Development and Characterization of a Novel Cell-based Model to Study MATE1- and MATE2-K-mediated Drug Uptake and Interactions

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Abstract

Transporter involved drug-drug interactions (DDIs) in the kidney can prolong the drug elimination half-life, leading to an accumulation of victim drugs in the body and causing pharmacological problems or renal toxicity. Multidrug and toxin extrusion transporters (MATEs), e.g., MATE1 and MATE2-K, localized on the apical membrane of proximal tubule cells are major transporters that mediate the excretion of important medications into the urine. Several studies have demonstrated that apical efflux by MATEs is one of the sites of DDI in the kidney. Therefore, the International Transporter Consortium (ITC) recommends studying the potential interaction of new molecular entities (NMEs) with MATE1 and 2K. A new "Thaw and Go" cell-based transporter model has been developed to evaluate MATE1- and MATE2-K-based interactions, which enables early *in vitro* evaluation of potential MATEs involved in drug induced renal toxicity. In the presence of oppositely directed H^+-gradient or ammonium chloride-induced intracellular acidification, the transport activity of various cations, such as tetraethylammonium (TEA), metformin, and 1-methyl-4-phenylpyridinium (MPP) were significantly stimulated in hMATE1 and hMATE2-K expressing cells more than 10-fold compared to the control cells expressing the empty vector. The concentration-dependent uptake of TEA and metformin in hMATE1 and hMATE2-K demonstrated "simple" Michaelis-Menten kinetics. The inhibitory parameters of known drug inhibitors for MATEs verapamil, quinidine, cimetidine, and ritonavir were also determined using this model, which are comparable to literature reports. The study demonstrates the model is a compliant and useful *in vitro* tool to screen MATE1 and MATE2-K involved in drug interaction and/or drug-induced renal toxicity in the early stages of drug development.

Introduction

Corning Life Sciences has recently launched a novel cell-based transporter model to support the study of regulatory agency recommended SLC transporters, (e.g., OATP1B1, OATP1B3, OAT1, OAT3, OCT2, and OCT1). This new model eliminates the need for intensive cell culture and maintenance, as well as providing consistent lot-to-lot performance. The 2013 International Transporter Consortium (ITC) Drug Transporter white paper published the proposed expansion of transporters for evaluation during drug development, among which, MATE1 (SLC47A1) and MATE2-K (SLC47A2) are being proposed for prospective investigation in drug development.[1]

MATE1 and MATE2-K are mainly localized in the liver and kidney (Figure 1). The oppositely directed H^+-gradient is the driving force for uptake or efflux. Apical efflux by the MATEs family is considered one of the sites of drug-drug interaction in the kidney in addition to OCTs at the basolateral membrane.[2] Several studies have demonstrated that drug-drug interaction between cimetidine and metformin happens at MATEs on the apical membrane of proximal tubular cells, instead of basolateral OCT.[3,4]

Figure 1. MATE1 and MATE2-K

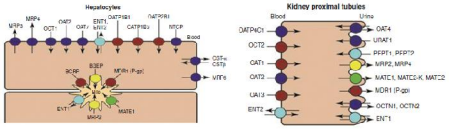


Figure 1: Human MATE1 (SLC47A1) is primarily expressed in the kidney and liver, where it is localized on the luminal/brush-border membranes of renal tubules and canalicular membrane of hepatocytes. Human MATE2-K is kidney-specific MATE2 and localizes at the brush-border membrane of proximal tubules.

Materials and Methods

Corning® TransportoCells™ product: human MATE1 (Cat. No. 354855); human MATE2-K (Cat. No. 354856), and control cells (Cat. No. 354854)

Chemicals: [^{14}C]Tetraethylammonium (TEA), [^{14}C]Metformin, [^{3}H]1-methyl-4-phenylpyridinium (MPP) were purchased from PerkinElmer (Waltham, MA). All the non-radiolabeled compounds were purchased from Sigma-Aldrich (St. Louis, MO)

Plates and Media: Corning BioCoat™ Poly-D-Lysine-coated plate (Cat. Nos. 354414, 354509, and 354461); Corning cellgro® DMEM high glucose (Cat. No. 10-017-CV); MEM non-essential amino acid solution (100X) (Cat. No. 25-025-CI); Fetal Bovine Serum (FBS) (Cat. No. 35-010-CV); Hank's Balanced Salt Solution with Ca^{2+} and Mg^{2+} (1X) (HBSS buffer) (Cat. No. 21-023-CV); Cell Lysis buffer (M-PER) (ThermoScientific Cat. No. 78503); Sodium butyrate (500 mM) (EMD-Millipore Cat. No. TR-1008-G)

Thawing and Plating. The cells were thawed in a 37°C water bath, then resuspended in plating media (DMEM, 1X non-essential amino acid, 10% FBS) with or without sodium butyrate and seeded in 24 well or 48 well Poly-D-Lysine-coated plates at a density of 400K or 200K cells per well, respectively. After 3-4 hours incubation, the cells were re-fed with fresh plating media supplemented with or without sodium butyrate. The cells were kept in a 37°C incubator with 8% CO_2 overnight (low humidity).

Uptake Assay. At 24 hours posting plating, after 10 min pre-incubation with assay buffer (HBSS with 10 mM Hepes, pH 7.4), the cells are incubated with 40 mM ammonium chloride for 20 min. Uptake was initiated by adding substrate or substrate/inhibitor solution. At designated time point, assay was terminated by washing twice with cold assay buffer. Cells were lysed for analysis.

Data Analysis. Kinetic parameters were determined by non-linear regression using SigmaPlot™. For inhibition assay, IC_{50} values were determined by using a Sigmoidal Hill three parameter equation.

References

1. Hillgren KM, et al., (2013) Clinical Pharmacology & Therapeutics 94(1):52-63
2. Motohashi H, et al., (2013) Molecular Aspects of Medicine 34:661-668
3. Ito S, et al., (2012) JPET 340:393-403
4. Tsuda M, et al., (2009) JPET 329:185-191
5. Tanihara Y, et al., (2007) Biochemical Pharmacology 74: 359-371
6. Masuda S, et al., (2006) Journal of the American Society of Nephrology 17:2127-2135

Figure 2: Summary of Corning® TransportoCells™ MATE1 and MATE2-K Performance

Transporters	Post Thaw Viability	Probe Substrate	Incubation Time (min)	Uptake Activity in Transporter Cells (pmol/mg/min)	Uptake Activity in control Cells (pmol/mg/min)	Uptake Ratio
MATE1	95%	30 µM TEA	2	1094	15.8	69
MATE2-K	92%	30 µM TEA	2	571	15.8	36

* 2 mM sodium butyrate (S.B.) is supplemented with plating media. See Figure 4.

Figure 3: Seeding Density Optimization

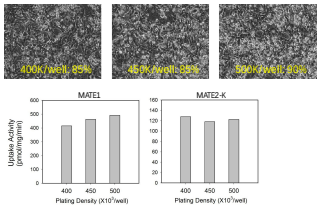


Figure 3: Seeding density optimization for MATE1 and MATE2-K cells. MATE1 and MATE2-K cells were plated on 24 well Poly-D-Lysine (PDL) plates at a density of 400K/well, 450K/well , and 500K/well. The 24-h confluency and uptake activity of 30 µM of TEA were comparable for all seeding densities. 2 mM sodium butyrate were supplemented at plating and the cells were re-fed at 3-4 hours post-plating and assayed at 24 hours. Plating density of 400K/well is recommended for MATE1 and MATE2-K for a 24 well plate assay.

Figure 4. The Effect of Sodium Butyrate on MATE1 and MATE2-K Uptake Activity

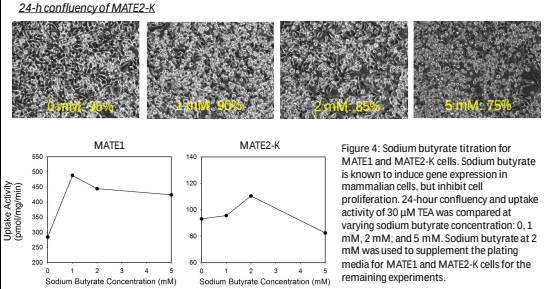


Figure 4: Sodium butyrate titration for MATE1 and MATE2-K cells. Sodium butyrate is known to induce gene expression in mammalian cells, but inhibit cell proliferation. 24-hour confluency and uptake activity of 30 µM TEA was compared at varying sodium butyrate concentration: 0, 1 mM, 2 mM, and 5 mM. Sodium butyrate at 2 mM was used to supplement the plating media for MATE1 and MATE2-K cells for the remaining experiments.

Figure 5. Intracellular Acidification by Ammonium Chloride Stimulates MATE1 and MATE2-K Uptake Activity

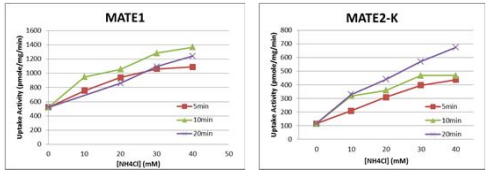


Figure 5. Generation of outward H^+ gradient by ammonium chloride titration. MATE1 and MATE2-K cells were pre-treated with 0, 10 mM, 20 mM, 30 mM, and 40 mM of ammonium chloride for 5 min, 10 min and 20 min, respectively, before assays were initiated. After which the cells were incubated with assay buffer containing 30 µM TEA for 2 min. The results show that 40 mM ammonium chloride and 20 min pre-incubation produces the highest activity. These conditions are recommended for routine studies.

Figure 6. pH-dependent Uptake by MATE1 and MATE2-K

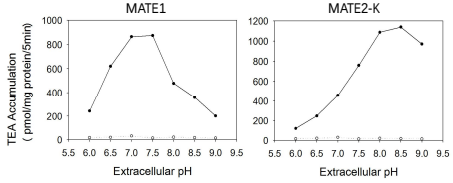


Figure 6: Oppositely directed H^+ gradient-dependent uptake of TEA by MATE1 and MATE2-K cells. The uptake of TEA was examined with the extracellular pH of 6.0 to 9.0, respectively. The cells were pre-incubated with assay buffer (pH7.4) supplemented with 20 mM ammonium chloride for 10 min. Then the cells were incubated with assay buffer (pH 6.0 to 9.0) containing 30 µM TEA for 2 min. The data is in agreement with the previous report that MATEs act as H^+ antiporters for organic cations such as TEA.

Figure 7: Time-dependent Uptake of MATE1 and MATE2-K

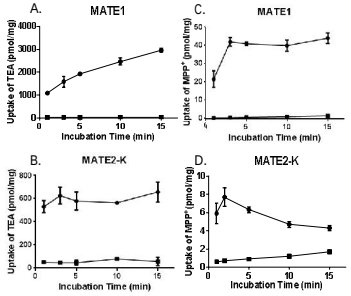


Figure 7: Time course of TEA and MPP$^+$ uptake by MATE1 and MATE2-K. Uptake of 30 µM TEA in MATE1 (A) and MATE2-K cells (B) were determined at 1, 2, 5, 10, and 15 min, respectively at 37°C. Uptake of 100 nM MPP$^+$ in MATE1 (C) and MATE2-K (D) were determined at 2, 5, 10, and 15 min respectively at 37°C. Control cells were used in all time course experiments to demonstrate the time-dependent uptake of selected cations via MATEs. The results represent the mean ± S.D. of three replicates.

Figure 8: Kinetic Analysis of Prototypical Substrate Uptake Activity in MATE1 and MATE2-K

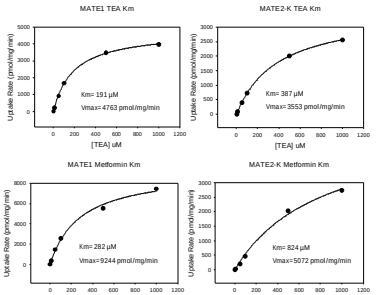
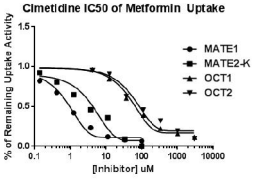


Figure 8: Concentration-dependent MATE1- and MATE2-K-mediated uptake of TEA, Metformin, and MPP+. MATE1 and MATE2-K cells were pre-incubated with ammonium chloride, then incubated with various concentrations of the indicated substrates for 2 min. Six substrate concentrations were used in each kinetic determination. The solid line represents the nonlinear fit of the uptake into the Corning TransportoCells corrected for the uptake in the control cells. MPP+ figure is not shown.

Corning TransportoCells SLC transporter				Reported Kinetic Parameters in Literature
Transporter	Substrate	K_m (µM)	K_m (µM)	Literature
MATE1	TEA	191	220	Otsuka M, et al., Proc Natl Acad Sci (PNAS), 2005
	Metformin	282	227	Chen Y, et al., Pharmacogenomics J, 2009
	MPP+	25.7	100	Tanihara Y, et al., Biochem Pharmacol, 2007
MATE2K	TEA	387	830	Masuda S, et al., J Am Soc Nephrol, 2006
	Metformin	824	1050	Masuda S, et al., J Am Soc Nephrol, 2006

All the reference data were generated using HEK293 based recombinant system

Figure 9: Comparison of Cimetidine Inhibition (IC_{50}) for MATEs and OCTs



	Corning IC_{50}	Ref K$_i$*
MATE1	0.7	3.8
MATE2-K	4.8	6.9
OCT1	57	104
OCT2	110	124

* Ito S, et al. (2012) JPET 340: 393-403

Figure 9: The inhibitory effect of cimetidine on MATE1-, MATE2-K-, OCT1-, and OCT2-mediated uptake of Metformin. IC_{50} was determined by co-incubating the cells with 10 µM Metformin in the presence of a range of concentration of cimetidine (0-100 µM for MATE1 and MATE2-K; 0-3 mM for OCT1, and OCT2 cells). Each point represents the mean value of three replicates and the solid lines represented the non-linear regression fitting. The curve represents one of 3-4 independent experiments. The IC_{50} values generated using Corning TransportoCells MATE1 and MATE2-K cells are comparable to those published in the literature.

Conclusions

- MATE1 and MATE2-K transiently overexpressing HEK293 cells were developed and validated
  - Uptake of cationic compounds by MATE1 and MATE2-K is oppositely directed H^+ gradient-dependent.
  - MATE1 and MATE2-K mediate uptake activity can be induced by sodium butyrate.
- Time- and concentration-dependent uptake of typical cationic substrates was evaluated. Kinetic and inhibition profiles are aligned with those generated using alternative SLC transporter models described in the literature.
- The Corning® TransportoCells™ Cryopreserved SLC transporter model is demonstrated to be an easy to use and robust assay platform that yields consistent results.