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 tubule.

We have previously identified specific human renal transporters responsible for drug, creatinine, and water secretion (OCT2, OCT3, SLC22A2, SLC22A3, SLC22A4, SLC22A5, SLC22A6, SLC22A7) within those transporters constitutively in the human Madin-Darby canine kidney (MDCK-II) cells, we have established an in vitro polarized tubule creatinine secretion model (quintuple-transporter model) [3]. The basolateral side of cells is the counterpart of the blood side in tubules, while the apical side resembles the urine side by adjusting T888. Therefore, BxA transcellular transport in the quintuple-transporter model simulates creatinine clearance (Ccr) in vivo. We have shown that cimetidine, a potent inhibitor of all five transporters, induced an almost complete inhibition of BxA creatinine transport while trimethoprim, lacking effects on OCT2, exerted 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 with their clinically relevant concentrations with their reported effects on creatinine clearance in patients.

RESULTS & DISCUSSION

1. ROLES OF INDIVIDUAL TRANSPORTERS IN CREATININE SECRETION: By expressing single, pairs, and quintuple transporters of renal epithelial cells, we confirmed previous findings that OCT1 is the main effector for creatinine secretion [3,4]. OCT2 and OCT3, were also functional in the quintuple transporter model. However, OCT2/OCT3/CRT may serve as a redundant mechanism for basolateral net creatinine secretion.

As such, when apical transporters, especially OCT1, were absent, creatinine was predominantly accumulated in the intracellular space with negligible BxA transport. OCT2 and OCT3 flux (Fig 2). On the opposite side, when basolateral transporters were absent, BxA transport was still significantly higher than GFP basal flux, with intracellular retention being minimal (Fig 2A 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 OCT1 (Fig 2 bottom). In contrast to OCT1, dialogs out one basolateral transporter from the quintuple combination did not have the dramatic effects on creatinine or cellular retention (Fig 3, in orange), further confirming redundancy in basolateral transport. Interestingly, the intracellular creatinine retention was lower in cells expressing OCT2 together with one or both OCTs, as compared to OCT2 alone (Fig 3 middle, dark vs light blue), suggesting that OCTs, which are facilitative transporters, may transport creatinine outwards as a result of OCT2’s ability in concentrating creatinine and thus changing the direction of trans-membrane creatinine concentration gradients. Such possibility may further confound the roles of OCTs in creatinine tubular secretion.

2. INHIBITION OF CREATININE Bx/A TRANSPORT IN QUINTUPLE-TRANSPORTER MODEL AND CORRELATION WITH CCR REDUCTION IN VIVO: By using the quintuple-transporter model, seven drugs from different therapeutic areas were tested for their effects on Bx/A transcellular creatinine transport. The fitted concentration-dependent inhibition curves of Bx/A flux were generated (Fig 4). For each drug, the fitting parameters were calculated to compare the corresponding inhibition at its clinical Cmax (Table 1). The inhibition values were then normalized to that of cimetidine and plotted against their clinically observed suppression on creatinine clearance (Fig 5).

CONCLUSION

Polarized MDCK-II cells expressing OCT2, OCTA2, OCT3, OCT1, and MATE2/K (quintuple-transporter) is a novel model to assess drug’s effects on creatinine secretion. The in vitro data obtained with the model form a dramatically 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.

REFERENCES