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Contribution of the organic anion transporter OAT2 to the renal active tubular secretion of creatinine and mechanism for serum creatinine elevations caused by cobicistat

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Many xenobiotics including the pharmacoenhancer cobicistat increase serum creatinine by inhibiting its renal active tubular secretion without affecting the glomerular filtration rate. This study aimed to define the transporters involved in creatinine secretion, applying that knowledge to establish the mechanism for xenobiotic-induced effects. The basolateral uptake transporters organic anion transporter OAT2 and organic cation transporters OCT2 and OCT3 were found to transport creatinine. At physiologic creatinine concentrations, the specific activity of OAT2 transport was over twofold higher than OCT2 or OCT3, establishing OAT2 as a likely relevant creatinine transporter and further challenging the traditional view that creatinine is solely transported by a cationic pathway. The apical multidrug and toxin extrusion transporters MATE1 and MATE2-K demonstrated low-affinity and high-capacity transport. All drugs known to affect creatinine inhibited OCT2 and MATE1. Similar to cimetidine and ritonavir, cobicistat had the greatest effect on MATE1 with a 50% inhibition constant of 0.99 μM for creatinine transport. Trimethoprim potently inhibited MATE2-K, whereas dolutegravir preferentially inhibited OCT2. Cimetidine was unique, inhibiting all transporters that interact with creatinine. Thus, the clinical observation of elevated serum creatinine in patients taking cobicistat is likely a result of OCT2 transport, facilitating intracellular accumulation, and MATE1 inhibition.

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KEYWORDS: antibiotics; Cockcroft–Gault; creatinine; creatinine clearance; glomerular filtration rate; proximal tubule

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A number of drugs from different therapeutic classes have been observed to reduce creatinine clearance without causing an effect on the rate of glomerular filtration (Supplementary Table S1 online).[1–13] The most prominent effects, increases in serum creatinine levels between 0.2 and 0.4 mg/dl and decreases in creatinine clearance of 15–34 ml/min per 1.73 m^2, have been noted with the antacid cimetidine,[1] a number of antibiotics including trimethoprim,[4] and the antiparasitic pyrimethamine.[5] Most recently, more modest effects (serum creatinine increases of ~0.1 mg/dl) have been observed for the antiretroviral drugs rilpivirine and dolutegravir,[6,7] and the pharmacoenhancers cobicistat and ritonavir.[8,13] Similar to other drugs, cobicistat was established to not have an adverse effect on renal function in a clinical study showing no change in the glomerular filtration rate, as measured by iohexol clearance.[8]

The effects on creatinine observed with these drugs have been attributed to the inhibition of the renal active tubular secretion component of creatinine clearance.[14] In subjects with normal renal function, active tubular secretion accounts for 10–40% of creatinine clearance.[15] In contrast to the passive filtration occurring at the glomerulus, active tubular secretion in the proximal tubule is mediated by specific transport proteins that can be subject to inhibition by xenobiotics.[16,17] Although creatinine has been shown to be a substrate for a number of transporters in the solute carrier (SLC) superfamily expressed in renal proximal tubules, the predominant pathway mediating creatinine secretion has not been fully elucidated. Creatinine has been shown to be a substrate for a cation transport pathway comprising the basolaterally expressed organic cation transporter 2 (OCT2; SLC22A2)[18–20] and the apically expressed multidrug and toxin extrusion (MATE) transporters 1 (SLC47A1) and 2-K (SLC47A2).[21] However, the absolute contribution of OCT2 to the basolateral uptake of creatinine has been called into question by recent studies in mice. Knockout of OCT2 did not affect serum creatinine in one report.[22] Another study has suggested a role for basolaterally expressed anionic transporters.[23]

In the current study, we sought to understand the molecular mechanism for increases in serum creatinine observed clinically with cobicistat. Cobicistat is a mechanism-based inhibitor of cytochrome *P*450 3A enzymes approved as part of a fixed-dose, once-daily, single-tablet regimen for the treatment of HIV (brand name STRIBILD) containing the nucleotide reverse transcriptase inhibitors tenofovir disoproxil fumarate and emtricitabine and the integrase inhibitor elvitegravir. Other antiviral combinations containing cobicistat are also being explored.[24,25] Studies were conducted in two parts: in part one, the mechanism of the active tubular secretion of creatinine was explored *in vitro* using refined transient transfection techniques, allowing for a better understanding of the relative contributions of individual transporters to creatinine transport. In part two, the interaction of cobicistat and other drugs with identified creatinine transporters was characterized. In addition to gaining novel insights regarding the mechanism of creatinine secretion, we elucidate the molecular mechanism for the effect of cobicistat on serum creatinine, providing a more comprehensive framework for understanding xenobiotic-induced changes in the renal handling of creatinine.

RESULTS

Identification of creatinine transporters

Madin–Darby canine kidney cells (MDCKII) were transfected with the human sequences encoding basolaterally expressed OCT1 (SLC22A1), OCT2, OCT3 (SLC22A3), organic anion transporters OAT1 (SLC22A6), OAT2 (SLC22A7), and OAT3 (SLC22A8), and apically expressed MATE1, MATE2-K, OAT4 (SLC22A11), and organic cation transporter novel (OCTN), type 1 (SLC22A4), and type 2 (SLC22A5). Transporter expression was confirmed by quantitative mRNA analysis and at the functional level with model substrates (Supplementary Figures S1 and S2 online).

Transporter-dependent and inhibitor-sensitive transport of creatinine was observed for the basolateral transporters OCT2, OCT3, and OAT2 (Figure 1a), and apical transporters MATE1 and MATE2-K (Figure 1b). Model inhibitors caused creatinine uptake into cells overexpressing these transporters to be reduced to levels observed in nontransfected cells. To facilitate comparison with background creatinine accumulation in control cells, data in Figure 1 are normalized to

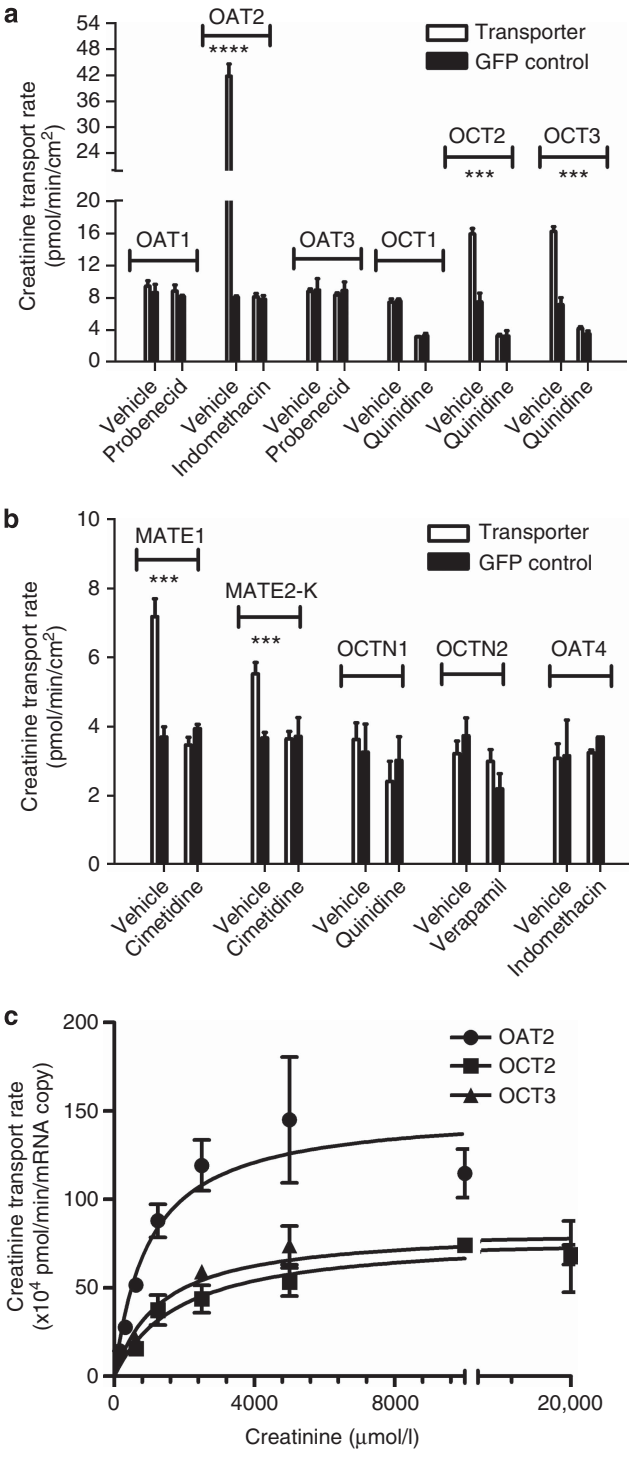

Figure 1 | Creatinine transport by renal transporters in polarized Madin–Darby canine kidney II (MDCKII) cells. Uptake of creatinine (100 μmol/l) by (**a**) basolateral and (**b**) apical renal transporters. Results are shown for transporter-transfected cells relative to matched cells transfected with green fluorescence protein (GFP) in the presence and absence of inhibitors incubated at ten times the respective concentrations required to inhibit transport by 50% for each transporter (100 μmol/l probenecid (OAT1 and OAT3), 100 or 200 μmol/l indomethacin (OAT2 and OAT4, respectively), 1 mmol/l quinidine (OCT1, OCT2, OCT3, and OCTN1), 100 μmol/l cimetidine (MATE1 and MATE2-K), and 500 μmol/l verapamil (OCTN2)). Each value represents the mean ± s.d. of three measurements. The statistical significance of the change in the ratio of accumulation in transporter-transfected cells relative to GFP control in the presence and absence of inhibitor was assessed using a Student's unpaired and two-tailed *t*-test assuming equal variance (***$P < 0.001$; and ****$P < 0.0001$). The concentration dependence of creatinine uptake by the basolateral transporters organic anion transporter OAT2 and organic cation transporters OCT2 and OCT3 is presented in panel **c**. Data fit by nonlinear curve fitting to a hyperbolic equation. MATE, multidrug and toxin extrusion; OAT, organic anion transporter; OCT, organic cation transporter.

surface area. Analysis of accumulation based on mRNA expression in units of pmol/min per mRNA copy number did not markedly change the rank order of transport, with the exception of OCT3 having an apparently twofold higher rate than OCT2 and MATE2-K having an apparently sixfold higher rate than MATE1 owing to correspondingly lower relative mRNA expression of OCT3 and MATE2-K in the transfection system (Supplementary Figure S1 online).

In contrast to our study, two prior studies have reported creatinine transport by human OAT3 in different transfection systems.[19,20] For example, Ciarimboli *et al.*[19] reported a 60% increase in creatinine accumulation over mock transfection in transiently transfected human embryonic kidney 293 (HEK293) cells. To assure that the background cell type did not affect our ability to detect OAT3-dependent creatinine transport, we assessed accumulation in transiently transfected HEK293 cells. In agreement with results from our MDCKII transfection system, no OAT3-dependent transport of creatinine was observed in HEK293 cells (data not shown).

Kinetics of creatinine transport

Kinetic constants for creatinine transport by the five identified transporters were determined (Table 1 and Figure 1c). Among the three basolateral transporters, OAT2 showed the highest affinity ($K_m = 986$ μmol/l) and most rapid maximal velocity ($V_{max} = 117 \times 10^4$ pmol/min per mRNA copy number) for creatinine. The transport rate at a physiological creatinine concentration (100 μmol/l) for OAT2 was 4.7- and 2.3-fold faster than that measured for OCT2 and OCT3, respectively. Kinetic constants for the apical transporters MATE1 and MATE2-K could not be accurately determined owing to their weak binding constants for creatinine ($K_m > 2000$ μmol/l) under the experimental conditions. The inefficient transport observed for these transporters may be related to the artificial experimental conditions where uptake is studied instead of physiologically relevant efflux by manipulating extracellular pH.

Inhibition of creatinine transporters by cobicistat and other drugs

Inhibition of the creatinine transporters identified above by cobicistat and other drugs reported to affect serum creatinine was studied using transfected cell lines and model substrates. Dose–response curves for each transporter are presented in Figure 2, and inhibition constants are summarized in Table 2. All compounds known to affect serum creatinine were found to inhibit OCT2 and MATE1. Similar to cimetidine and ritonavir, cobicistat most potently inhibited MATE1 (half-maximal inhibitory concentration (IC_{50}) = 1.87 μmol/l) with a weaker effect on the other transporters. Of the basolateral uptake transporters, cobicistat showed weak inhibition of OCT2 ($IC_{50} = 24$ μmol/l), with little or no inhibition of OCT3 and OAT2 ($IC_{50} > 100$ μmol/l). Dolutegravir was found to be one of the most potent OCT2 inhibitors reported to date based on its effect on tetraethylammonium transport ($IC_{50} = 0.066$ μmol/l). The potent inhibition by dolutegravir was confirmed in a separate assay by the observation of similar inhibition of OCT2-dependent metformin transport ($IC_{50} = 0.11$ μmol/l). These results are consistent with the relatively potent OCT2 inhibition by dolutegravir reported elsewhere.[26] Trimethoprim was found to be a relatively potent inhibitor of MATE2-K. Cimetidine was unique in its inhibition of all creatinine transporters.

To establish the physiological relevance of results obtained with model substrates, IC_{50} values for cimetidine and cobicistat were determined for OAT2-, OCT2-, and MATE1-mediated creatinine transport (Supplementary Figure S3 online). The observation of substrate-dependent inhibition would not be anticipated on the basis of the lack of a marked difference in the cimetidine inhibition constants (K_i) for a panel of cationic transporters measured with five different substrates reported in a recent comprehensive study.[27] Studies with creatinine confirmed results obtained with model substrates showing MATE1 to be the most sensitive transporter to inhibition by cobicistat and cimetidine (IC_{50} values 0.99 and 0.20 μmol/l, respectively). The increased potencies observed for the tested transporters relative to assays completed with model substrates incubated at their respective K_m values are consistent with the observation of a competitive mode of inhibition and a creatinine concentration substantially below K_m.

Uptake of cobicistat by renal transporters

To explore the possibility that cobicistat accumulation in proximal tubule cells has a role in serum creatinine elevations observed clinically, we assessed the uptake of cobicistat into

Table 1 | Kinetic constants for creatinine transport by renal transporters tested in transiently transfected cell models

	K_m (μmol/l)	V_{max} ($\times 10^4$ pmol/min per mRNA copy number)	Efficiency (V_{max}/K_m)	Physiologic rate ($\times 10^4$pmol/min per mRNA copy number)[a]
OAT2	986 ± 177	117 ± 6	0.119	9.25 ± 1.44
OCT2	1860 ± 370	42.1 ± 2.4	0.023	1.97 ± 0.30
OCT3	1320 ± 380	68.2 ± 7.1	0.052	3.99 ± 0.52
MATE1	>2000	>2	ND	0.23 ± 0.04
MATE2-K	>2000	>20	ND	1.32 ± 0.41

Abbreviations: MATE, multidrug and toxin extrusion; MATE1 and MATE2-K, multidrug and toxin extrusion transporters 1 and 2-K; ND, not determined; OAT2, organic anion transporter 2; OCT2 and OCT3, organic cation transporters 2 and 3.
Kinetic constant derived from nonlinear curve fitting plus/minus s.e. based on the concentration dependence of creatinine transport from $n \geqslant 3$ independent experiments.
[a]Rate determined at 100 μmol/l creatinine.

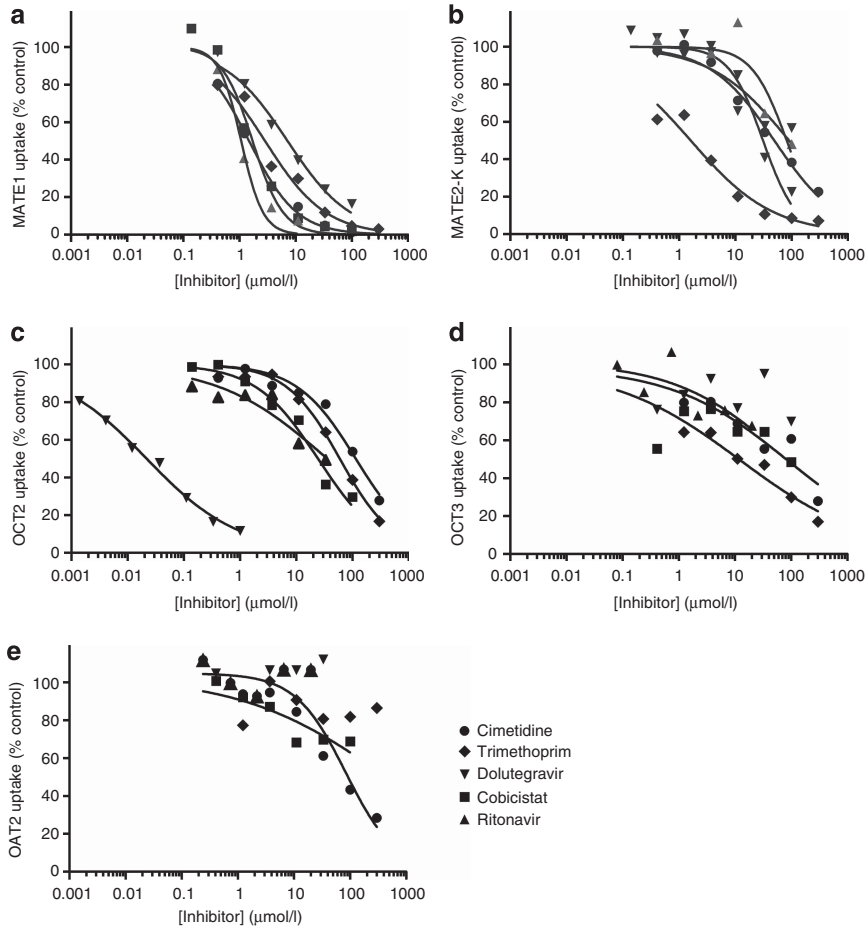

Figure 2 | Inhibition of creatinine transporters. Inhibition of multidrug and toxin extrusion transporters (**a**) MATE1 and (**b**) MATE2-K, organic cation transporters (**c**) OCT2 and (**d**) OCT3, and organic anion transporter (**e**) OAT2 mediated transport of model substrates by cimetidine, trimethoprim, dolutegravir, cobicistat, and ritonavir. The concentration-dependent inhibition data are fit by nonlinear curve fitting to a sigmoidal equation. Curves could not be generated for inhibition of OCT3-dependent transport by cobicistat and dolutegravir, and OAT2-dependent transport by dolutegravir, ritonavir, and trimethoprim, owing to a lack of concentration-dependent inhibition. Data represent the average of two to three independent determinations of the concentration-dependent inhibition by each inhibitor. MATE, multidrug and toxin extrusion.

Table 2 | Inhibition of the transport of model substrates by transporters identified to transport creatinine by drugs that reduce the active tubular secretion of creatinine

	IC_{50} (μmol/l)				
	OAT2	OCT2	OCT3	MATE1	MATE2-K
Cimetidine	72.8 ± 17.0	135 ± 16	87.7 ± 55.1	1.46 ± 0.11	46.6 ± 7.23
Trimethoprim	> 300	68.0 ± 5.2	12.3 ± 5.2	3.31 ± 0.67	1.87 ± 0.57
Cobicistat	> 100	24.0 ± 4.6	> 100	1.87 ± 0.22	33.5 ± 4.2
Ritonavir	> 20	~ 20	> 20	1.34 ± 0.23	> 20
Dolutegravir	> 100	0.066 ± 0.003	> 100	4.67 ± 1.11	> 100

Abbreviations: IC_{50}, half-maximal inhibitory concentration; MATE1 and MATE2-K, multidrug and toxin extrusion transporters 1 and 2-K; OAT2, organic anion transporter 2; OCT2 and OCT3, organic cation transporters 2 and 3.
Inhibition constants were measured for OAT2 transport of 3′, 5′-cyclic GMP, OCT3 transport of 1-methyl-4-phenylpyridinium, and OCT2, MATE1, and MATE-2K transport of tetraethylammonium.
Inhibition by compounds was studied by threefold serial dilution up to 20 μmol/l (ritonavir), 100 μmol/l (cobicistat and dolutegravir), and 300 μmol/l (cimetidine and trimethoprim). The highest concentrations tested were based on aqueous solubility limits.
IC_{50} values represent the average plus/minus s.e.m. from curve fitting of at least two measurements per inhibitor concentration.

fresh human primary proximal tubule cells and cell lines overexpressing individual transporters. Assessing cobicistat accumulation in cells was technically challenging because of the high levels of nonspecific membrane association observed for this lipophilic drug. Addition of protein to the incubation and isolation of cells by spinning through oil reduced the background to allow observation of transporter-dependent uptake (see Materials and Methods). Although transporter activity is variable and progressively declining after isolation, evidence for transporter-dependent uptake of cobicistat was observed in freshly isolated primary proximal tubule cells based on small but reproducible decreases in accumulation in the presence of the transport inhibitors cimetidine or probenecid (Figure 3a). To better characterize the molecular mechanism for cobicistat accumulation in proximal tubule cells, uptake studies were completed in cells overexpressing individual basolateral uptake transporters. OCT2 and OAT3 were selected on the basis of the apparent sensitivity of

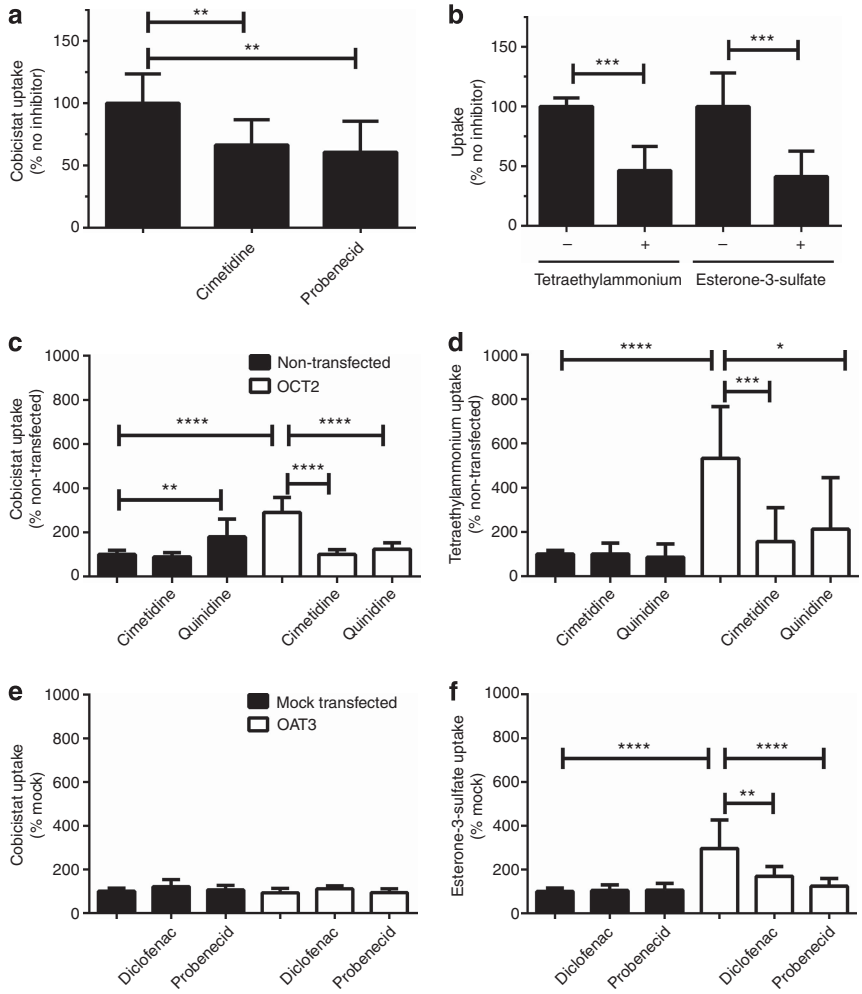

Figure 3 | Uptake transport of cobicistat by renal transporters. (**a**) The uptake of cobicistat (1 μmol/l) was assessed in fresh isolated primary proximal tubule cells in the presence of a model cationic transport inhibitor cimetidine (1500 μmol/l) and anionic transport inhibitor probenecid (90 μmol/l). (**b**) The cimetidine-sensitive uptake of the model cationic substrate tetraethylammonium or probenecid-sensitive anionic substrate esterone-3-sulfate was determined by establishing transporter expression in the primary proximal tubule cells. The effect of OCT2 or OAT3 expression on the intracellular accumulation of cobicistat (1 μmol/l) was assessed in transfected cell lines. (**c**) The effect of inhibitors on levels of cobicistat in parental (nontransfected) or Madin–Darby canine kidney II (MDCKII) cells transfected with the organic cation transporter OCT2 were assessed in the presence or absence of the OCT2 inhibitors cimetidine (1500 μmol/l) or quinidine (200 μmol/l). (**d**) The inhibitor-sensitive accumulation of the known OCT2 substrate tetraethylammonium was determined, establishing appropriate OCT2 expression. (**e**) The effect of inhibitors on levels of cobicistat in human embryonic kidney 293 (HEK293) cells either mock-transfected or transfected with the organic anion transporter OAT3 were assessed in the presence or absence of the OAT3 inhibitors diclofenac or probenecid both incubated at 90 μmol/l. (**f**) The inhibitor-sensitive accumulation of the known OAT3 substrate esterone-3-sulfate was determined, establishing appropriate OAT3 expression. Data represent the average ± s.d. of three independent assays performed in triplicate. The statistical significance of differences between conditions was assessed using the Student's unpaired and two-tailed *t*-test assuming equal variance (*$P<0.05$; **$P<0.005$; ***$P<0.001$; and ****$P<0.0001$).

cobicistat accumulation to cimetidine and probenecid in proximal tubule cells and evidence for some molecular interaction provided by the weak inhibition of these transporters by cobicistat reported here and elsewhere.[28] A 2.9-fold increase in cobicistat accumulation was observed in cells transfected with OCT2 (Figure 3c). Uptake in OCT2-expressing cells was reduced to that of nontransfected cells in the presence of OCT2 inhibitors. Although quinidine caused a significant increase in cobicistat accumulation in non-transfected cells, likely related to the inhibition of p-glycoprotein-mediated cobicistat efflux, it completely inhibited the uptake transport in OCT2-expressing cells, reducing accumulation to levels observed in nontransfected cells in the absence of inhibitor. In contrast, no inhibitor-sensitive accumulation of cobicistat was observed in cells transfected with OAT3 (Figure 3e). Transporter expression was established in the different systems with the use of model substrates (Figure 3b, d, and f).

DISCUSSION

In patients with normal renal function, creatinine is primarily eliminated by glomerular filtration, and it can be

used as a convenient means for estimating the glomerular filtration rate. Therefore, measurement of serum creatinine levels is the most common method used clinically for the routine monitoring of renal function. It is important to understand which xenobiotics have the potential to elevate serum creatinine without causing an effect on the rate of glomerular filtration to interpret correctly changes upon initiation of therapy. In elucidating the molecular mechanism for the elevation of serum creatinine with cobicistat, we have obtained novel insights that should help in the prediction of the potential of other drugs to affect creatinine clearance.

The active tubular secretion of creatinine has traditionally been believed to be mediated by an entirely cationic pathway, with the basolateral transporter OCT2 serving as the primary means of uptake into proximal tubules.[18–20] Eisner *et al.*[22] called into question the relative role of Oct2 in mice by showing inhibition of creatinine secretion by the anion *para*-aminohippurate and the lack of an effect of Oct1/Oct2 knockout. This result has generated controversy, with another group reporting a decrease in creatinine secretion in the same knockout system and suggesting that the contrasting results may be due to the use of ketamine anesthesia in the earlier report, as ketamine itself has the potential to interfere with creatinine secretion.[19] Supporting a role of anionic transporters in creatinine secretion across species, inhibitors of anionic transporters have been noted to affect renal creatinine secretion in a number of species including humans.[22,23,29–34] Adding further support for the role of anion transporters in creatinine secretion, Vallon *et al.*[23] found that mouse Oat1 and Oat3 can transport creatinine *in vitro*, that this transport was inhibited by cimetidine, and that Oat3-knockout mice had blunted creatinine secretion *in vivo*. While providing a more comprehensive understanding of the role of Oat3 in creatinine secretion in mice, species differences in transporter expression and substrate specificity may affect the relevance of this finding to humans. Indeed, it is more problematic to propose OAT3 as the predominant basolateral transporter in humans given the minimal transport activity detected by human OAT3 by others,[19,20] and the complete lack of OAT3-mediated transport observed in well-controlled assays in two separate cell backgrounds in the current study. OAT2 is expressed on the basolateral membrane of the proximal tubule in humans, and it has been reported to have threefold higher mRNA expression levels than OCT2.[35] Therefore, our observation that OAT2 facilitates the most efficient transport of creatinine *in vitro* of any of the human creatinine transporters identified, coupled with high expression levels in proximal tubules, suggests that it may make a substantial contribution to creatinine secretion in man and further establishes the more general involvement of anionic transporters in creatinine secretion.

A recent paper also reported OAT2-dependent transport of creatinine.[19] However, in contrast to our observation of relatively efficient creatinine transport, OAT2 was found to cause markedly less accumulation than that facilitated by OCT2 or OCT3. However, transporter expression and the kinetics of creatinine transport were not determined in this study. Stably transfected cell lines generated in different laboratories are known to have variable transporter expression. Therefore, we used highly optimized transient transfection techniques and characterized cells for mRNA and functional transporter expression. This rigorous characterization of the test system makes us confident in the observations described in the current report.

Although the mechanism of basolateral uptake of creatinine into the proximal tubule is being debated, MATE transporters have emerged as the likely mechanism for apical efflux into the urine across species. Serum creatinine levels are increased in mice lacking MATE1.[36] In addition to findings in mice, the availability of a potent and relatively selective inhibitor of MATE transporters, something unfortunately unavailable for basolateral transporters, provides the most compelling evidence for their role in creatinine secretion. Pyrimethamine is a potent and selective inhibitor of MATE1 and MATE2-K (K_i values of 93 and 59 nmol/l, respectively),[37] and it causes large increases in serum creatinine clinically.[5] In the current investigation, we found MATE1 and MATE2-K to be the only apical transporters able to transport creatinine. It was notable that all drugs known to increase serum creatinine were potent inhibitors of MATE1 (IC_{50} <5 μmol/l), with only trimethoprim and dolutegravir more potently inhibiting another creatinine transporter. Although cimetidine most potently inhibited MATE1, the observation of inhibition of all creatinine transporters provides a possible explanation for why high-dose cimetidine can completely block creatinine secretion, reducing its clearance to a level that approximates the glomerular filtration rate.[1]

Clinically, cobicistat achieves a maximum plasma concentration of 1.4 μmol/l, over 90% of which is bound to plasma proteins. Therefore, cobicistat would have to achieve concentrations in proximal tubule cells that are substantially above free concentrations in plasma, approaching total, to inhibit MATE1-dependent creatinine transport in a manner consistent with clinically observed elevations in serum creatinine. Our results show that cobicistat is taken up by proximal tubule cells, mediated at least in part by OCT2. Furthermore, the limited renal elimination of cobicistat (approximately 8% in humans) suggests that accumulation within cells is possible. The proposed mechanism for creatinine secretion and inhibition by cobicistat is presented in Figure 4. Cellular accumulation resulting in the inhibition of efflux transport is likely a more general mechanism for drugs that inhibit creatinine secretion. The same mechanism has been proposed previously for the inhibition of the active tubular secretion of metformin by cimetidine, whereby intracellular levels of cimetidine are increased by OCT2-dependent uptake.[27]

In summary, we have further elucidated the transporters involved in creatinine secretion and delineated a common mechanism of MATE1 inhibition for xenobiotic-induced

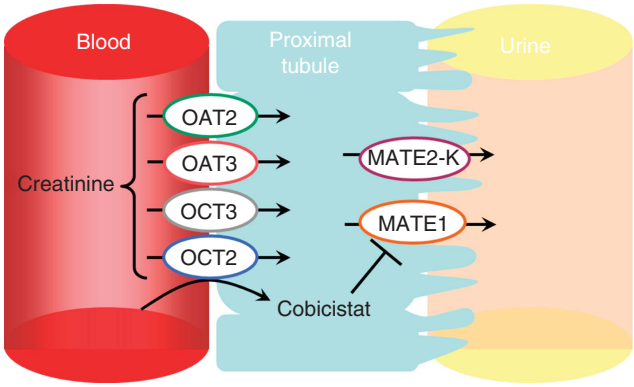

Figure 4 | Proposed mechanism for serum creatinine elevations observed clinically with cobicistat. The active tubular secretion of creatinine is mediated by basolateral uptake by organic anion transporters OAT2 and OAT3, and organic cation transporters OCT2 and OCT3, and apical efflux by multidrug and toxin extrusion transporters MATE1 and MATE2-K. Although no transport was observed with the human transporter in the current study, OAT3 is included given its established role in creatinine secretion in mice.[23] OCT2-mediated uptake of cobicistat into proximal tubule cells facilitates the inhibition of the MATE1 component of creatinine secretion.

changes in creatinine elimination. The novel insight that an organic anion transporter, OAT2, facilitates creatinine transport with relatively high efficiency furthers the evidence for the involvement of anionic transporters in creatinine secretion. For cobicistat, uptake into the proximal tubule, mediated at least in part by OCT2, facilitates intracellular accumulation and inhibition of apically expressed MATE1. These findings focus attention on the importance of determining uptake transport and resulting cellular accumulation when predicting the potential for clinically relevant transporter inhibition, and further elucidate the mechanism whereby some drugs artificially affect creatinine-based measurements that are routinely used clinically to assess renal function.

MATERIALS AND METHODS

See Supplementary Material online for more detailed description of methods including reagents, cell lines, and culturing conditions.

Creatinine transport assays

Transiently transfected MDCKII cells were preincubated with Hank's balanced salt solution in the presence (MATE1 and MATE2-K only) or absence (all other transporters) of 30 mmol/l NH_4Cl for 20 min. Transport assays were initiated by adding the substrate in the presence or absence of inhibitor in the appropriate chamber of the insert plate based on apical or basolateral expression of the respective transporter. The kinetics of uptake by identified creatinine transporters was determined by using a concentration range of 31 µmol/l to 20 mmol/l. After the incubation, the cells were washed with ice-cold, phosphate-buffered saline four times, and then extracted using 1:1 acetonitrile:water mixture. The amount of intracellular substrate was subsequently quantified by radiometric counting.

Transporter inhibition assays

Model substrate inhibition studies were conducted in stably transfected Chinese hamster ovary cells (OCT2 and MATE1), HEK293 cells (MATE2-K), or transiently transfected MDCKII cells (OAT2 and OCT3). Inhibition of the transport of the radiolabeled model substrates 3′,5′-cyclic GMP (2 µmol/l; OAT2), 1-methyl-4-phenylpyridinium (10 µmol/l; OCT3), and tetraethylammonium (3.6 µmol/l; OCT2 and MATE1; 5 µmol/l; MATE2-K) was determined. Control inhibitors were tested in each assay: indomethacin (OAT2), lansoprozole (OCT3), verapamil (OCT2), quinidine (MATE1), and cimetidine (MATE2-K). Positive control inhibitors were incubated at concentrations exceeding their reported IC_{50} for the respective transporter by at least 10-fold.

Uptake into human proximal tubule cells and cell lines transfected with OCT2 or OAT3

Briefly, the uptake of cobicistat into recently isolated human proximal tubule cells or cell lines transfected with OCT2 or OAT3 was studied in medium containing 5% bovine serum albumin and in the presence or absence of transport inhibitors. Before analysis, cells were isolated by centrifugation through an oil layer.

DISCLOSURE

E-IL, JH, RB, BPM, GB, TC, and ASR are employed by and are shareholders of Gilead Sciences, which is marketing cobicistat as part of the combination pill STRIBILD for the treatment of HIV. XZ, JH, AK, and YH are employed by Optivia Biotechnology a contract service provider of transporter-related assays including the proprietary Opti-Expression transfection technology used in these studies.

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SUPPLEMENTARY MATERIAL

Table S1. Effects of drugs on serum creatinine and creatinine clearance compiled from studies completed in subjects with normal renal function.

Figure S1. Transporter mRNA levels in transiently transfected MDCKII cells.

Figure S2. Uptake of model substrates by renal transporters in the presence and absence of reference inhibitors.

Figure S3. Inhibition of creatinine uptake by cimetidine and cobicistat.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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