide novel evidence that active renal tubular secretion of flecainide was not mediated by hMATE1 in the apical membrane of renal proximal tubules though it inhibited hMATE1-mediated transport. This finding...
is important to understand the molecular mechanisms underly-
ing drug–drug interactions in the renal elimination of fle-
cainide because co-administration of several drugs decreases
total clearance of flecainide.7,8

The interaction of flecainide with hOCT2, hMATE1, and
hMATE2-K was examined using an uptake transporter inhibi-
tion assay of the probe substrate metformin. Flecainide did not
sufficiently inhibit the hOCT2-mediated metformin transport
at concentrations over \( C_{\text{max}} \) of 1.1 \( \mu \text{M} \) (Fig. 1). It showed a
weak inhibition of hMATE2-K-mediated metformin transport
even at a higher concentration of 25 \( \mu \text{M} \) (Fig. 1). In contrast,
flecainide inhibited hMATE1-mediated metformin transport
with an IC\(_{50}\) value of 6.7 \( \mu \text{M} \) (Figs. 1, 2). Unbound \( C_{\text{max}} /\ IC_{50} \), which was 0.07 based on unbound fraction of 0.42,4 was
over 0.02 as the cutoff value for further in vivo evaluation.10

Moreover the reported renal tissue concentration of flecainide
is considerably higher (50 \( \mu \text{M} \)) than the serum concentra-
tion.11 Therefore, the in vivo drug–drug interaction study of
flecainide with substrates of hMATE1 may be needed.

We further examined the accumulation of flecainide in
hMATE1-CHO cells to clarify whether flecainide was a
substrate of hMATE1. Flecainide was a weak substrate for
hMATE1 because the hMATE1-mediated uptake of flecainide
did not reach the threshold of fold accumulation >2\( ^{10} \) (Fig.
3). This result may be attributed to passive accumulation of
flecainide which is a lipophilic drug. Interestingly, flecainide
was accumulated by quinidine-sensitive unknown transport
system because the presence of quinidine decreased the ac-
cumulation of flecainide, irrespectively of the cell line (Fig.
3). These findings suggest that hMATE1 is not involved in the
renal tubular secretion of flecainide.

A previous in vitro study has reported that flecainide was
transported selectively in a basolateral-to-apical direction in
MDR1-expressing renal epithelial LLC-GA5-COL150 cell
monolayers.6 The renal clearance of flecainide is decreased by
co-administration of amiodarone, an MDR1 inhibitor.7,12

Because amiodarone does not show in vitro inhibition of
hOCT2 and hMATE1,13,14 the drug–drug interaction may be
attributable to the inhibition of MDR1. This hypothesis may
also be supported by a decrease in total clearance of flecainide
with co-administration of quinidine or verapamil,15,16 which
are another MDR1 inhibitors.12,15 However, both quinidine
and verapamil are MATE1 and MATE2-K inhibitors.16 In
contrast, the renal clearance of flecainide is not affected by ci-
metidine,17 which is an MATE1 and MATE2-K inhibitor.14,16

These findings suggest that MDR1 is involved primarily in the
pharmacokinetic drug–drug interaction in renal clearance of
flecainide.

In conclusion, we revealed that flecainide was a weak
substrate of hMATE1, which is involved in the renal tubu-
lar secretion of cationic drugs, and the transport activity of
hMATE1 may be less important in the pharmacokinetic
drug–drug interaction for renal excretion of flecainide. How-
ever, in vivo drug–drug interaction studies of flecainide with
substrates of hMATE1 may be needed because flecainide has
the potential to inhibit hMATE1.

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Conflict of Interest The authors declare no conflict of interest.

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