Minireview

The Complexities of Interpreting Reversible Elevated Serum Creatinine Levels in Drug Development: Does a Correlation with Inhibition of Renal Transporters Exist?

Xiaoyan Chu, Kelly Bleasby, Grace Hoyee Chan, Irene Nunes, and Raymond Evers

Department of Pharmacokinetics, Pharmacodynamics and Drug Metabolism (X.C., K.B., G.H.C., R.E.), and Global Regulatory Affairs, Oncology, Immunology, Biologics & Devices (I.N.), Merck Sharp & Dohme Corporation, Kenilworth, New Jersey

ABSTRACT

In humans, creatinine is formed by a multistep process in liver and muscle and eliminated via the kidney by a combination of glomerular filtration and active transport. Based on current evidence, creatinine can be taken up into renal proximal tubule cells by the basolaterally localized organic cation transporter 2 (OCT2) and the organic anion transporter 2, and effluxed into the urine by the apically localized multidrug and toxin extrusion protein 1 (MATE1) and MATE2K. Drug-induced elevation of serum creatinine (SCr) and/or reduced creatinine renal clearance is routinely used as a marker for acute kidney injury. Interpretation of elevated SCr can be complex, because such increases can be reversible and explained by inhibition of renal transporters involved in active secretion of creatinine or other secondary factors, such as diet and disease state. Distinction between these possibilities is important from a drug development perspective, as increases in SCr can result in the termination of otherwise efficacious drug candidates. In this review, we discuss the challenges associated with using creatinine as a marker for kidney damage. Furthermore, to evaluate whether reversible changes in SCr can be predicted prospectively based on in vitro transporter inhibition data, an in-depth in vitro–in vivo correlation (IVIVC) analysis was conducted for 16 drugs with in-house and literature in vitro transporter inhibition data for OCT2, MATE1, and MATE2K, as well as total and unbound maximum plasma concentration (C_{max} and $C_{max,u}$) data measured in the clinic.

Introduction

Serum creatinine (SCr), an endogenous cation produced mainly by muscle metabolism, is the most widely used marker to assess renal injury (Tschuppert et al., 2007). Traditional monitoring for nephrotoxicity relies upon SCr measurements (Waikar et al., 2012). Creatinine is primarily filtered through the kidney through the glomeruli, but depending on a number of factors, ~10–40% is actively secreted by the proximal tubule cells through transporter-mediated active uptake and efflux (Levey et al., 1988; Breyer and Qi, 2010). Therefore, alterations in glomerular filtration rate (GFR) and/or proximal tubular secretion of creatinine can lead to increases in SCr and decreases in the estimated creatinine clearance. Elevation of SCr often results in reduction of drug dose (Arya et al., 2013, 2014) and may lead to discontinuation of the development of potentially promising drug candidates. Therefore, it is critical to distinguish clinically relevant increases in SCr due to renal toxicity from the nonpathologic increase in SCr attributed to the inhibition of renal transporters. Mild to moderate and reversible elevation of SCr and decrease in creatinine renal clearance

(CL_{cr}) has been reported, which can be attributed to inhibition of creatinine transporters without affecting renal function per se (Arya et al., 2013, 2014). This is supported by the clinical observation that several drugs, such as cobicistat (Lepist et al., 2014), pyrimethamine (Opravil et al., 1993), cimetidine (Dubb et al., 1978), and trimethoprim (Berglund et al., 1975), lead to increased levels of SCr without affecting kidney function. Such observations have also been reported for several recently approved drugs, including crizotinib (Brosnan et al., 2014; Camidge et al., 2014) and dolutegravir (Koteff et al., 2013). Understanding the mechanism of active secretion of SCr and how drugs may interfere with this process is therefore important from both a drug development and clinical practice perspective, where SCr is used as a marker of kidney injury.

Acute kidney injury (AKI) is a common condition that complicates up to 7% of all hospital admissions and 25% of intensive care unit admissions (Klevens et al., 2007; Vaidya et al., 2008; Minejima et al., 2011). Although progress has been made in understanding the pathophysiology of AKI and in the clinical care of patients with AKI, mortality rates have remained unchanged at 50–70% over the past 50 years (Minejima et al., 2011). Despite routine monitoring of systemic drug levels and renal function using traditional blood and urinary

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ABBREVIATIONS: AGAT, L-arginine-glycine amidinotransferase; AKI, acute kidney injury; CL_{cr}, creatinine renal clearance; DDI, drug-drug interaction; DX-619, 7-[(3R)-3-(1-aminocyclopropyl)pyrrolidin-1-yl]-1-[(1R,2S)-2-fluorocyclopropyl]-8-methoxy-4-oxoquinoline-3-carboxylic acid; FR, fraction reabsorbed; GFR, glomerular filtration rate; IVIVC, in vitro–in vivo correlation; MATE, multidrug and toxin extrusion protein; NMN, N-methylnicotimide; OAT, organic anion transporter; OCT, organic cation transporter; QD, every day; SCr, serum creatinine; SNP, single nucleotide polymorphism.

markers of kidney injury (e.g., creatinine, blood urea nitrogen, tubular casts, urinary concentrating ability), 10–20% of patients receiving aminoglycoside therapy, for instance, will develop AKI (Rybak et al., 1999). The lack of sensitive and specific markers of AKI limits the ability for early detection and intervention in drug-induced nephrotoxicity.

In the kidney, the elimination of drugs and endogenous compounds, such as creatinine, is the net result of passive glomerular filtration and reabsorption, as well as transporter-mediated active tubular secretion and/or reabsorption. The major transporters in human proximal tubule cells that play a role in the uptake of drugs and endogenous compounds from blood into proximal tubule cells are the organic cation transporter 2 (OCT2) and the organic anion transporters 1 and 3 (OAT1 and OAT3; Fig. 1). In the apical membrane, major efflux transporters involved in the excretion of drugs into the urine are the multidrug and toxin extrusion protein 1 (MATE1) and MATE2K, and the multidrug-resistance protein MDR1 P-glycoprotein. Inhibition of these transporters may alter systemic and tissue exposure of drugs, metabolites, and endogenous compounds, which may subsequently lead to clinically significant drug-drug interactions (DDIs). This can be of concern from a drug efficacy or safety perspective (Giacomini et al., 2010; Hillgren et al., 2013). Other transporters, such as the breast cancer resistance protein, are also expressed in the proximal tubule (Fig. 1), but their clinical significance is less well defined (Giacomini et al., 2010; Giacomini and Huang, 2013; Hillgren et al., 2013). In general, drug transporters are promiscuous in substrate recognition, and in addition to the charge of drugs, other factors, such as polar surface area, molecular weight, and number of hydrogen bond donors and acceptors, contribute to substrate specificity (Chang et al., 2006). Excellent reviews on renal transporters have been published previously, and the reader is referred to these for further details (Masereeuw and Russel, 2010; Morrissey et al., 2013).

In this review we provide: 1) an overview of the biosynthesis and disposition of creatinine in humans; 2) the current knowledge of transporters involved in the active renal secretion of creatinine; 3) a discussion of potential mechanisms that could result in increased levels of SCr; 4) a retrospective analysis to assess the correlation of elevation of SCr and inhibition of the renal transporters OCT2, MATE1, and MATE2K, and a discussion on the challenges associated with the identification of reliable biomarkers for AKI; and 5) a discussion on whether creatinine is a predictive and sensitive biomarker for DDIs attributed to inhibition of OCT2 and MATEs.

Markers of Renal Function

GFR is generally accepted as the best index of renal function in health and disease (Levey et al., 2015), and it can be accurately assessed by measuring the clearance of an exogenous substance, such as inulin, ^{99m}Tc-diethylenetriamine pentaacetic acid, ^{125}I-othalamate, or ^{51}Cr-EDTA (Korhonen, 2015). However, as these methods are expensive and inconvenient for use in the clinical setting, GFR is routinely estimated from the measurement of SCr using a variety of equations, such as those recommended by the Modification of Diet in Renal Disease study (National Kidney Foundation, 2002) and the Chronic Kidney Disease Epidemiology Collaboration (Levey et al., 2009), which take into account the impact of age, gender, and race on SCr.

The accuracy of the GFR estimate relies heavily upon the laboratory measurement of SCr. The interlaboratory differences in the measurement of SCr have been widely documented (Miller et al., 2005; Seronie-Vivien et al., 2005). Miller et al. compared 50 methods of creatinine measurements in 5624 laboratories with an isotope-dilution mass spectrometry reference method and reported large bias and discrepancies between the methods and laboratories (Miller et al., 2005). For example, measurements ranged from 0.87 to 1.21 mg/dl for the 0.90-mg/dl creatinine reference sample. To put this into context, using the Modification of Diet in Renal Disease equation, a 0.1-mg/dL change in creatinine for a 60-year-old woman causes a 10% change in calculated GFR. More recently, the introduction of calibration standards, which can be traced to the "gold standard" isotope-dilution mass spectrometry method, has helped resolve these concerns (Korhonen, 2015).

During the last decade, there has also been increasing interest in cystatin C as an additional endogenous marker of renal function. Cystatin C, produced at a constant rate by human nucleated cells, is freely filtered, not actively secreted, or dependent on muscle mass or diet (Nyman et al., 2015). Equations combining serum cystatin C and creatinine have been proposed to provide a more accurate estimate of GFR (Inker et al., 2012).

Biosynthesis and Disposition of Creatinine

Creatinine is a product of the degradation of creatine, which is an organic nitrogenous compound that plays an important role in cellular energy metabolism. Creatine is derived from dietary sources and de novo synthesis. As illustrated in Fig. 2, the biosynthesis of creatine in humans accounts for ~50% of the daily requirement and is a two-step process: first guanidinoacetate is formed from arginine and glycine precursors, under the control of L-arginine-glycine amidinotransferase (AGAT), followed by the guanidoacetate methyl transferase–catalyzed transfer of a methyl group from S-adenosyl-methionine to produce creatine. AGAT and guanidoacetate methyl transferase activities have been reported in many tissues. However, they are most highly expressed in the kidney and liver, respectively (Edison et al., 2007; Beard and Braissant, 2010). Creatine synthesis is balanced with that of dietary intake through feedback inhibition of AGAT. On a creatine-free diet, this pathway is fully active. However, when creatine is ingested through the diet, AGAT is partially repressed, and guanidinoacetate synthesis
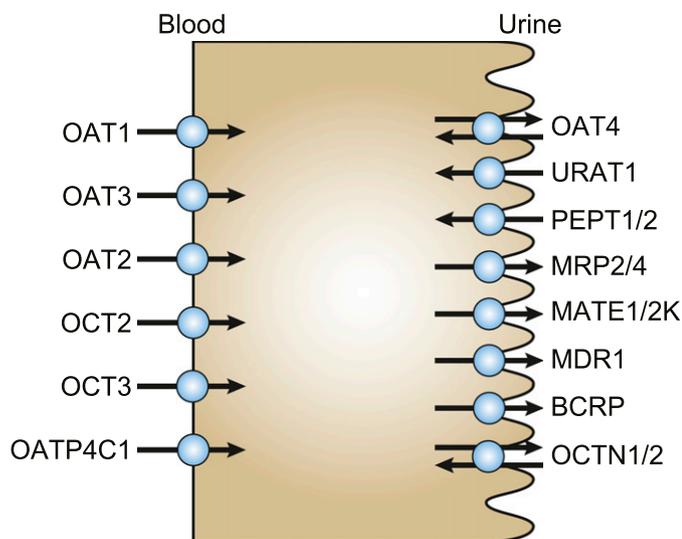

Fig. 1. Membrane transporters expressed on human renal proximal tubule cells. The transporters located in the basolateral plasma membrane include OAT1 (SLC22A6), OAT3 (SLC22A8), OAT2 (SLC22A7), OCT2 (SLC22A2), OCT3 (SLC22A3), and organic anion transporting polypeptide 4C1 (OATP4C1; SLCO4C1). Transporters located in the apical membrane include P-glycoprotein (MDR1, ABCB1), MATE1 (SLC47A1), MATE2K (SLC47A2), breast cancer resistance protein (BCRP; ABCG2), multidrug resistance protein 2 (MRP2; ABCC2), MRP4 (ABCC4), OAT4 (SLC22A11), urate transporter 1 (URAT1; SCL22A12), peptide transporter 1 (PEPT1; SLC15A1), PEPT2 (SLC15A2), organic cation/carnitine transporter 1 (OCTN1; SLC22A4) and OCTN2 (SLC22A5).
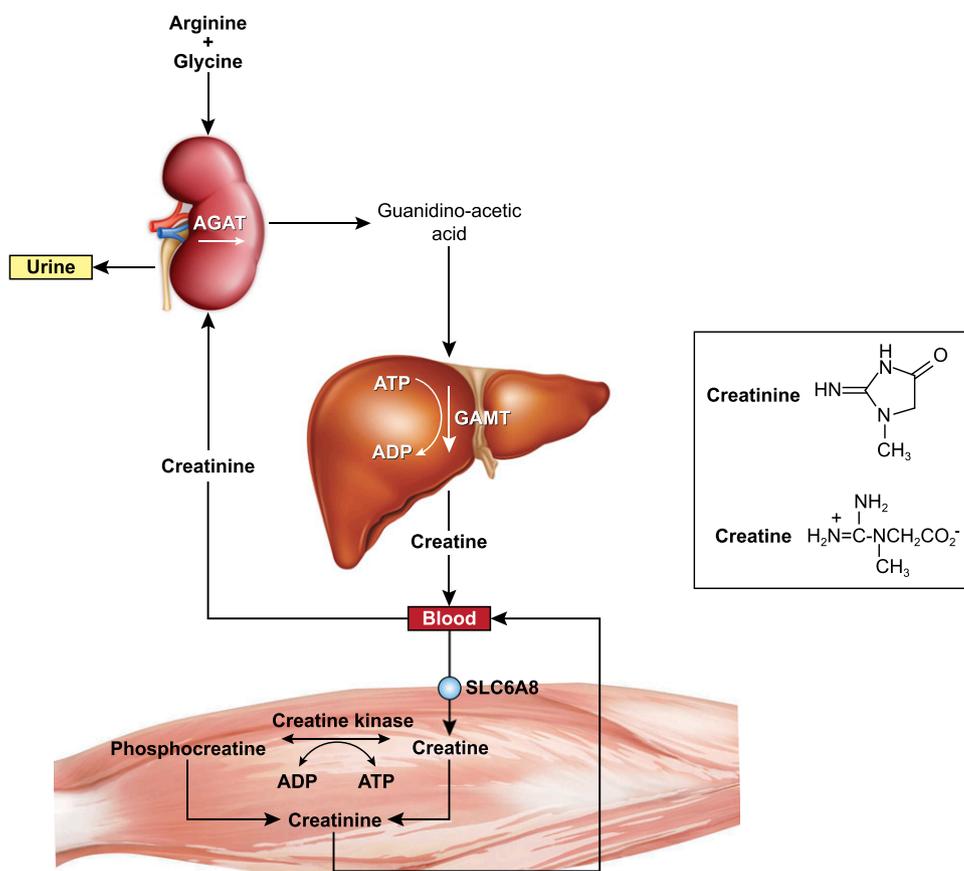
Fig. 2. Schematic representation of the biosynthesis and disposition of creatine and creatinine. GAMT, guanidoacetate methyl transferase.

(and thus subsequent creatine synthesis) is reduced (Heymsfield et al., 1983). Once synthesized, creatine is released into blood circulation, where it is taken up into muscle and other tissues by the Na^+-Cl^-–dependent creatine transporter SLC6A8 (Verhoeven et al., 2005). The majority (98%) of the total body creatine pool is found in skeletal muscle, with small amounts also found in the brain, kidney, and liver (Heymsfield et al., 1983). Approximately 1.7% of the total creatine pool (creatine and phosphocreatine) dehydrates to creatinine per day (Edison et al., 2007) and permeates through the cell plasma membrane into the blood circulation.

As a low-molecular-weight cation (molecular weight = 113), creatinine is eliminated solely by renal excretion through a combination of glomerular filtration and tubular secretion, with minimal binding to plasma proteins and metabolism (Fig. 3). Glomerular filtration, the passive process of ultrafiltration of plasma from blood as it crosses the glomerular capillaries, accounts for the large majority of the renal elimination of creatinine (Levey et al., 2015), whereas the secretory component is estimated to be 10–20% of total creatinine elimination in some reports (Breyer and Qi, 2010) and up to 40% in others, under normal conditions (Levey et al., 1988). Net tubular reabsorption of creatinine is uncommon, but may occur in infants and the elderly (Musso et al., 2009). During chronic renal failure, the proportion of creatinine excreted by glomerular filtration decreases, and the fraction undergoing tubular secretion may increase to 50–60%. In addition, under conditions of greatly reduced GFR, up to 60% of the daily creatinine generated may be eliminated by extrarenal routes, such as degradation by intestinal microflora (Shemesh et al., 1985; Levey et al., 1988).

Beyond renal injury or disease, several factors are known to impact the formation and elimination of creatinine, including exercise, diet, emotional stress, age, fever, and trauma, as well as inhibition of the secretory component by drugs (as discussed later) (Heymsfield et al., 1983; Levey et al., 1988). For example, creatinine excretion declines in the elderly, and this is likely the result of several factors, including reduced muscle mass, decreased dietary protein consumption, and the net tubular reabsorption of creatinine (Heymsfield et al., 1983; Musso et al., 2009).

Mathematical Concepts of Renal Clearance of Creatinine

The renal clearance of creatinine is determined by its glomerular filtration, tubular secretion, and reabsorption:

$$CL_{cr} = CL_{filtration} + CL_{secretion} - CL_{reabsorption} \qquad (1)$$

where $CL_{filtration}$, $CL_{secretion}$, and $CL_{reabsorption}$, represent creatinine clearance by renal filtration, tubular secretion, and reabsorption, respectively.

CL_{cr} can be described by eq.2 (Shitara et al., 2005):

$$\begin{aligned}CL_{cr} &= (1-FR) \cdot (f_u \cdot GFR + CL_{secretion}) \\ &= (1-FR) \cdot \left(f_u \cdot GFR + \left(Q_R \cdot f_u \cdot CL_{ser,int} / Q_R + f_u \cdot CL_{ser,int}\right)\right)\end{aligned}$$
$$(2)$$

where FR, f_u, Q_R, and $CL_{ser,int}$ represent the fraction reabsorbed, protein unbound fraction in the blood, renal blood flow rate, and intrinsic clearance of tubular secretion, respectively.

As described later, tubular secretion of creatinine involves transporter-mediated active uptake and efflux (Fig.3). Therefore, $CL_{ser,int}$ is saturable and may be inhibited by drugs that are inhibitors of these transporters. FR may be in part saturable (Shitara et al., 2005),

but the mechanism(s) contributing to reabsorption of creatinine, in particular, the role of transporters, is not well understood.

Imamura et al. (2011) established mechanistic models to describe the renal elimination of creatinine. The model analysis suggested that active tubular secretion contributed significantly to the renal elimination of creatinine (30–60%), whereas the significance of reabsorption depended on the models used.

Transporters Involved in Active Renal Secretion of Creatinine

Several drugs are reported to impact creatinine secretion, thereby causing transient increase in SCr without altering GFR (Table 2 and discussed later). The current hypothesis is that these changes are explained by the reversible inhibition of transporters involved in tubular secretion of creatinine (German et al., 2012). In the following sections, we summarize the current knowledge of the role of transporters in the uptake of creatinine into the proximal tubular cells of the kidney and efflux into the urine.

Transporters Involved in Creatinine Uptake in the Kidney

Renal uptake of creatinine has been studied by members of the SLC22A family, such as the organic cation transporters OCT2 and OCT3, and organic anion transporters OAT1, OAT2, and OAT3. Comprehensive reviews on these organic cation and anion transporters can be found in several publications (Jonker and Schinkel, 2004; Koepsell et al., 2007; Burckhardt, 2012; Nigam et al., 2015).

Expression and Function of OCT2, OCT3, OAT1, OAT2, and OAT3. OCT2 is a renal organic cation uptake transporter primarily localized in the basolateral membrane of the whole segment of the renal proximal tubule cells. It plays a major role in renal uptake of mostly cationic compounds, but also transports some anionic and zwitterionic compounds (Jonker and Schinkel, 2004). On the contrary, OCT3 is recognized as an extraneuronal monoamine transporter (Jonker and Schinkel, 2004). It is widely expressed in many tissues, such as liver, kidney, skeletal muscle, placenta, and heart, as well as in glial cells and epithelial cells of the choroid plexus, and neurons. OCT3 transports a wide range of monoamine neurotransmitters, hormones, and steroids (Wu et al., 1998). OCT3 mRNA was detected in the human kidney cortex; however, its level was much lower compared with OCT2 (Motohashi et al., 2002). Therefore, at least based on mRNA analysis, the importance of OCT3 in transport of cationic compounds in the kidney is much less compared with OCT2 (Motohashi et al., 2002). Nevertheless, recent studies in Oct3 knockout mice demonstrate that deletion of Oct3 has an impact on the pharmacokinetic and pharmacological effects of its substrates, such as metformin (Chen et al., 2015).

OAT1, OAT2, and OAT3 are renal organic anion uptake transporters located in the basolateral membrane of proximal tubules (Motohashi et al., 2002). OAT1 and OAT3 have overlapping substrate specificities, and are responsible for the uptake of many anionic drugs, such as antibiotics, antivirals, diuretics, uricosurics, statins, angiotensin-converting enzyme inhibitors, and antineoplastic drugs (Burckhardt, 2012). In contrast to OAT1 and OAT3, the role of OAT2 is less well characterized. More studies have emerged this decade focusing on OAT2 expression in the human kidney as well as its role in renal tubular handling of drugs (Cheng et al., 2012; Lepist et al., 2014; Shen et al., 2015). OAT2 is expressed in the basolateral membrane of renal proximal tubule cells as well as in the sinusoidal membrane of hepatocytes (Kobayashi et al., 2005; Cheng et al., 2012). One group showed that OAT2 was localized in both basolateral and apical membranes of human and cynomolgus monkey renal proximal tubules, but only in the apical membrane of rat proximal tubules (Shen et al., 2015). These findings suggest species differences for OAT2/Oat2 localization and possibly a role in reabsorption of OAT2 in primates.

Species differences in OAT2/Oat2 localization make rodents a poor translatable model to predict effects in primates for substrates of this transporter. OAT2 has many substrates in common with OAT1 and OAT3. However, several antiviral drugs eliminated exclusively in the urine were preferentially transported by OAT2 and not by OAT1 and OAT3 (Cheng et al., 2012). OAT1, OAT2, and OAT3 mRNAs are present in the human kidney cortex, with the highest mRNA level observed for OAT3, and different mRNA levels for OAT1 and OAT2 in two separate reports (Motohashi et al., 2002; Cheng et al., 2012). However, it is not known whether these differences in mRNA levels translate into different amount of transporter protein.

Transporters Involved in Creatinine Uptake. Creatinine has been reported to be an in vitro substrate for OCT2 (Urakami et al., 2004; Imamura et al., 2011; Ciarimboli et al., 2012; Lepist et al., 2014), with a Michaelis constant (K_m) ranging from 2 to 56 mM, suggesting low-affinity transport. Based on the physiologic concentration of creatinine in plasma (30–85 μM), OCT2-mediated transport of creatinine will not be saturable, which is especially important for patients with reduced GFR (Urakami et al., 2004). In vivo studies using Oct1/2 double-knockout mice showed the significance of Oct in creatinine secretion; creatinine clearance and renal accumulation of exogenous creatinine were 35- and 23-fold lower in Oct1/2 knockout mice compared with wild-type mice, respectively (Ciarimboli et al., 2012). One group, however, questioned the role of Oct2 in creatinine transport, as they did not observe a significant difference in creatinine secretion in Oct1/2 knockout mice compared with control mice (Eisner et al., 2010). This discrepancy may be explained by the use of ketamine by Eisner et al. (2010), which has the potential to interfere with creatinine secretion (Ciarimboli et al., 2012). It should be noted that species differences may complicate the translation of the OCT2/Oct2 contribution in creatinine transport from rodents to humans. For instance, in mice, both Oct1 and Oct2 are expressed in the kidney, whereas in humans, only OCT2 is expressed in the kidney and OCT1 is predominantly expressed in the liver (Jonker and Schinkel, 2004). More direct evidence of creatinine as an OCT2 substrate came from genome-wide association studies showing acute elevation of SCr (24% increase) in cancer patients following treatment with cisplatin, a known substrate and inhibitor of OCT2. The effect of cisplatin on creatinine secretion is attributed primarily to competitive inhibition of OCT2 transport (Ciarimboli et al., 2012).

In a genetic association study, an intergenic single nucleotide polymorphism (SNP) (rs2504954; T>G), located on chromosome 6 in the region between the OCT2 and OCT3 genes, was significantly associated with higher SCr level (Ciarimboli et al., 2012). Another noncoding SNP (rs2279463; T>C) in the OCT2 gene was associated with creatinine metabolism (Kottgen et al., 2010). On the other hand, a coding SNP in OCT2 (rs316019; S270A) has been associated with reduced cisplatin-induced nephrotoxicity (Filipski et al., 2009) and reduced renal elimination of metformin (Wang et al., 2008), but not with altered SCr levels. An intronic OCT2 SNP (rs316009; G>A), which highly correlated to rs316019, showed a strong association with tubular creatinine secretion and end-stage renal disease (Reznichenko et al., 2013). Taken together, both in vitro and in vivo data indicate a role of OCT2 in tubular secretion of creatinine.

Creatinine has also been reported to be an in vitro substrate for OCT3 (Imamura et al., 2011; Ciarimboli et al., 2012; Lepist et al., 2014). Similar to OCT2, OCT3 transports creatinine with a K_m in the millimolar range (~1.9 mM for OCT2 and ~1.3 mM for OCT3) (Lepist et al., 2014). Clinically significant polymorphisms have been identified in OCT3. It is unknown, however, whether or not these SNPs have an impact on creatinine secretion (Aoyama et al., 2006; Sakata et al., 2010). Based on current evidence, OCT3 is likely less important than OCT2 for creatinine uptake in the kidney.
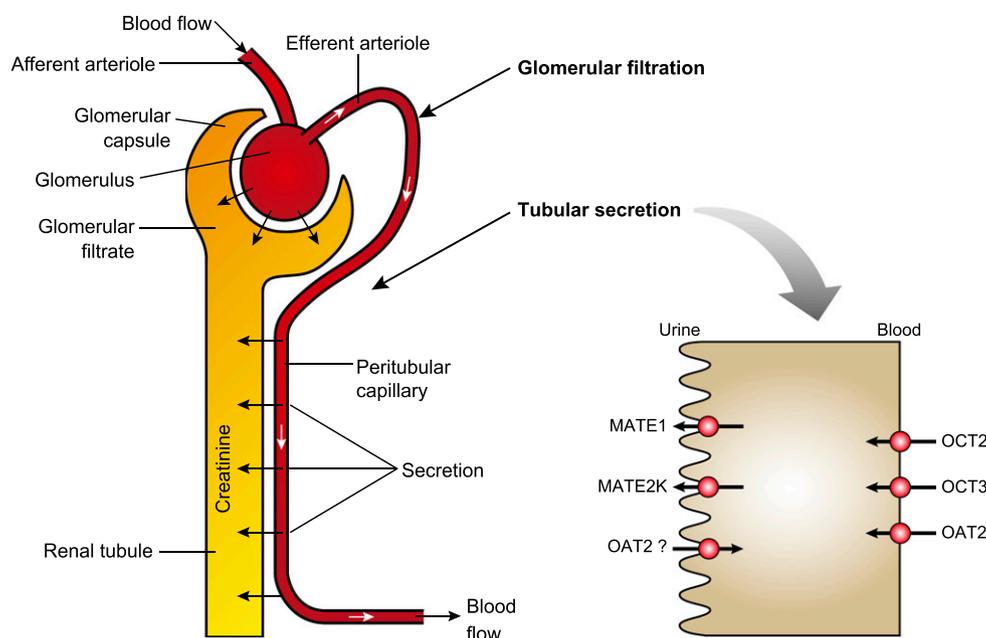
Fig. 3. Schematic representation of renal elimination of creatinine and the transporters known to transport creatinine in vitro.

Opposing the traditional view of organic cationic pathways as the sole mechanism of creatinine secretion in the kidney, creatinine has been reported to be an in vitro substrate for OAT2 (Ciarimboli et al., 2012; Lepist et al., 2014; Shen et al., 2015). A comprehensive analysis to identify the transporters for creatinine was performed, in which each transporter's mRNA and function were measured (Lepist et al., 2014). Creatinine showed a somewhat higher affinity toward OAT2 ($K_m = 986$ μM) as compared with OCT2 and OCT3. Other groups also observed higher affinity transport of creatinine by OAT2 compared with that by other transporters (Shen et al., 2015). OAT2 might contribute to creatinine secretion, and possibly reabsorption in human renal proximal tubules, but clinical data are needed to support this hypothesis.

The role of OAT3 in creatinine secretion is unclear. Contradictory findings were observed in vitro in OAT3-transfected cell lines (Urakami et al., 2004), and kinetic data have not been reported. The involvement of mouse Oat3 in creatinine secretion is also unclear. Vallon et al. (2012) used *Xenopus laevis* oocytes to show that creatinine was transported by mouse Oat3, and renal creatinine clearance was significantly reduced in Oat3 knockout compared with wild-type mice. However, Ciarimboli et al. (2012) did not observe any creatinine uptake by mouse Oat3 in a transfected cell line. The contribution of OAT3 to renal creatinine uptake in humans was estimated to be very low based on a relative activity factor evaluation (Imamura et al., 2011). For OAT1, several reports showed that creatinine was not a substrate for this transporter (Urakami et al., 2004; Imamura et al., 2011; Ciarimboli et al., 2012; Lepist et al., 2014).

Transporters Involved in Creatinine Efflux in the Kidney

Creatinine transport has been studied by members of the SLC47A family, such as MATE1 and MATE2K, and SLC22A family members, such as organic cation and carnitine transporters OCTN1 and OCTN2, and organic anion transporter OAT4.

Expression and Function of MATE1, MATE2K, OCTN1, OCTN2, and OAT4. MATEs are proton/organic cation antiporters. MATE1 is highly expressed in the kidney, liver, adrenal gland, skeletal muscle, and several other tissues, whereas MATE2K is specifically expressed in the kidney (Masuda et al., 2006). Both MATE1 and MATE2K play a role in the renal tubular secretion of cationic drugs and

endogenous compounds in humans (Yonezawa and Inui, 2011). OCTN1 and OCTN2 are organic cation transporters expressed in many tissues. They are localized at the brush border membrane of the proximal tubules in the kidney and play a role in L-carnitine tissue distribution and renal reabsorption (Wu et al., 1999; Tamai, 2013). OAT4 is also located at the brush border membrane of proximal tubules and mediates the bidirectional transport of urate and some organic anions in a substrate-dependent manner (Miyazaki et al., 2005; Hagos et al., 2007).

Transporters Involved in Creatinine Efflux. Creatinine has been reported to be a substrate for MATE1 and MATE2K (Tanihara et al., 2007). Although MATEs function as efflux transporters in vivo, they are often evaluated as uptake transporters by manipulating extracellular pH in vitro. In interpreting in vitro data for MATEs, it is assumed that the intra- and extracellular binding sites have an equal affinity for substrates and inhibitors. In vitro studies suggest that MATE1 and MATE2K are involved in tubular secretion of creatinine (Tanihara et al., 2007; Lepist et al., 2014; Shen et al., 2015). The uptake window of creatinine by MATEs at extracellular pH 8.4 was relatively low, and only ~2- to 3-fold higher in MATE1-transfected cells and 1.3- to 3-fold higher in MATE2K-transfected cells compared with control cells (Tanihara et al., 2007; Lepist et al., 2014; Shen et al., 2015). Intracellular acidification by pretreatment with ammonium chloride enhanced the uptake of creatinine by MATE1 and MATE2K (Tanihara et al., 2007). Kinetic analyses showed that creatinine has low affinity toward MATE1 and MATE2K, with K_m values of ~10 and ~21 mM, respectively (Shen et al., 2015). Orthologs of human MATE1, but not MATE2K, have been identified in rats and mice (Yonezawa and Inui, 2011). When studying the nephrotoxicity of cisplatin, a significant increase in creatinine was observed in cisplatin-treated Mate1 knockout mice compared with control mice. In addition, the combination of pyrimethamine, a selective inhibitor of mouse Mate1, with cisplatin significantly increased creatinine levels compared with cisplatin alone in wild-type mice. Both studies indirectly suggested a role of Mate1 in creatinine transport, at least in mice (Nakamura et al., 2010).

Several polymorphisms have been identified in MATE1 (rs111060524-G64D, rs111060526-A310V, rs111060527-D328A, rs111060528-N474S) and MATE2K (rs111060529-K64N and

rs111060532-G211V) in Japanese subjects, and these variants were associated with loss of transport activity of TEA and metformin in vitro (Kajiwara et al., 2009). Other MATE1 SNPs (rs35646404 -T159M and rs35790011-V338I) have also been described with similar reduction in transport activity of TEA and metformin in other subjects from various ethnic groups (Meyer zu Schwabedissen et al., 2010). The effect of these MATE1 and MATE2K variants on creatinine transport remains to be elucidated.

To date, no reports have demonstrated creatinine transport by OCTN1 and/or OCTN2. It is unclear whether creatinine is reabsorbed by proximal tubular cells through OAT4, as OAT4 functions as a bidirectional transporter (Hagos et al., 2007), suggesting that it could be involved in excreting substrates into urine and/or reuptake of substrates from urine into cells. Using different transfected cell lines, some researchers observed creatinine uptake by OAT4 (Imamura et al., 2011), but this was not confirmed by others (Lepist et al., 2014).

In summary, based on current evidence, OCT2, MATE1, and MATE2K are the major transporters involved in renal creatinine secretion. OAT2 also could be involved based on in vitro evidence, but its in vivo relevance in humans is not clear yet (Fig. 3).

Inhibition of Renal Transporters and Elevation of SCr: An In Vitro–In Vivo Correlation Analysis

In drug development, it is desirable to develop approaches to understand underlying mechanisms for interactions of drug candidates with active renal secretion of creatinine and to subsequently distinguish clinically relevant increases in SCr due to impairment of renal function from nonpathologic increases in SCr caused by inhibition of renal transporters. We therefore conducted a retrospective analysis to evaluate whether an in vitro–in vivo correlation (IVIVC) exists between inhibition of the renal transporters OCT2, MATE1, MATE2K, OAT2, and OCT3 and elevations of SCr and/or decreases in CL_{cr}. In this analysis (Tables 1 and 2), a total of 16 compounds were identified that showed 1) ≥10% reversible elevation of SCr without a significant change in measured GFR (cimetidine, pyrimethamine, trimethoprim, dronedarone, 7-[(3R)-3-(1-aminocyclopropyl)pyrrolidin-1-yl]-1-[(1R,2S)-2-fluorocyclopropyl]-8-methoxy-4-oxoquinoline-3-carboxylic acid (DX-619), dolutegravir, cobicistat, ritonavir,

ranolazine, rilpivirine, and telaprevir); 2) >10% reversible elevation of SCr, without reported data on changes in GFR (amiodarone, vandetanib); and 3) no significant elevation of SCr and GFR and/or other renal toxicity markers at clinically relevant exposure as negative in vivo controls (famotidine, ranitidine, and raltegravir). In vitro inhibition data (IC_{50} or K_i values) for human OCT2, MATE1, MATE2K, OAT2, and OCT3 were collected for these compounds from the University of Washington DDI database (https://www.druginteractioninfo.org). The range of IC_{50} or K_i values is summarized in Table 1. To better understand the correlation between in vitro inhibition of OCT2 and MATEs and the elevation of SCr, in vitro IC_{50} values for inhibition of OCT2, MATE1, and MATE2K for 15 compounds listed in Table 1 were measured at Merck Research Laboratories (Kenilworth, NJ) using metformin as the probe substrate and the method described by Rizk et al. (2014) in CHO-K1-OCT2, CHO-K1-MATE1, and MDCKII-MATE2K cells. Although creatinine is an ideal in vitro probe for IVIVC evaluations, its assay window in OCT2 and MATE uptake assays is relatively low (our unpublished observations) (Lepist et al., 2014; Shen et al., 2015), and therefore, it is unsuitable for measuring IC_{50} values.

IVIVC analysis in this review will be focused on OCT2 and MATEs. In vitro inhibition data for OAT2 and OCT3, which were recently identified as renal creatinine transporters, are currently available only for a few compounds (Table 1). These compounds generally show weak inhibition of OAT2 and OCT3 compared with MATEs and/or OCT2, suggesting that inhibition of these transporters might be clinically less relevant. Indomethacin is a relatively potent in vitro inhibitor of OAT2 (IC_{50} = 2.1 μM) (Shen et al., 2015). However, the effect of indomethacin on elevation of SCr in several clinical studies is controversial (Prescott et al., 1990; Al-Waili, 2002). In female healthy volunteers, indomethacin (150 mg daily for 3 days) had no significant effect on SCr, GFR, or renal blood flow (Prescott et al., 1990). However, indomethacin was reported to increase SCr in neonates (Al-Waili, 2002). As indomethacin is a potent prostaglandin synthesis inhibitor, it is likely that mechanisms other than transporter inhibition could result in the observed elevation of blood creatinine (Al-Waili, 2002).

In vitro inhibition data on OCT2, MATE1, and MATE2K in the literature showed high variability for several compounds (Table 1). For

TABLE 1

In vitro inhibition (IC_{50} or K_i) of selected compounds on human OCT2, MATE1, MATE2K, OAT2, and OCT3

Inhibitors	OCT2 IC_{50} or K_i (μM)		MATE1 IC_{50} or K_i (μM)		MATE2K IC_{50} or K_i (μM)		OAT2 IC_{50} or K_i (μM)	OCT3 IC_{50} or K_i (μM)
	Probe Metformin (10 μM)[a]	Reported in the Literature[b]	Probe Metformin (5 μM)[a]	Reported in the Literature[b]	Probe Metformin (5 μM)[a]	Reported in the Literature[b]	Reported in the Literature[b]	Reported in the Literature[b]
Cimetidine	2.9 ± 0.7	16.6–1650	0.6 ± 0.05	0.2–16.3	5.6 ± 0.7	2.1–46.6	22–72.8	9.8–111
Pyrimethamine	0.61 ± 0.04	4.8–23.6	0.02 ± 0.002	0.077–0.63	0.045 ± 0.003	0.046–0.52	—	—
Famotidine	21.6 ± 3.4	36.1–1800	0.45 ± 0.03	0.6–0.76	6.6 ± 0.9	9.7–36.2	—	6.7–14
Ranitidine	11.7 ± 1.3	30.5–79	8.2 ± 0.6	8.3–25.4	21 ± 2	25	—	62–290
Trimethoprim	19.8 ± 1.5	13.2–1327	0.51 ± 0.03	3.31–29.1	0.14 ± 0.02	0.61–28.9	NI	12.3
Dronedarone	1.9 ± 0.3	—	0.46 ± 0.02	—	8.9 ± 1.7	—	—	—
DX-619	—	0.94–1.29		0.82–4.32		0.1	—	—
Dolutegravir	0.21 ± 0.04	0.066–1.93	3.6 ± 0.7	4.67	12.5 ± 1.6	>100	>100	>100
Cobicistat	37.9 ± 4.9	8.24–33	0.98 ± 0.19	0.99–1.87	20.5 ± 3.0	33.5	>100	>100
Ritonavir	24.8 ± 3.4	20–25	0.28 ± 0.05	0.08–15.4	40.1 ± 6.5	23.7	>20	300
Ranolazine	47 ± 9	—	16.8 ± 1.5	—	50 ± 8	—	—	—
Rilpivirine	0.38 ± 0.05	5.13	0.25 ± 0.04	—	0.28 ± 0.08	—	—	—
Amiodarone	4.7 ± 1.1	>1000	1.0 ± 0.2	—	>50	—	—	>1000
Raltegravir	>100	>100	>100	>100	>100	>100	—	—
Telaprevir	>100	6.35	62 ± 5	22.98	>100	—	—	—
Vandetanib	0.4 ± 0.05	5.5–73.4	0.06 ± 0.01	0.16–1.23	0.04 ± 0.01	0.3–1.26	—	—

—, Data are not reported or available; NI, no inhibition.
[a]Data generated at Merck & Co. for inhibition of OCT2, MATE1, and MATE2K using metformin as the probe substrate in CHO-K1-OCT2, CHO-K1-MATE1, and MDCKII-MATE2K cells using the method described by Rizk et al. (2014).
[b]Data obtained from the University of Washington DDI database (https://www.druginteractioninfo.org).

TABLE 2

Effect of selected compounds on SCr, CL$_{cr}$, and GFR in humans and the correlation with in vitro inhibition of OCT2, MATE1, and MATE2K

Inhibitors	Dosing Regimen	SCr↑	CL$_{cr}$↓	GFR↓	Markers for GFR	C$_{max}$	f$_u$	C$_{max}$/IC$_{50}$ [a]			C$_{max,u}$/IC$_{50}$ [a]			References
		%	%			μM		OCT2	MATE1	MATE2K	OCT2	MATE1	MATE2K	
Cimetidine	400 mg (QDS)	13–26	20	NS	^{51}Cr–EDTA, inulin	8–12	0.8	4.14	20.00	2.14	3.31	16.00	1.71	Dutt et al., 1981; Hilbrands et al., 1991
Pyrimethamine	50–100 mg SD	18–26	25–27	NS	inulin	2.3[a]	0.13	3.77	115.00	51.11	0.49	14.95	6.64	Opravil et al., 1993; Kusuhara et al., 2011
Famotidine	40 mg QD, 7days	NS	NS	—	—	0.39	0.8	0.02	0.87	0.06	0.01	0.69	0.05	Ishigami et al., 1989
Famotidine	200 mg SD; 160 mg q4h	SI	SI	—	—	1.25	0.8	0.06	2.78	0.19	0.05	2.22	0.15	Hibma et al., 2015
Ranitidine	300 mg QD	NS	NS	—	—	3.72	0.85	0.32	0.45	0.18	0.27	0.39	0.15	Motyl, 2004
Trimethoprim	20 mg/kg/day (10 days); 200 mg BID	31	16	NS	^{51}Cr–EDTA iothalamate	3.4–6.9	0.56	0.35	13.53	49.29	0.20	7.58	27.60	Naderer et al., 1997; Arya et al., 2014
Dronedarone	400 mg BID 7days	10–15	18	NS	Sinistrin, PAH	0.30	0.02	0.16	0.65	0.03	0.003	0.013	0.001	Tschuppert et al., 2007
DX-619	800 mg (QD) (4 days)	30–40	26	NS	Iohexol	20.5–22	0.29–0.35[b]	23.40	26.83	220.0	8.19	9.39	77.0	Sarapa et al., 2007
Dolutegravir	50 mg (QD or BID, 14 days)	9–17	10 –14	NS	Iohexol Cystatin C	6.7–13.1	0.01	62.38	3.64	1.05	0.62	0.04	0.01	Koteff et al., 2013
Cobicistat	150 mg QD, 7 days, PO	10.5, 23	8–14, 9 – 20	NS	Iohexol	1.55	0.03	0.04	1.58	0.08	0.001	0.05	0.002	Cohen et al., 2011; German et al., 2012; Arya et al., 2014
Ritonavir	100 mg QD	—	25	NS	Iohexol	2.16	0.015	0.09	7.71	0.05	0.001	0.12	0.001	Deray et al. 1998; German et al., 2012; Lepist et al., 2014
Ranolazine	1000 mg BID, 5 days, PO	12	10	NS	Sinistrin	6.01	0.38	0.13	0.36	0.12	0.05	0.14	0.05	Arya et al., 2014
Rilpivirine	25 mg (QD, 96 weeks)	10	—	NS	Cystatin C	0.6	0.005	1.58	2.40	2.14	0.01	0.01	0.01	Drug label; Maggi et al., 2014
Amiodarone	400 mg QD	11	—	—	—	0.8–2.3	0.04	0.49	2.30	0.05	0.02	0.09	0.002	Pollak et al., 1993
Raltegravir	400 mg BID	NS	NS	—	—	3.38	0.17	<0.03	<0.03	<0.03	<0.01	<0.01	<0.01	Drug label; Maggi et al., 2014; Rizk et al., 2014
Telaprevir	750 mg q8h	SI	—	NS	Cystatin C, L–FABP, NAG	5.82	0.04–0.24[b]	<0.06	0.09	<0.06	<0.06	0.02	<0.01	Suzuki et al., 2013; Matsui et al., 2015
Vandetanib	300 mg QD	15	—	—	—	0.33	0.1	0.83	5.50	8.25	0.08	0.55	0.83	Shen et al., 2013

—, data are not reported or available; BID, twice daily; L-FABP, liver-type fatty acid binding protein; NAG, N-acetyl-β-Dglucosaminidase; NS, not significant (either statistically or clinically); PAH, para-amino-hippurate; PO, by mouth; QDS, four times a day; q4h, every 4 hours; q8h, every 8 hours; SD, single dose; SI, significantly increased compared with baseline level.

[a]IC$_{50}$ values used were generated at Merck & Co. and shown in Table 1, except for DX-619, for which the lowest IC$_{50}$ value obtained from the literature is used (see Table 1).

[b]Highest f$_u$ values are used to estimate C$_{max,u}$/IC$_{50}$ as the worst-case scenario.

instance, the in vitro IC_{50} or K_i for ritonavir with MATE1 showed a 193-fold variability, and inhibition of OCT2 by trimethoprim and cimetidine showed a 101- and 99-fold variability, respectively. The reasons for this high variability are not understood, but it could be caused by the use of different probe substrates, and differences in in vitro systems and assay conditions. For example, remarkable substrate-dependent differences in IC_{50} values for inhibition of MATE2K by trimethoprim were reported (47-fold, metformin vs. N-methylnicotina-mide as probes) (Muller et al., 2015) and for OCT2 inhibition by vandetanib (13-fold, N-methyl-4-phenylpyridinium iodide vs. metfor-min as probes) (Shen et al., 2013) when the studies were conducted in the same laboratory using the same in vitro system. Substrate-dependent inhibition of OCT2, MATE1, and MATE2K has been systemically evaluated with several prototypic substrates (Belzer et al., 2013; Martinez-Guerrero and Wright, 2013), suggesting that both OCT2 and MATEs have multiple drug binding sites. In contrast to such substrate-dependent inhibition, several other studies have shown consistent K_i or IC_{50} values with selected OCT2/MATE inhibitors across different probe substrates. For instance, Ito et al. (2012b) reported no marked substrate dependence in cimetidine K_i values for OCT2, MATE1, and MATE2K with five probe substrates. Likewise, similar IC_{50} values were obtained with cobicistat for OCT2 and MATE1 using TEA and creatinine as probe substrates (Lepist et al., 2014). Nevertheless, development of predictive DDI models for OCT2 and MATEs needs to take into account the potential for substrate dependence of ligand interactions with these proteins. Furthermore, different in vitro systems and assay conditions may have a marked effect on IC_{50} variability. For example, in studies where metformin was used as probe substrate, the ritonavir IC_{50} for MATE1 was 0.08 μM when preincubating MATE1-transfected human embryonic kidney 293 cells for 30 minutes in a 30 mM NH_4Cl buffer to create an artificial pH gradient (Wittwer et al., 2013), whereas the IC_{50} was 15.4 μM when using MATE1-transfected HeLa cells without preincubation with NH_4Cl (Meyer zu Schwabedissen et al., 2010).

In Table 2, the risk for in vivo inhibition of OCT2, MATE1, and MATE2K was assessed by comparing total and unbound maximal plasma concentrations (C_{max} and $C_{max,u}$) of test compounds with in vitro IC_{50} values (C_{max}/IC_{50} and $C_{max,u}/IC_{50}$). A cutoff of $C_{max}/IC_{50} \geq$ 0.1 or $C_{max,u}/IC_{50} \geq 0.1$ was used to predict the risk for in vivo inhibition of respective transporters. As the relative contribution of these transporters (fraction transported) and the rate-determining step for renal secretion of creatinine are not well known, we assume that OCT2, MATE1, and MATE2K contribute equally to the renal secretion of creatinine. Therefore, in assessing the existence of an IVIVC, inhibition of any of the aforementioned transporters was considered as an indication of in vivo inhibition of creatinine secretion as the worst case scenario. As shown in Table 2, using our in-house IC_{50} data, C_{max}/IC_{50} (≥ 0.1) provided a reasonably good prediction for the elevation of SCr for this set of compounds, as there were no false-negative predictions. Use of $C_{max,u}/IC_{50}$ (≥ 0.1) resulted in four false negatives (dronedarone, cobicistat, rilpivirine, and telaprevir). Both C_{max}/IC_{50} and $C_{max,u}/IC_{50}$ resulted in a false-positive prediction for famotidine [40 mg every day (QD) for 7 days] and ranitidine.

Considering the variability of IC_{50} and K_i values reported in the literature, using the lowest IC_{50} or K_i values for OCT2, MATE1, and MATE2K available for 11 compounds (Table 1), C_{max}/IC_{50} (≥ 0.1) provided a reasonably good prediction for the elevation of SCr, whereas $C_{max,u}/IC_{50}$ (≥ 0.1) resulted in a false-negative prediction for cobicistat (data not shown). Likewise, using the highest IC_{50} or K_i values reported for OCT2, MATE1, and MATE2K, C_{max}/IC_{50} (≥ 0.1) still provided a good prediction of the elevation of SCr, whereas $C_{max,u}/IC_{50}$ (≥ 0.1) resulted in a false-negative prediction for cobicistat, dolutegravir,

ritonavir, and vandetanib. Use of either the lowest or highest IC_{50} literature values, C_{max}/IC_{50} and $C_{max,u}/IC_{50}$ both resulted in false-positive predictions for famotidine (40 mg QD for 7 days) and ranitidine (data not shown). However, Hibma et al. (2015) have recently reported an elevation of SCr and a reduction in CL_{cr} by famotidine in humans at a single dose of 200 mg and multiple doses of 160 mg, which were 4- to 5-fold higher than in a previous report (Ishigami et al., 1989) (Table 2). The reason for the lack of IVIVC for these two compounds at clinically relevant exposure is unclear. As there are no major circulating metabolites for ranitidine and famotidine, it is less likely for metabolites to cause transporter inhibition. An effect on reabsorption of creatinine cannot be excluded, however.

Currently, $C_{max,u}/IC_{50} \geq 0.1$ is being recommended by the Food and Drug Administration for OCT2 (U.S. Department of Health and Human Services et al., 2012) and the International Transporter Consortium for OCT2 and MATEs (Hillgren et al., 2013) as the cutoff value to assess the risk for DDIs with OCT2/MATEs transporters. For prediction of transporter-related DDIs, it is critical to use relevant inhibitor concentrations, which are unbound inhibitor concentrations at the site of interactions with the transporter of interest. As such, $C_{max,u}$ will be the relevant concentration for predicting DDI with OCT2, which is localized in the basolateral plasma membrane of renal proximal tubule cells, whereas it may not be adequate to predict DDIs for efflux transporters, such as MATEs, as these are localized in the apical plasma membrane. For example, if the inhibitor is actively taken up by the proximal tubule cells, $C_{max,u}$ may underestimate the inhibitory effects for efflux transporters. Thus, unbound intracellular inhibitor concentrations in relevant tissues would be more relevant for prediction of efflux transporter–related DDIs. However, the methodologies to measure and/or predict such values are currently still limited (Chu et al., 2013).

Is Creatinine a Sensitive Biomarker for Renal Cationic Transporter–Related DDIs?

Determining the impact of perpetrator drugs on plasma concentration or urinary excretion of suitable endogenous biomarkers is a valuable tool to assess the risk for drug interactions early in drug development (e.g., phase I clinical trials). Recently, some endogenous probes for studying renal cationic transporter–related DDIs have been identi-fied. Ito et al. (2012a) have found that the endogenous metabolite N-methylnicotimide (NMN), a substrate for OCT2, MATE1, and MATE2K, could be used as an endogenous probe to study the DDIs related to OCT2/MATE inhibition in humans. Pyrimethamine, a potent inhibitor of MATE1 and MATE2K, almost completely diminished tubular secretion of NMN (renal clearance 403 vs. 119 ml/min), but had a minimal effect on plasma exposure of NMN. Furthermore, Muller et al. (2015) reported that trimethoprim, another OCT2/MATE inhibitor, decreased NMN renal clearance by 19.9% without a significant impact on NMN plasma area under the curve. The magnitude of trimethoprim-induced renal clearance reduction was positively correlated between NMN and metformin in 12 subjects, suggesting the potential use of NMN as an endogenous probe for DDIs involving OCT2/MATEs. Using untargeted metabolomics analysis of urine specimens from healthy subjects and mice treated with or without pyrimethamine, Kato et al. (2014) found that thiamine, a vitamin B1, which is essential for carbohydrate metabolism and neural function, is also a potential biomarker for inhibition of MATE1 and MATE2K.

To evaluate if creatinine can be used as a biomarker to assess OCT2/MATE-related DDIs, we searched the literature for examples where clinical DDIs can be mechanistically explained by inhibition of OCT2 and/or MATEs, and the changes in SCr or CL_{cr} were measured in the same clinical studies. As shown in Table 3, observed DDIs with several OCT2/MATE inhibitors (cimetidine, pyrimethamine, trimethoprim, and vandetanib) at the dose indicated correlated with a 10–30% elevation

TABLE 3

Examples of transporter-related DDIs involving OCT2, MATE1, and/or MATE2K and correlation with transient elevation of SCr

Perpetrator	Perpetrator Dose Regimen	Victim	Victim Dose Regimen	% Change in AUC	% Change in Renal CL	Elevation of SCr and/or % Decrease of CLcr	References
Cimetidine	400 mg QID (8 days)	Gabapentin	1200 mg QD [4 days]	23.7	−17.8	Yes; CLcr↓10%	Lal et al., 2010
Cimetidine	800 mg BID (6 days)	Glycopyrronium	100 µg inhalation SD	19.2	−22.1	—	Dumitras et al., 2013
Cimetidine	400 mg BID (6.5 days)	Metformin	500 mg SD	54.2	−44.6	No; CLcr NS	Wang et al., 2008
Cimetidine	400 mg BID (5 days)	Metformin	250 mg QD (10 days)	46.2	−28.3	No[a]	Somogyi et al., 1987
Cimetidine	300 mg TID (5 days)	Varenicline	2 mg SD	29.7	−25.1	Yes; CLcr↓5–10%	Feng et al., 2008
Dolutegravir	50 mg BID (7 days)	Metformin	500 mg BID (12 days)	145	—	Yes; SCr ↑	Zong et al., 2014
Pyrimethamine	50 mg SD	Metformin	250 mg SD	35.3	−35	Yes; CLcr↓20%	Kusuhara et al., 2011
Ranitidine	150 mg BID	Procainamide	1 g SD	13.7	−18.5	—	Somogyi and Bochner, 1984
Ranitidine	150 mg BID (4 days)	Triamterene	100 mg/day QD (8 days)	−24	−51	No; NS Clcr	Muirhead et al., 1988
Trimethoprim	200 mg TID (6 days)	Metformin	500 mg TID (10 days)	37	−32	Yes; CLcr↓20%; SCr ↑23%	Grun et al., 2013
Trimethoprim	200 mg TID(5 days)	Metformin	850 mg QD (2 doses)	29.5	−26.4	Yes; CLcr↓16.9%	Muller et al., 2015
Vandetanib	100 mg QD (three 21-day cycles)	Cisplatin	75 mg/m^2 SD (three 21-day cycles)	32.7	—	—	Blackhall et al., 2010
Vandetanib	800 mg SD	Metformin	1000 mg SD	73.3	−52	Yes; SCr ↑ 8–29%	Johansson et al., 2014

—, Data are not reported or available; AUC, area under the curve; BID, twice daily; NS, not significant statistically; QID, four times a day; SD, single dose; TID, three times a day.
[a]A time-dependent variation of SCr was observed.

in SCr or a decrease in CL$_{cr}$. This was not the case for ranitidine, however, as it caused DDIs with procainamide and triamterene without affecting SCr levels. Interestingly, famotidine, a recently reported MATE1 selective inhibitor, significantly increased SCr (200 mg QD and 160 mg every 4 hours) without affecting the plasma exposure of metformin (Hibma et al., 2015). The latter is likely explained by the opposing effects of the famotidine-induced increase in both metformin absorption and renal clearance. Elevation of SCr by cimetidine was variable and less sensitive in some DDI studies at the clinically relevant dose of 300–400 mg. Considering the weak to moderate change in SCr associated with OCT/MATE-related DDIs, and that a range of other factors may potentially impact SCr exposure, as we have discussed elsewhere in this review, SCr does not appear to be a biomarker with sufficient sensitivity to assess the risk, either qualitatively or quantitatively, of inhibition of OCT2 or MATEs in humans. However, follow-up mechanistic studies, such as transporter inhibition experiments, are still useful in cases where increases in SCr exposure are observed.

Is Serum Creatinine an Appropriate Marker for Renal Injury?

Traditional monitoring for nephrotoxicity relies upon the measurement of SCr. However, SCr retains poor specificity for AKI and is insensitive to the degree of AKI for three reasons. First, a large amount of nephron loss can occur without significant changes in SCr due to residual renal reserve. This fact is most clearly evident in kidney donors in whom no significant change in SCr occurs despite a 50% loss of functioning renal mass (Bosch et al., 1983). Second, the rate of rise in SCr following a renal insult is delayed due to the kinetics of creatinine production from muscle turnover and accumulation secondary to reduced glomerular filtration. At a normal GFR of 120 ml/min, the serum half-life of creatinine is approximately 4 hours; however, at a GFR of 30 ml/min, the half-life extends to 16 hours and will therefore not reach steady state for nearly 3 days (Waikar and Bonventre, 2009). Third, as previously discussed, SCr is influenced by a number of other factors, including inhibition of tubular secretion by drugs, weight, gender, age, muscle metabolism, hydration state, and protein intake (Blantz, 1998). Reduced muscle mass secondary to malnutrition or immobility is a frequently observed clinical problem that severely limits the utility of SCr as a marker of kidney function. Based on the limitations of SCr, there has been great interest in the identification of alternate markers of renal function. To date, a number of promising biomarker candidates have been identified, characterized, and validated

using models of kidney injury in animals or described for various clinical settings in humans, such as sepsis, cardiac bypass surgery, and contrast media exposure (Fuchs and Hewitt, 2011; Waring and Moonie, 2011; Vanmassenhove et al., 2013). Importantly, the utility of these new biomarkers in clinically detecting drug-induced AKI in either the patient-care or drug-development setting has not been established. Presently, regulatory agencies have agreed that urine biomarkers should be used for nonclinical phases of drug development, and on a case-by-case basis for clinical drug development research investigation (Dieterle et al., 2010). Clinical qualification of novel AKI urine biomarkers for use during clinical drug development is currently ongoing.

Conclusions

Based on the in vitro and pharmacogenomic evidence available, OCT2 is one of the transporters involved in the uptake of creatinine into kidney proximal tubule cells, but its quantitative involvement is unknown. More recent in vitro data suggest that OAT2 also transports creatinine efficiently, but to what extent this is relevant in humans is not yet clear. Following uptake into the kidney, MATE1 and MATE2K mediate the efflux of creatinine into the urine. Important questions that remain are whether uptake or efflux is rate-determining in the active secretion of creatinine, what the relative contribution is of each transporter in this process, and whether there are yet-unidentified transporters involved in creatinine excretion and/or reabsorption. Similar to hepatobiliary transport, it is generally hypothesized that uptake is the rate-limiting step for active tubular secretion, if the luminal efflux is markedly greater than the basolateral efflux. In this case, the inhibition of the luminal efflux should have less impact on the overall systemic intrinsic clearance. However, this cannot explain the significant elevation of SCr by pyrimethamine, a selective inhibitor of MATEs, relative to OCT2.

Currently, the effect of drugs on creatinine transport is measured in cell lines transfected with individual transporters. Recently, a quintuple in vitro transporter model expressing OAT2/OCT2/OCT3/MATE1/MATE2K was explored to evaluate the impact of test compounds on creatinine transport (Zhang et al., 2015), but more data are needed to establish the predictive value of this model. Development and use of holistic models and integrated systems, such as immortalized cell lines derived from human kidney with preserved activity of transporters and drug-metabolizing enzymes, may provide more physiologically relevant models to study the interaction of drugs with the renal secretion of creatinine in the future (Schophuizen et al., 2015).

Overall, a high variability of in vitro transporter inhibition data, not limited to OCT2/MATEs, has become a significant concern and may limit in vitro to in vivo extrapolation using universal cutoff values for transporter perpetrator decision trees, which trigger clinical DDI studies (Bentz et al., 2013). Although the underlying mechanisms for IC_{50} variability can be complex, proper standardization of in vitro inhibition assays using, for example, clinically relevant probe substrates, standardized incubation conditions, and cell lines will be helpful for improving in vitro to in vivo extrapolation.

In an attempt to establish an IVIVC between inhibition of OCT2, MATE1, and MATE2K, several false negatives were identified using a cutoff for the ratio of $C_{max,u}/IC_{50}$ or $C_{max,u}/K_i$ of ≥ 0.1. The true-positive rate was higher if total (bound plus unbound) drug concentrations were used for the analyses. Since only unbound drug will be available for interactions with transporters, this suggests that the free drug concentration measured in plasma is lower than in the proximal tubule cells, or that mechanisms other than inhibition of MATEs and OCT2 contribute to the effects on creatinine. For example, although cobicistat is an inhibitor of MATE1 in vitro, this inhibition is not predicted to be clinically significant based on $C_{max,u}/IC_{50}$ data. Remarkably, famotidine and ranitidine were identified as inhibitors of MATE- and OCT2-mediated creatinine transport in vitro, whereas no effect on creatinine was observed at clinically relevant exposures. Currently, we have no good explanations for the lack of IVIVC for these compounds. In the future, use of mechanistic models may improve the prediction of in vivo interaction of drug molecules with creatinine renal transporters.

Due to potential interactions of drug molecules with creatinine secretion along with several other limitations, an alternative method to estimate GFR is desirable. Despite ongoing efforts to identify more sensitive and specific markers for renal function and injury, use of creatinine to estimate GFR is currently still a practical approach. As such, if a transient and/or reversible elevation of SCr was observed during drug development, understanding the potential interaction of drug molecules with active renal secretion of creatinine and carefully monitoring renal function with alternate markers, such as cystatin C, would be recommended.

Since organic cation transporters such as OCT2 and MATEs are known to transport endogenous compounds (Jonker and Schinkel, 2004), it would be valuable from a drug development perspective if changes in these compounds could be used as biomarkers for assessing DDIs involving inhibition of these transporters. In the case of the kidney, such biomarkers would need to be excreted to a significant extent by active transport (as opposed to GFR), their levels should not be affected by secondary factors such as diet and disease, they should not be sensitive to diurnal effects, and they would need to be selective for the transporter(s) of interest. Based on these criteria and our retrospective analysis of in vitro and clinical data, creatinine is not an optimal biomarker, as its synthesis involves multiple steps, external factors such as diet and exercise affect plasma levels, and the contribution of active transport to clearance is relatively small and not consistent between patient populations. However, mechanistic studies to explain increases in creatinine in the absence of a decrease in GFR will continue to be important.

Authorship Contributions

Conducted experiments: Chan.

Performed data analysis: Chu, Bleasby, Chan, Evers.

Wrote or contributed to the writing of the manuscript: Chu, Bleasby, Chan, Nunes, Evers.

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Address correspondence to: Dr. Xiaoyan Chu, Merck & Co., RY800-D211, 126 East Lincoln Avenue, Rahway, NJ 07065. E-mail: xiaoyan_chu@merck.com