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Development and Characterization of a Quintuple Transporter Model for Studying Tubular Creatinine Secretion

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ABSTRACT

Serum creatinine level is a commonly used surrogate measure for glomerule damage and kidney function evaluation. It is well known that creatinine clearance in the kidney is through free glomerular filtration and transporter mediated excretion in proximal tubules. Recently, we have identified that, in addition to OCT2, MATE1 and MATE2K, OAT2 and OCT3, which are predominantly expressed on the basolateral membranes of renal tubular cells, play important roles on creatinine secretion. This new finding led to the development of a novel cellular model co-expressing all five transporters, for modeling active creatinine secretion in the kidney.

We hereby present functional characterization data from this quintuple transporter model, including assay dose- and time-dependence of transcellular transport and intracellular accumulation of [^{14}C]creatinine. The contribution of individual transporters or their combinations was evaluated by studying creatinine transport and intracellular accumulation in cells without these transporter(s). Furthermore, drugs that cause serum creatinine increase clinically were studied using the model by looking at their effects on both transcellular flux and intracellular accumulation of creatinine.

Our data suggest that the apical efflux of creatinine, which is mediated primarily by MATE1, is the rate limiting step of transcellular creatinine flux; while on the basal side, OAT2 could be the major transporter for removing creatinine from blood, which is augmented by OCT2 and OCT3. Most of the drugs we tested inhibited transcellular transport, interestingly they exhibited distinct effects on intracellular creatinine accumulation. Such difference is likely attributed to distinct transporter inhibition profiles. For example, cimetidine, a unique pan-inhibitor of all five transporters, was able to inhibit both transcellular flux and intracellular accumulation of creatinine through blocking both basal uptake and apical efflux of creatinine; whereas trimethoprim was able to block transcellular flux primarily via inhibiting MATE1, which in turn leaded to cellular creatinine build-up as trimethoprim has less effect on basal creatinine transporters. Such mechanistic difference might help understand the discordance between serum creatinine increase and creatinine clearance caused by drugs.

Our results demonstrate that the quintuple transporter model can be a useful *in vitro* tool to study a drug's effect on tubular creatinine secretion.

BACKGROUND

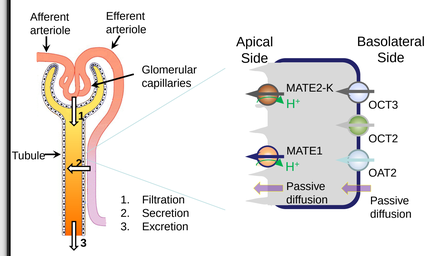

Fig 1. Schematic showing creatinine is excreted by glomerular filtration and proximal tubular secretion (Left) and the major transporters involved in the secretion (Right). There might also exist diffusion at the proximal tubules due to the moderate passive permeability of creatinine (Right).

Creatinine is formed non-enzymatically from creatine in muscle and is completely cleared by renal excretion when renal function is normal. Creatinine is eliminated by glomerular filtration as well as active proximal tubular secretion with the latter accounting for 10-40 % of total creatinine clearance. Due to moderate passive permeability of creatinine (P_{app}=3.2±1.8 nm/s in MDCK cells), a small portion of creatinine may also be cleared through passive diffusion at the proximal tubules (Fig 1).

Serum creatinine (sCr) levels is the most common parameter used clinically for routine monitoring of renal function. However, using sCr to estimate glomerular filtration rate (GFR) results in an overestimation of the actual GFR due to the tubular secretion component of creatinine elimination. A number of drugs from different therapeutic classes have been observed to reduce creatinine clearance without affecting renal function. It has been observed that the sCr increase is not always parallel to the extent of reduction of creatinine clearance, suggesting these drugs have different pharmacological properties in blocking tubular creatinine secretion (Table 1).

Table 1. Examples of drugs affecting serum creatinine and creatinine clearance in patients with normal renal function. The increase in serum creatinine is not always parallel to the extent of reduction of creatinine clearance, suggesting these drugs have different pharmacological properties in blocking the tubular creatinine secretion.

Drug	Δserum creatinine (mg/dL)	Δcreatinine clearance (mL/min/1.73 m^2)
Cimetidine	↑0.37	↓15
Trimethoprim	↑0.28	↓16.1
Pyrimethamine	↑0.24	↓34
Ritonavir	↑0.14	↓14

To understand the roles of transporters in mediating tubular creatinine secretion and to assess a compound's effect on creatinine clearance, we propose to establish a novel cell based creatinine secretion model with the expression of major responsible transporters. This model will provide a convenient tool to analyze the contribution of each transporter and to explore the integrated effect of a drug on creatinine secretion. The relevance of the data generated from this model to the clinical effect of a drug will also be analyzed.

MATERIALS AND METHODS

Millipore Millicell 24-well Cell Culture Plate Assembly (PET-1 μm) with 24-well receiver tray, and 96-well insert plate (PCF-0.4 μm) with 96-well receiver tray were used for transcellular and uptake assays respectively. [^{14}C]-Creatinine was purchased from Moravek Biochemicals.

MDCK-II cells were maintained in DMEM with low glucose and 10% FBS. Cells were seeded and then transfected using a novel *in situ* transfection technology, Opti-Expression™, which allows consistent and effective transfection of polarized cell monolayers. Cells were either transfected with plasmids encoding the SLC transporters or a plasmid encoding GFP as a control.

The cells were used in uptake assays 48 hours after transfection. The cells were pre-incubated with assay buffer (except for MATE1 and MATE2-K) for the appropriate amount of time. Transport was initiated by adding [^{14}C]creatinine or [^{14}C]creatinine/inhibitor mixture in the appropriate chamber of the transwell plate. Following the incubation, the cells were washed and solubilized using a 1:1 acetonitrile:water mixture to measure the intracellular accumulation of substrate. The amount of substrate was quantified by radiometric counting. For MATE1 and MATE2-K uptake assays, the intracellular compartment was initially acidified by preincubation with 30 mM NH_4Cl for 20 min. This treatment reversed the direction of transport for the MATEs from efflux to influx.

Transcellular transport assays were conducted 48 hours after transfection. The cells were pre-incubated with HBSS-HEPES buffer (pH 7.4) with the inhibitor on both apical and basolateral sides. Then the basolateral preincubation mixture were replaced by the substrate or substrate/inhibitor mixture in HBSS-HEPES, and the apical buffer was replaced by HBSS-Bis-Tris (pH 6.0) with the inhibitor/vehicle for the proper functioning of MATEs. The apical buffer was sampled for transcellular transport measurement. The intracellular contents were also analyzed.

RESULTS

1. Determination of the major transporters responsible for creatinine transport

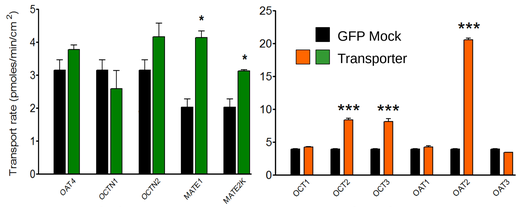

Fig 2. Uptake of 100 μM creatinine in polarized MDCK-II cells expressing various basolateral and apical renal transporters. Data are expressed as mean±SD of triplicate measurements.

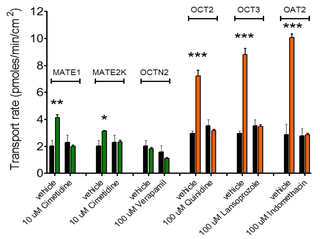

Fig 3. Uptake of 100 μM creatinine by renal transporters in the presence and absence of reference inhibitors.

a) Among the 11 transporters screened, the apical MATE1 and MATE2K, and the basolateral OCT2, OCT3 and OAT2 are the major transporters responsible for creatinine transport (See Ref 6 for details).
b) Contribution of individual creatinine transporters to creatinine secretion is evaluated by selectively expressing transporters in polarized MDCK monolayers.

2. Contribution of each transporter to creatinine transport

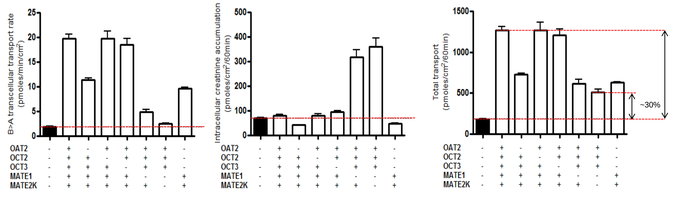

Fig 4. Contribution of individual transporters to creatinine transcellular flux (left), intracellular retention (middle) and total basal clearance (right) in 60 min in MDCK-II cells transfected with different combinations (quintuple (5), 5 minus 1, or 5 minus 2) of transporters.

RESULTS (cont'd)

a) OAT2 is a major transporter for basal creatinine uptake, and MATE1 is the dominant transporter for apical creatinine efflux.
b) Leaving out the apical MATE1 and MATE2-K abolished the transcelular transport but augmented the intracellular accumulation, resulting in a partial inhibition of basal clearance.
c) Taking off the three basolateral transporters did not eliminate the transcellular flux while the intracellular retention was even lower than GFP mock, suggesting the expression of apical MATE1 and MATE2-K may boost the passive diffusion.
d) Overall, omission of the apical two or the basolateral three transporters did not block the basal clearance completely, leaving approximately 30% residue activities (Fig 4. right panel).

3. Drugs' effects on the quintuple-transporter model and clinical relevance

Transporter (substrate) / IC50 (μM)	OAT2 cGMP	OCT2 Metformin	OCT3 MPP$^+$	MATE1 Metformin	MATE2K Metformin
Cimetidine	72.8	135	87.8	0.3	2.3
Trimethoprim	NI	68	12.3	3.3	1.9

Table 2. IC_{50} of cimetidine and trimethoprim on the 5 transporters. Cimetidine is a pan-inhibitor with the most potent inhibition in MATE1. Trimethoprim did not affect OAT2 (Ref 5 & 6 and internal data). NI: no inhibition.

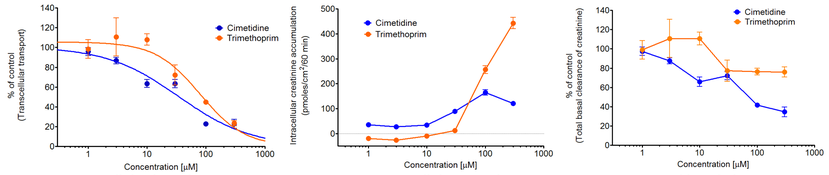

Fig 5. Evaluating the tubular secretion and intracellular retention of creatinine with the quintuple-transporter model by using cimetidine and trimethoprim which elevate serum creatinine without affecting renal function.

a) At high concentration, cimetidine and trimethoprim significantly reduced B→A flux by inhibiting MATEs. Trimethoprim induced much more intracellular accumulation of creatinine as it failed to affect OAT2.
b) The difference in basal clearance of creatinine by cimetidine and trimethoprim is consistent with the clinical observation that at high dose, trimethoprim causes less increase of serum creatinine than cimetidine, possibly due to their difference in blocking basal uptake. (note: the fraction of intracellular creatinine in total basal clearance is time-dependent, less contribution of intracellular creatinine is expected under *in vivo* conditions as it would reach steady state quickly).
c) The discrepancy between serum creatinine increase and creatinine clearance observed in clinical studies (Ref. Table 1) may be due to discounting intracellular creatinine retention in renal tubular cells.

CONCLUSIONS

Polarized MDCK-II cells expressing OAT2, OCT2, OCT3, MATE1 and MATE2-K (Quintuple-transporter) is a novel model providing a more complete, integrated and accurate assessment of drug's effects on creatinine secretion than other *in vitro* models that are currently available. The data obtained from this model may be used to correlate drugs' clinical effects on creatinine secretion.

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