Abstract ID: 762
ASCPT
Mar 6th, 2015
New Orleans, LA

In vitro-In vivo Correlation (IVIVC) of Drug Induced Inhibition of Creatinine Tubular Secretion using MDCK Cells Expressing OCT2/OAT2/OCT3/MATE1/MATE2K Transporters

Xuexiang Zhang, Wenjie Jiang, Chienming Li, Jane Huang, Yong Huang

OPTIVIA BIOTECHNOLOGY

ABSTRACT

BACKGROUND: Serum creatinine (SCr) levels or creatinine clearance (CrCl) are commonly measured to estimate renal function in clinical practices. A number of drugs have been reported to affect SCr/CrCl without nephrotoxicity, attributed to the reduction of tubular creatinine secretion by inhibiting specific transporters. We have identified that OCT2, OAT2, OCT3, MATE1 and MATE2K are the major creatinine transporters, and established a novel creatinine secretion model by co-expressing the quintuple transporters in polarized MDCK-II cells. We explored whether an IVIVC exists between the clinical CrCl reduction by drugs and the inhibition of creatinine transport in our model.

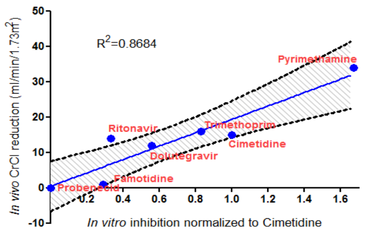

Fig 1: IVIVC between the normalized drug-induced inhibition of creatinine transport at $C_{max,u}$ in the quintuple-transporter model and the clinical reduction of creatinine clearance

METHODS: The inhibition of drugs from different therapeutic categories on the creatinine transport were measured at a wide range of concentrations including the clinical C_{max}. **RESULTS**: All drugs except probenecid (a negative control) attenuated the transcellular transport of creatinine. Inhibition at the clinical C_{max} was quantified and correlated with the corresponding clinical CrCl reduction documented in literature, leading to the appearance of a clear IVIVC (Fig 1). **CONCLUSION:** The quintuple-transporter model of creatinine tubular secretion can be a useful *in vitro* tool to evaluate and predict a drug's effect on creatinine tubular secretion *in vivo*.

INTRODUCTION

Creatinine elimination has been the gold standard of renal function for decades in clinical practice. A number of drugs have been reported to reduce creatinine clearance without adversely affecting renal function. For example, cimetidine completely blocks creatinine clearance at high doses without causing nephrotoxicity. Trimethoprim, an antibiotic, induces partial inhibition of serum creatinine clearance. These effects have been attributed to the reduction of tubular secretion by inhibiting specific transporters in the proximal tubules.

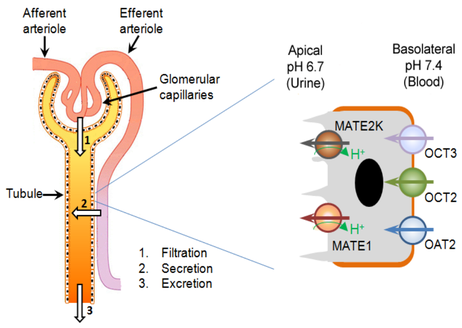

Fig 2. Quintuple-transporter model by expressing OAT2, OCT2, OCT3, MATE1 and MATE2K in the polarized MDCK-II cells to study tubular creatinine secretion in the kidney.

We have previously identified five human renal transporters responsible for creatinine secretion, namely OCT2 (SLC22A2), OAT2 (SLC22A7), OCT3 (SLC22A3), MATE1 (SLC47A1), and MATE2K (SLC47A2) [1]. By expressing these transporters concomitantly in the polarized Madin-Darby canine kidney (MDCK-II) cells, we have established an *in vitro* proximal tubule creatinine secretion model (quintuple-transporter model) [2,3] (Fig 2). The basolateral side of cells is the counterpart of the blood side in tubules, while the apical side resembles the urine side by adjusting pH to 6.7. Therefore, B>A transcellular transport in the quintuple-transporter model simulates creatinine clearance (CrCl) *in vivo*. We have shown cimetidine, a pan-inhibitor of all five transporters, induced an almost complete inhibition of B>A creatinine transport; while trimethoprim, lacking effects on OAT2, evoked only partial suppression on creatinine transport [3].

In this study, we further characterized the role of each transporter in the quintuple-transporter model, by expressing part of five transporters and measuring transcellular flux, intracellular retention and apical efflux of creatinine under the assay conditions mimicking *in vivo* milieu. Moreover, by utilizing the quintuple-transporter model, we evaluated the inhibitory effects of seven drugs from different therapeutic areas on creatinine secretion and correlated the drugs' inhibition obtained in the model at their clinically relevant concentrations with their reported effects on creatinine clearance in patients.

MATERIALS AND METHODS

CELL CULTURE AND TRANSFECTION: MDCK-II cells were seeded in Millipore Millicell 96-well insert plate (PCF-0.4 μm). Approximately 24 hr later, cells were transfected using a proprietary *in situ* transfection technology, Opti-Expression™, which allows consistent and effective transfection of polarized cell monolayers. Cells were transfected with a mixture of plasmids encoding all (quintuple-transporter model) or part of five SLC transporters including OCT2, OAT2, OCT3, MATE1, and MATE2K, at a final concentration of 24, 12, 4, 8, 4 ng/μL, respectively. Relative DNA levels of individual transporters were chosen based on surveying reported relative *in vivo* mRNA or protein levels of these transporters in tubular cells.

TRANSCELLULAR CREATININE TRANSPORT ASSAY: Assays were conducted 48 hr after transfection to allow the cells to become polarized and transporters being appropriately localized. Briefly, cells were pre-incubated with HBSS for 15 min at 37°C. Then the basolateral side was supplied with 100 μM [^{14}C]creatinine in HBSS-HEPES, pH7.4, containing [^{3}H]mannitol as a paracellular permeability marker, mixed with a series concentrations of a testing drug. The apical buffer was replaced by HBSS-MES (pH 6.7) with the same matching concentrations of the drug. Sixty or 90 minutes was allowed for transporters to work in concert to transport creatinine from the basolateral side to the intracellular space, and then sequentially for creatinine to be secreted to the apical space. Creatinine and mannitol contents in the apical, intracellular and basolateral compartments were quantified by radiometric detection on a 1450 Microbeta counter.

***IN VITRO* INHIBITION CURVE FITTING**: The percent inhibition was calculated by dividing B>A flux of creatinine in the presence of a drug against the corresponding value for vehicle, after subtracting the paracellular diffusion surrogated by mannitol based on Stokes-Einstein relationship. Initial free drug concentration on the dosing side was measured by LC-MS/MS to exclude the possible non-specific binding of drugs to assay wares. The concentration-inhibition curve was then fitted by using Graphpad Prism nonlinear variable bottom Hill Equation:

$$\%\ \text{inhibition} = \frac{Top - Bottom}{1 + (\frac{[x]}{IC_{50}})^{n}} + Bottom$$

where [x] is the inhibitor concentration. IC_{50} represents the concentration where transport rate is inhibited by 50%. Top is a fit variable and bottom represents a non-inhibitable component. n is Hill coefficient.

***IN VITRO IN VIVO* CORRELATION (IVIVC)**: With all the parameters in the fitting equation were obtained for each drug, the clinical unbound C_{max} of a drug was used to calculate its corresponding inhibition in the quintuple-transporter model. The calculated inhibition was then normalized to that of cimetidine and plotted against its clinically observed creatinine clearance suppression.

RESULTS & DISCUSSION

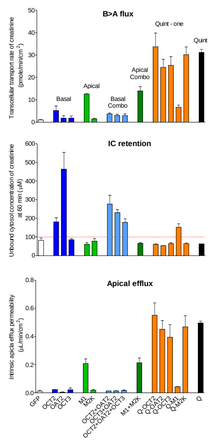

Fig 3. Contribution of individual transporters to B>A transcellular flux, intracellular retention, and apical efflux of creatinine. Red dotted line showing the initial dosing concentration of creatinine on the basolateral side.

1. ROLES OF INDIVIDUAL TRANSPORTERS IN CREATININE SECRETION: By expressing and assaying individual or different combinations of apically or basolaterally expressed transporters, we confirmed our previous findings that MATE1 is the main efflux transporter on the apical side, and OAT2 is the major basolateral transporter. However, OAT2/OCT2/OCT3 may serve as a redundant mechanism for basolateral creatinine transport.

As such, when apical transporters, especially MATE1, were absent, creatinine was predominantly accumulated in the intracellular space with negligible B>A transcellular flux (Fig 3 top and middle, in blue). On the opposite side, when basolateral transporters were omitted, B>A transport was still significantly higher than GFP mock but the intracellular retention was minimal (Fig 3 top and middle, in green). These findings were confirmed by calculating the apical efflux permeability based on the free intracellular concentration of creatinine. Robust apical efflux existed only in the presence of apical transporters, especially MATE1 (Fig 3 bottom). In contrast to MATE1, dialing out one basolateral transporter from the quintuple combination did not have the dramatic effects on creatinine transport or cellular retention (Fig 3, in orange), further confirming redundancy in basolateral transport. Interestingly, the intracellular creatinine retention was lower in cells expressing OAT2 together with one or both OCTs, as compared to OAT2 alone (Fig 3 middle, dark vs light blue), suggesting that OCTs, which are facilitative transporters, may transport creatinine outwards as a result of OAT2's ability in concentrating creatinine and thus changing the direction of cross-membrane creatinine concentration gradient. Such possibility may further confound the roles of OCTs in creatinine tubular secretion.

RESULTS & DISCUSSION (CONT'D)

2. INHIBITION OF CREATININE B>A TRANSPORT IN QUINTUPLE-TRANSPORTER MODEL AND CORRELATION WITH CrCl REDUCTION *IN VIVO*: By using the quintuple-transporter model, seven drugs from different therapeutic areas were interrogated for their effects on B>A transcellular creatinine transport. The fitted concentration-dependent inhibition curves of B>A flux were generated (Fig 4). For each drug, the fitting parameters were used to calculate the corresponding inhibition at its clinical Cmax,u (Tab 1). These inhibitions were then normalized to that of cimetidine and plotted against their clinically observed suppression on creatinine clearance (Fig 1).

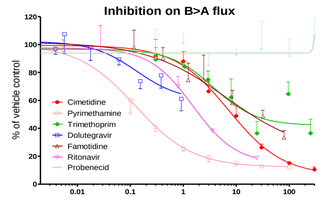

Fig 4. Concentration-dependent inhibition of creatinine B>A flux by drugs in quintuple-transporter model. Free drug concentrations were measured to exclude possible nonspecific binding to the assay wares.

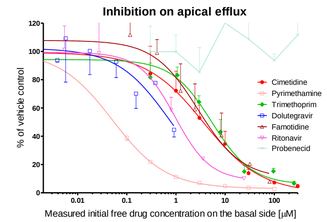

Fig 5. All drugs tested except for probenecid suppressed the apical efflux of creatinine in quintuple-transporter model.

Tab 1. Calculated inhibition on B>A transcellular transport in quintuple-transporter model of seven drugs at their Cmax,u and clinically observed effects on creatinine clearance.

					IVIVC	In vitro inhibition				
	In vivo clinical observations					Calculated inhibition at Cmax,u	Inhibition curve fitting parameter			
Drug	Mean Cmax (μM)	Plasma protein binding (%)	Unbound mean Cmax (μM)	Mean sCr increase (μM)	Mean CrCl change (ml/min/1.73m²)	Normalized to cimetidine	Calculated inhibition in Quint model at Cmax, u (%)	In vitro Hill curve Top (%)	In vitro Hill curve bottom (%)	Fitted IC_{50} (uM)	Fitted n
Cimetidine	4	20	3.2	33	15	1.000	31.09	99.44	6.25	7.23	0.849
Pyrimethamine	1.87	90	0.187	21.2	34	1.673	52.00	100.00	11.80	0.12	0.757
Trimethoprim	5.9	44	3.304	17	16	0.831	25.82	96.78	42.00	3.68	1.066
Dolutegravir	8.32	98.9	0.09152	11	12	0.559	17.37	102.30	59.84	0.13	1.042
Famotidine	0.24	20	0.192	11.5	1	0.291	9.05	101.70	22.07	6.56	0.582
Ritonavir	16	98.5	0.24	12.4	14	0.332	10.33	97.65	14.53	1.51	1.061
Probenecid				0	0	0	0	NA (no inhibition)			

3. INHIBITION OF MATE TRANSPORTERS IN EFFLUX MODE: MATE1/2K in the quintuple-transporter model operated as efflux transporters, which was different from a non-physiological uptake mode that is adopted conventionally just for assay convenience. All drugs tested except for probenecid inhibited the apical efflux of creatinine in quintuple-transporter model (Fig 5). Interestingly, dolutegravir exhibited moderate inhibition of MATE efflux activity at clinical relevant concentrations (<200 nM), although it had only minimal effects on inhibiting MATE1 uptake activity (IC_{50}=4.7 μM) as reported in [1]. Such discrepancies may be attributed to the difference in MATE transporters when they operate in uptake vs. efflux modes as previously shown by us [4], raising the caution of using kinetic or inhibition constants of MATE1/2K, obtained with the artificial uptake assay mode, in modeling Drug-Drug Interactions mediated by MATEs, which are efflux transporters under physiological conditions.

CONCLUSION

Polarized MDCK-II cells expressing OCT2, OAT2, OCT3, MATE1 and MATE2K (quintuple-transporter) is a novel model to assess drug's effects on creatinine secretion. The *in vitro* data obtained with the model for seven drugs nicely correlated to their clinical reducing effects on renal creatinine clearance. With further validation with more drugs, such model could serve as an inexpensive surrogate to *in vivo* studies of drug's inhibition of creatinine secretion.

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