Down-Regulation of Hepatic CYP3A and CYP2C Mediated Metabolism in Rats with Moderate Chronic Kidney Disease

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ABSTRACT:
Expression and activity of drug-metabolizing enzymes are decreased in severe kidney disease; however, only a small percentage of patients with chronic kidney disease (CKD) are at the final stage of the disease. This study aimed to determine the changes in drug-metabolizing enzyme function and expression in rats with varying degrees of kidney disease. Sprague-Dawley rats were subjected to surgical procedures that allowed the generation of three distinct models of kidney function: normal kidney function, moderate kidney function, and severe kidney disease. Forty-two days after surgery, rats were sacrificed and hepatic CYP3A and CYP2C expression was determined. In addition, enzymatic activity was determined in liver microsomes by evaluating midazolam (CYP3A), testosterone (CYP3A and CYP2C), and tolbutamide (CYP2C) enzyme kinetics. Both moderate and severe kidney disease were associated with a reduction in CYP3A2 and CYP2C11 expression (p < 0.05). Likewise, moderate kidney disease resulted in more than a 60% decrease in enzyme activity (Vmax) for CYP2C11 and CYP3A, compared with controls (p < 0.05). When the degree of kidney disease was correlated with metabolic activity, an exponential decline in CYP2C- and CYP3A-mediated metabolism was observed. Our results demonstrate that CYP3A and CYP2C expression and activity are decreased in both moderate and severe CKD. Our data suggest that drug metabolism is significantly decreased in the earlier stages of CKD and imply that patients with moderate CKD may be subject to unpredictable pharmacokinetics.

Introduction
Chronic kidney disease (CKD) is a progressive condition that is characterized by a decrease in glomerular filtration rate over time. The incidence of CKD is increasing at an alarming rate, and these patients require more than seven medications to manage both their CKD and associated comorbidities (Talbert, 1994). Drug therapy in patients with CKD is complicated because these patients frequently experience unpredictable pharmacokinetics resulting in an increased incidence of medication-related adverse events (Manley et al., 2005). Although previous research showed that renal clearance of drugs is compromised in CKD, recent studies have demonstrated that kidney failure also affects nonrenal drug clearance (Leblond et al., 2001; Naud et al., 2008; Nolin et al., 2009). The nonrenal clearance of drugs is principally mediated by hepatic drug metabolism. CYP3A4, a member of the cytochrome P450 (P450) family of drug-metabolizing enzymes, is involved in the oxidative metabolism of up to 50% of all drugs on the market (Wrighton et al., 1996). Elegant studies in experimental animal models of severe CKD have demonstrated a reduction in the function and expression of CYP3A and other members of the P450 family (Leblond et al., 2000, 2002).

The Kidney Early Evaluation Program (2000–2010) and the National Health and Nutritional Examination Survey (NHANES; 1996–2006) have estimated the prevalence of kidney disease to be greater than 14% in the United States [McCullough et al., 2011; Stevens et al., 2011; Kidney Early Evaluation Program (KEEP) (2011)]. The majority of animal and clinical studies investigating the effect of CKD on nonrenal drug clearance have focused solely on severe CKD. Patients studied in the majority of these publications are on dialysis, despite the fact that severe CKD patients comprise approximately 3% of the total number of patients with CKD (KEEP 2000–2009; NHANES 1999–2006). According to KEEP, 11, 19, and 67% of patients with CKD are in stages 1, 2, and 3, respectively [Kidney Early Evaluation Program (KEEP) (2011)]. Therefore, patients with mild and moderate kidney disease comprise the majority (97%) of the population of patients with CKD. Few studies have evaluated pharmacokinetics and drug metabolism in varying degrees of kidney disease despite the fact that CKD is known to be a progressive disorder whereby kidney function declines over time. Clinical studies in patients with varying

ABBREVIATIONS: CKD, chronic kidney disease; P450, cytochrome P450; KEEP, Kidney Early Evaluation Program; NHANES, National Health and Nutrition Examination Survey; PCR, polymerase chain reaction; Ct, cycle threshold; HRP, horseradish peroxidase; UPLC-PDA, ultraperformance liquid chromatography with photodiode array detection; ESRD, end-stage renal disease; NF, nuclear factor.
degrees of CKD have demonstrated altered lidocaine and nicardipine pharmacokinetics; however, these drugs are metabolized by multiple P450 enzymes and are influenced by other pharmacokinetic factors in kidney disease, such as changes in plasma protein binding (Ahmed et al., 1991; De et al., 2006). Consequently, there is a lack of data investigating the effects of CKD on specific drug-metabolizing enzymes in the majority of the patient population. This void in the literature has been highlighted in a recent review (Naud et al., 2012).

The objective of this study was to determine whether similar decreases in hepatic drug-metabolizing enzymes observed in severe CKD are also seen at earlier stages of disease progression. To address this, we used a combination of surgical kidney resection and partial renal artery ligation in rats to establish a novel model of CKD with two different degrees of kidney disease. Upon establishing this model, we assessed hepatic expression and function of the predominant P450 isoforms, CYP3A and CYP2C. To fully elucidate the effect of CKD progression on enzyme function, full enzyme kinetics of selected probe drugs was used to characterize the metabolic activity of these enzymes across the continuum of disease progression.

**Materials and Methods**

Chemical Reagents and Drugs. Midazolam, 1’OH-midazolam, 4’OH-midazolam, OH-tolbutamide, and ketoconazole were purchased from Toronto Research Chemicals (Toronto, ON). Testosterone, 6βOH-testosterone, and 16αOH-testosterone were purchased from Steraloids Inc. (Newport, RI). Flurazepam and carbamazepine were purchased from Cerilliant (Round Rock, TX), and tolbutamide was purchased from Sigma-Aldrich (St. Louis, MO).

Experimental Model. Seventeen male Sprague-Dawley rats, weighing 150 g, were obtained from Charles River Laboratories, Inc. (Wilmington, MA). The animals were maintained on standard rat chow and water ad libitum on a 12-h light cycle. Rats were allowed to acclimatize for at least 3 days before any experimental procedure was undertaken. The animal protocols were approved by the University of Western Ontario Animal Care Committee.

Surgical Induction of Kidney Disease. Studies were performed in three experimental groups, which simulated normal kidney function (n = 6) as well as moderate (n = 7) and severe (n = 4) kidney disease. Moderate and severe kidney diseases were surgically induced by modification of a standard two-stage partial nephrectomy. Atropine (0.04 mg/kg s.c.) was given before anesthesia in addition to intramuscular xylazine (5 mg/kg) and ketamine (100 mg/kg). Anesthesia was maintained with 2% isoflurane in oxygen via face mask throughout all surgical procedures. On day zero, rats in the moderate kidney disease group underwent a one-third partial nephrectomy of the left kidney. Rats in the severe kidney disease group were subjected to the same procedure plus ligation of one branch of the left renal artery to mitigate the perfusion of blood to the remaining kidney. Seven days later, a complete right nephrectomy was performed on both moderate and severe kidney disease groups as previously reported (Leblond et al., 2000). Rats in the control group underwent sham laparotomies. All rats were given ketoprofen (5 mg/kg i.m.) preoperatively and every 24 h postoperation for 3 days. All rats were weighed daily to monitor health. Control and moderate kidney disease rats were pair-fed the same amount of standard rat chow that was ingested by severe kidney disease rats on the previous day. Forty-two days after the initial surgery, rats were sacrificed and liver tissue was harvested, flash frozen in liquid nitrogen, and stored at −80°C. Serum creatinine and urea concentrations were determined by the London Laboratory Services Group (London, ON, Canada) by standard methods.

Real-Time PCR Analysis. Total RNA was extracted using TRIzol Reagent (Life Technologies Inc., Burlington, ON, Canada) according to the manufacturer’s instructions. RNA concentration and integrity were measured by spectrophotometry. Total RNA was reverse-transcribed using iScript RT-qPCR Supermix (Bio-Rad Laboratories, Hercules, CA) in a 20-µl reaction volume. Primer pairs used were as follows: CYP3A2 (forward) 5’-GCTCTTGTAGTGCATGGTTAAAGATTTG-3’ and (reverse) 5’-ATCACAGACCTTGCCAACTCTTT-3’ (Baldwin et al., 2006); CYP2C11 (forward) 5’-CCCTGAAGACGTTTTGAGATGGC-3’ and (reverse) 5’-AGGGCGACCTTTGCTCTTCT-3’; and β-actin (forward) 5’-ACGGACCGAGCAAGCACAGA-3’ and (reverse) 5’-TTGGTACACTGCGGTTCA-3’ (Sohi et al., 2011). Real-time PCR was performed using SsoFast Evagreen (Bio-Rad Laboratories), and expression was analyzed using the ∆∆Ct normalized to β-actin.

Hepatic Microsome Isolation. Liver microsomes were isolated by differential centrifugation according to a slightly modified method by Kurosawa et al. (2009). In brief, liver tissue was rinsed in 0.9% NaCl solution and homog-
anized in 1.15% KCl containing 1 mM EDTA using a Potter-Elvehjem tissue grinder. The homogenate was centrifuged at 105,000 g for 20 min at 4°C, and the subsequent supernatant was centrifuged at 105,000 g for 60 min at 4°C. The microsomal pellet was resuspended in 100 mM potassium phosphate buffer containing 2 mM MgCl₂ (pH 7.4) with 1 mg/ml NADPH. At the end of the incubation, reactions were terminated with 50 mM potassium phosphate buffer and 2 mM MgCl₂ (pH 7.4) with 1 mg/ml hepatic microsomal protein. Before experimentation, linear rate of production of metabolites was determined by varying time, protein, and relevant substrate concentrations. Metabolism was determined to be linear at 10 min for formation of testosterone metabolites (6β-OH testosterone and 16α-OH testosterone) and 30 min for formation of midazolam (1-OH midazolam and 4-OH midazolam) and tolbutamide (OH-tolbutamide) metabolites. All reactions were conducted at 37°C and were started by the addition of a relevant concentration of substrate drug (midazolam, testosterone, or tolbutamide) followed by 1 mM NADPH. At the end of the incubation, reactions were terminated with 50 μl of ice-cold acetonitrile followed by a 15-min incubation on ice and centrifugation to pellet precipitated protein (Chovan et al., 2007). Reactions (250 μl final volume) were conducted in 50 mM potassium phosphate buffer and 2 mM MgCl₂ (pH 7.4) with 1 mg/ml hepatic microsomal protein. Before experimentation, linear rate of production of metabolites was determined by varying time, protein, and relevant substrate concentrations. Metabolism was determined to be linear at 10 min for formation of testosterone metabolites (6β-OH testosterone and 16α-OH testosterone) and 30 min for formation of midazolam (1-OH midazolam and 4-OH midazolam) and tolbutamide (OH-tolbutamide) metabolites. All reactions were conducted at 37°C and were started by the addition of a relevant concentration of substrate drug (midazolam, testosterone, or tolbutamide) followed by 1 mM NADPH. At the end of the incubation, reactions were terminated with 50 μl of ice-cold acetonitrile followed by a 15-min incubation on ice and centrifugation to pellet precipitated protein (Chovan et al., 2007).

Metabolite Analysis by UPLC-PDA. Metabolite analysis was conducted by solid-phase extraction followed by UPLC-PDA. Solid-phase extraction cartridges (C18, Strata-X Polymeric Reverse Phase 33 μm; Phenomenex, Torrance, CA) were conditioned according to manufacturer’s specifications. Flurazepam was used as an internal standard for midazolam and tolbutamide quantification, and carbamazepine was used as an internal standard for testosterone quantification. Analytes and internal standard were passed across the
packing by gravity, after which the cartridges were washed with 1 ml of nano-pure water followed by 1 ml of 50:50 methanol/water (for midazolam and testosterone extraction) or 1 ml of 5% methanol/water (for tolbutamide extraction). Analytes were eluted into clean glass test tubes with 1 ml of methanol containing 0.1% triethylamine and 0.1% trifluoroacetic acid. The eluent was dried, reconstituted in mobile phase, and injected on either a Waters ACQUITY UPLC PDA detector (Waters) was used to detect midazolam and gradients for each assay are summarized in Supplemental Table 1. An ACQUITY UPLC BEH Shield RP C18 column (1.7-μm particle size, 50 × 2.1 mm; Waters, Milford, MA) was used to detect tolbutamide (230 nm), and testosterone (245 nm for testosterone and 254 nm), tolbutamide (230 nm), and testosterone (245 nm for testosterone and 254 nm) for midazolam and tolbutamide analyte separation. The columns were maintained at 40°C in a Waters ACQUITY UPLC H-Class System. The mobile phase flow was maintained at 0.8 ml/min and consisted of 10 mM KH2PO4 (pH 3.0), acetoni-trol, and methanol. Mobile phase ratios and gradients for each assay are summarized in Supplemental Table 1. An ACQUITY UPLC PDA detector (Waters) was used to detect midazolam (254 nm), tolbutamide (230 nm), and testosterone (245 nm for testosterone and 290 nm for carbamazepine) for quantification. The interassay coefficients of variation for 1′OH-midazolam, 4′OH-midazolam, 6β-OH testosterone, 16α-OH testosterone, and OH-tolbutamide were 10.7, 23.1, 3.1, 3.2, and 5.6%, respectively. The intra-assay coefficients of variation for 1′OH-midazolam, 4′OH-midazolam, 6β-OH testosterone, 16α-OH testosterone, and OH-tolbutamide were 2.7, 5.0, 3.2, 3.2, and 8.6%, respectively.

Data Analysis and Statistical Procedures. A Michaelis-Menten model was used to fit the formation of midazolam, testosterone, and tolbutamide metabolites. Maximum reaction rate of enzyme (Vmax) and Michaelis-Menten constant (Km) were calculated using GraphPad Prism (version 5.00 for Windows; GraphPad Software Inc., San Diego, CA). Correlations were performed using Spearman’s rank test. Statistical analysis between control, moderate, and severe kidney disease groups was performed using one-way analysis of variance followed by Tukey’s multiple comparison tests. Data are presented as mean ± S.E.M., and a p value less than 0.05 was considered significant.

Results

Serum Biochemistry and Body Weight. A rat 5/6 nephrectomy model has been used extensively as an animal model for severe kidney disease (Leblond et al., 2000; Michaud et al., 2006, 2010). We used variations of this surgical procedure to generate rats with moderate or severe CKD. Rats that underwent sham laparotomy (control), 2/3 nephrectomy, and 2/3 nephrectomy plus vessel ligation surgeries resulted in three distinct degrees of kidney function (control, moderate CKD, and severe CKD, respectively). This is represented by 1.65- and 4.78-fold higher serum creatinine levels in moderate and severe CKD, respectively, compared with control (p < 0.05) (Table 1). Rats in the severe kidney disease group also had significantly higher serum creatinine levels than those with moderate kidney disease (p < 0.05). Serum urea concentrations were higher in severe kidney disease compared with control and moderate CKD rats (p < 0.05). There was no difference in body weight between the groups (Table 1).

CYP3A2 and CYP2C11 mRNA and Protein Expression. An 88 and 99.6% decrease in hepatic CYP3A2 mRNA expression was demonstrated in moderate and severe kidney disease rats, respectively, compared with controls (p < 0.05; Fig. 1A). Likewise, CYP2C11 mRNA expression in moderate and severe CKD was decreased by 77 and 95%, respectively, compared with controls (Fig. 1B). When mRNA expression was correlated with serum creatinine, there was a significant exponential decrease in both CYP3A and CYP2C mRNA expression as serum creatinine levels increased [r = −0.785 and r = −0.809 (p < 0.05); Fig. 1, C and D, respectively].

Similar to mRNA expression, CYP3A2 and CYP2C11 protein expressions were significantly decreased in both moderate and severe CKD. CYP3A2 protein expression was decreased by 75 and 91% in moderate and severe kidney disease rats, respectively, compared with controls (p < 0.05; Fig. 2A). Likewise, a reduction in CYP2C11 expression was also demonstrated in moderate (41%) and severe (68%) kidney disease compared with controls (p < 0.05; Fig. 2B). Both CYP3A2 and CYP2C11 protein expression showed a significant inverse exponential relationship with serum creatinine levels [r = −0.820 and r = −0.665 (p < 0.05); Fig. 2, C and D, respectively].

Hepatic CYP3A- and CYP2C-Mediated Drug Metabolism. To determine the effect of varying degrees of kidney disease on hepatic
CYP3A function, we evaluated the metabolism of CYP3A probe drugs, midazolam, and testosterone, using rat liver microsomes. Both 1'-OH midazolam and 4'-OH midazolam are produced by CYP3A (Chovan et al., 2007), and full enzyme kinetics of these metabolites were determined in this study. $V_{max}$ values for 1'-OH midazolam production was significantly reduced by 62 and 72% in moderate and severe kidney disease groups, respectively, compared with controls ($p < 0.05$; Fig. 3A). 4'-OH-midazolam production was reduced by 63 and 75% in moderate and severe CKD, respectively, compared with controls ($p < 0.05$; Fig. 3B). The formation of 6βOH-testosterone is also specifically catalyzed by CYP3A-dependant mechanisms (Chovan et al., 2007). A 66 and 68% decrease in 6βOH-testosterone $V_{max}$ was demonstrated in moderate and severe kidney disease groups, respectively, compared with controls (Fig. 3C). Michaelis-Menten kinetic parameters for 1'-OH midazolam, 4'-OH midazolam, and 6βOH-testosterone are summarized in Table 2. A significant inverse correlation was found between $V_{max}$ values for 1'-OH midazolam, 4'-OH midazolam, and 6βOH-testosterone formation and serum creatinine levels ($r_s = -0.818$, $r_s = -0.659$, and $r_s = -0.750$ ($p < 0.05$); Fig. 3, D, E, and F, respectively).

Hepatic CYP2C enzyme activity was evaluated using the probe drugs testosterone and tolbutamide. Testosterone is specifically metabolized to 16αOH-testosterone by CYP2C11. CYP2C11 $V_{max}$ was significantly decreased by 67 and 82% in moderate and severe kidney disease rats, respectively (Fig. 4A). An inverse correlation was also demonstrated between $V_{max}$ values for 16αOH-testosterone production and serum creatinine ($r_s = -0.742$, $p < 0.05$; Fig. 4C). Hepatic CYP2C function was also characterized by evaluating tolbutamide metabolism. Formation of OH-tolbutamide was unchanged between control, moderate, and severe kidney disease (Fig. 4B), and there was no correlation between OH-tolbutamide $V_{max}$ and serum creatinine (Fig. 4D). Michaelis-Menten kinetic parameters for 16αOH-testosterone and OH-tolbutamide are presented in Table 2.

**Discussion**

A number of studies have shown altered hepatic P450 expression in severe CKD (Uchida et al., 1995; Leblond et al., 2000, 2001). Investigations of major rat drug-metabolizing isoforms included CYP1A2, CYP2C6, CYP2C11, CYP2D, CYP2E1, and CYP3A1/2. Of these isoforms, CYP2C11 and CYP3A1/2 continually appear to be down-regulated in severe CKD (Uchida et al., 1995; Leblond et al., 2001; Guévin et al., 2002; Rege et al., 2003). Our results indicate that CYP3A2 and CYP2C11 protein expressions are decreased by 75 and 40%, respectively, in moderate kidney disease. This profound decrease in protein expression is similar to the decrease seen in severe kidney disease. Clinically, this suggests that patients with earlier stages of CKD are likely to experience variable and unpredictable pharmacokinetics and drug response when taking standard doses of drugs that are substrates for CYP3A and CYP2C. These results may help explain the increased incidence of medication-related, adverse events in patients with CKD (Manley et al., 2005).

Previous studies have suggested that protein down-regulation is the result of a reduction in mRNA for hepatic CYP3A2 and CYP2C11 (Leblond et al., 2001). Our study provides further evidence of this notion because both moderate and severe kidney disease resulted in reduced CYP3A2 and CYP2C11 protein expression that is secondary to decreased mRNA. A possible cause for this decrease may be due to uremic toxin accumulation in moderate and severe CKD. Recent studies have shown that uremic toxins can cause direct inhibition or down-regulation of drug-metabolizing enzymes and drug transporters (Tsujimoto et al., 2010; Reyes and Benet, 2011). In this study, it appears that uremic toxins are interfering with the regulation of drug-metabolizing enzyme transcrip-

### Table 2

<table>
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<th></th>
<th>Control</th>
<th>Moderate CKD</th>
<th>Severe CKD</th>
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<tr>
<td><strong>OH-Midazolam</strong></td>
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<td>$V_{max}$ ($\mu$M/min/mg protein)</td>
<td>39.80 ± 7.73</td>
<td>16.06 ± 2.85</td>
<td>10.20 ± 0.34</td>
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<tr>
<td><strong>4'-OH Midazolam</strong></td>
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<tr>
<td>$V_{max}$ ($\mu$M/min/mg protein)</td>
<td>20.80 ± 2.78</td>
<td>5.48 ± 3.19</td>
<td>3.10 ± 0.11</td>
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<td><strong>16α-OH Testosterone</strong></td>
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<tr>
<td>$V_{max}$ ($\mu$M/min/mg protein)</td>
<td>15.64 ± 2.18</td>
<td>2.20 ± 0.10</td>
<td>0.72 ± 0.34</td>
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<tr>
<td><strong>6β-OH Testosterone</strong></td>
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<td>$V_{max}$ ($\mu$M/min/mg protein)</td>
<td>18.16 ± 1.76</td>
<td>5.07 ± 0.53</td>
<td>2.14 ± 0.01</td>
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Data are presented as means ± S.E.M.

$p < 0.05$ compared with control.

* $p < 0.01$ compared with control.

** $p < 0.001$ compared with control.
The uremic toxin, indoxyl sulfate, has been shown to activate the well established probe substrates, midazolam and testosterone (Sun et al., 2004). Although erythromycin is metabolized by CYP3A enzymes, it is also metabolized by many other P450 isoforms, such as CYP1A2 and CYP3A (Tanaka and Breimer, 1997). These data support other studies which demonstrate that CYP2C11 is not a substrate for uptake or efflux drug transporters (Nolin et al., 2000). A similar inverse correlation was also demonstrated for the uremic toxin 16αOH-testosterone, a specific metabolite of CYP2C11. The formation of 16αOH-testosterone was decreased in hepatic microsomes of both moderate and severe CKD. These data support other studies which demonstrate that CYP2C11 function is diminished in severe kidney disease (Uchida et al., 1995; Leblond et al., 2000). This also suggests that earlier stages of kidney disease result in a significant decrease in CYP2C11 function, similar to the outcome seen in severe kidney disease.

Few studies have evaluated the correlation between the progression of kidney disease and the extent of drug metabolism. After demonstrating that CYP3A and CYP2C11 enzyme function and expression were decreased in moderate CKD, we examined the correlation of this affect with the degree of kidney function. Our study shows a significant inverse exponential correlation between kidney function and CYP3A and CYP2C11 protein and mRNA expression in patients with varying degrees of kidney function have also used a variety of nonselective probe substrates for P450 enzymes (Ahmed et al., 1991; De et al., 2006). Although these studies clearly highlight that drug metabolism is differentially affected as kidney function declines, it is impossible to draw mechanistic conclusions about which specific P450 isozymes are affected. An alternate approach is to use midazolam, a selective phenotypic probe for CYP3A, which is not a substrate for uptake or efflux drug transporters (Nolin et al., 2009). In the present study, we evaluated the function of CYP3A using the well established probe substrates, midazolam and testosterone (Guengerich, 1999; Dostalek et al., 2011). Our results demonstrate that as kidney function declines, there is a rapid decrease in CYP3A enzymatic activity.

CYP2C function has been assessed in severe CKD using the amino-pyrine breath test (Leblond et al., 2000). Although aminopyrine is metabolized by CYP2C enzymes, it is also metabolized by many other P450 isoforms, such as CYP1A2 and CYP3A (Tanaka and Breimer, 1997). In our study, CYP2C-mediated metabolism of tolbutamide to OH-tolbutamide was unchanged in CKD. Tolbutamide is a CYP2C family drug probe but is not specific for CYP2C11 (Cribb et al., 1995; Brown et al., 2007; Dostalek et al., 2007). Therefore, other CYP2C isoforms that are unaffected by CKD may have accounted for the lack of effect observed with regard to tolbutamide metabolism. This theory is supported by studies that show no effect of severe CKD on CYP2C6 protein expression (Leblond et al., 2001). To characterize the effect of progressive decline in kidney function on CYP2C11 activity, we analyzed the formation of 16αOH-testosterone, a specific metabolite of CYP2C11. The formation of 16αOH-testosterone was decreased in hepatic microsomes of both moderate and severe CKD.

Few studies have evaluated the correlation between the progression of kidney disease and the extent of drug metabolism. After demonstrating that CYP3A and CYP2C11 enzyme function and expression were decreased in moderate CKD, we examined the correlation of this affect with the degree of kidney function. Our study shows a significant inverse exponential correlation between kidney function and CYP3A and CYP2C11 protein and mRNA expression. A similar inverse correlation was also demonstrated between kidney function and CYP3A and CYP2C11 enzyme function. We were surprised to find that these correlations are not linear because our data clearly demonstrates that a small decrease in kidney function during earlier stages of CKD produces a pronounced decrease in enzyme function and expression. The mechanism by which this occurs is unknown but may be due to the exponential increase in serum uremic mediators as CKD progresses. For example, the uremic toxin indoxyl sulfate increases exponentially as kidney disease progresses (Niwa and Ise, 1994; Barreto et al., 2009). A similar trend has also been demonstrated for the uremic toxin p-cresylsulfate (Liabeuf et al., 2010). Our results suggest that the rapid decrease in kidney function may coincide with the exponential increase in uremic toxins that occurs in the earlier stages of CKD.
Altered nonrenal drug clearance has been studied in patients with varying levels of kidney disease including ESRD (Nolin et al., 2006, 2009; Sun et al., 2010). In a recent study by Nolin et al. (2009), ESRD patients on hemodialysis were given oral midazolam. We were surprised to find that the metabolism of midazolam was unchanged in ESRD patients compared with healthy controls. The authors suggest that as ESRD patients undergo routine hemodialysis, it is possible that uremic toxins that modulate the expression and/or direct inhibition of CYP3A4 are removed and restore drug metabolism to levels observed in healthy controls (Nolin et al., 2009). This is supported by other clinical studies that show no change in nicardipine metabolism in ESRD patients on dialysis, yet show significant reductions in patients with CKD who do not require dialysis (Ahmed et al., 1991).

In conclusion, this study demonstrates that changes in drug metabolism are not restricted to severe CKD, and that milder forms of CKD significantly decrease the expression and activity of CYP3A4 and CYP2C enzymes. To our knowledge, this is the first study to systematically evaluate the effect of varying degrees of CKD on hepatic drug metabolism using variations of the commonly used remnant kidney model. In addition, this is the first study to evaluate CYP3A4 and CYP2C activity in CKD by performing full enzyme kinetics of substrate drugs. The results of our study suggest that metabolism and disposition of drugs that are substrates of CYP3A4 or CYP2C may be altered in earlier stages of kidney disease. This may have profound implications in the variable pharmacokinetics of medications in kidney disease. A recent assessment by the U.S. Food and Drug Administration highlights the importance of evaluating the impact of kidney impairment on the pharmacokinetics of drugs (Zhang et al., 2009). Our data are in agreement with this assessment and suggest that the pharmacokinetics of drugs in all stages of kidney impairment, not just ESRD, should be investigated.

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Authorship Contributions

Participation in research design: Velenosi, Fu, Luo, Wang, and Urquhart. Conducted experiments: Velenosi, Fu, and Luo.

Contributed new reagents or analytic tools: Urquhart.

Performed data analysis: Velenosi, Fu, Luo, Wang, and Urquhart.

Wrote or contributed to the writing of the manuscript: Velenosi and Urquhart.

References


Wrote or contributed to the writing of the manuscript: Velenosi and Urquhart.
**Supplemental Table 1.** UPLC gradient conditions for drug metabolite quantification. Solvents are expressed as mobile phase percentage.

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<th></th>
<th>Time (min)</th>
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<th>Methanol</th>
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Supplemental Figure 1 Specificity of anti-rat P450 antibodies tested on recombinant cDNA-expressed P450 enzymes isoforms prepared from the baculovirus-infected insect cell system (BD Supersomes™). Western blots were performed with 2 μg of each Supersome™ preparation.