Decreased nuclear receptor activity and epigenetic modulation associates with down-regulation of hepatic drug-metabolizing enzymes in chronic kidney disease

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ABSTRACT Patients with chronic kidney disease (CKD) require many medications. CYP2C and CYP3A drug-metabolizing enzymes play a critical role in determining the pharmacokinetics of the majority of prescribed medications. These enzymes are transcriptionally regulated by the nuclear receptors pregnane X receptor (PXR) and hepatic nuclear factor 4α (HNF-4α). Expression of CYP2C and CYP3A is decreased in CKD; however, the mechanisms by which this occurs is unknown. We induced CKD in rats by 5/6 nephrectomy and used chromatin immunoprecipitation (ChIP) to determine nuclear receptor and epigenetic alteration-mediated differences in the promoter region of the CYP2C and CYP3A genes. RNA polymerase II and HNF-4α binding was decreased 76 and 57% in the CYP2C11 promoter and 71 and 77% in the CYP3A2 promoter, respectively (P<0.05). ChIP also revealed a 57% decrease in PXR binding to the CYP3A2 promoter in CKD rats (P<0.05). The decrease in PXR and HNF-4α binding was accompanied by diminished histone 4 acetylation in the CYP3A2 promoter (48%) and histone 3 acetylation in the CYP2C11 (77%) and CYP3A2 (77%) promoter loci for nuclear receptor activation (P<0.05). This study suggests that decreased nuclear receptor binding and histone acetylation may contribute to the mechanism of drug-metabolizing enzyme down-regulation and altered pharmacokinetics in CKD.—Velenosi, T. J., Feere, D. A., Sohi, G., Hardy, D. B., Urquhart, B. L. Decreased nuclear receptor activity and epigenetic modulation associates with down-regulation of hepatic drug-metabolizing enzymes in chronic kidney disease. FASEB J. 28, 5388–5397 (2014). www.fasebj.org

Key Words: cytochrome P450 · chromatin immunoprecipitation · pharmacokinetics · uremia · histone modification

Patients with chronic kidney disease (CKD) take many medications to manage their CKD and comorbidities (1). Subtherapeutic dosing and overdosing result in adverse drug events commonly associated with CKD, which translate into therapeutic ineffectiveness or drug-induced toxicity. It is estimated that one medication-related problem occurs with every 2.7 medication exposures in patients undergoing dialysis (2). Several reports have suggested that altered pharmacokinetics in CKD at least partially mediates this increase in adverse drug events (3–5). Although it is well known that the renal clearance of drugs is altered in CKD, the effect on nonrenal drug clearance is not well understood.

Hepatic drug metabolism is the major route of drug elimination and is mediated predominantly by the cytochrome P450 (CYP) superfamily of oxidizing enzymes. The CYP isoforms, CYP2C and CYP3A, metabolize the majority (43%) of clinically used drugs (6). Several studies have reported down-regulation of CYP2C and CYP3A enzymes in CKD (3, 7–9). In addition, in vitro studies in which rat primary hepatocytes were incubated with uremic serum demonstrate a decrease in expression of CYP2C11 and CYP3A2, which are rat orthologs for human CYP2C9 and CYP3A4, respectively (10). Consistent with these studies, surgical induction of CKD in rats results in a pronounced decrease of hepatic CYP2C and CYP3A function and expression (3, 7, 11). Although decreased CYP2C and CYP3A function and protein expression appear to be secondary to decreased mRNA expression, the molecular mechanism(s) by which this occurs in CKD is unclear.
Hepatic CYP enzymes are transcriptionally regulated by nuclear receptors. These xenosensing and hormonal regulators of gene expression include the pregnane X receptor (PXR) and hepatocyte nuclear factor (HNF)-4α (12, 13). PXR contains a promiscuous ligand-binding domain that can be activated by chemically diverse compounds to regulate CYP3A expression. When activated, PXR translocates into the nucleus and heterodimerizes with the retinoid X receptor (RXR). This heterodimer binds to regulatory regions on DNA to modulate transcription. HNF-4α is an essential transcriptional regulator of CYP3A and CYP2C expression (12, 14). In contrast to PXR, HNF-4α binds to the promoter of these enzymes as a homodimer to activate transcription (12, 14). In addition to altered CYP expression, numerous clinical and animal studies suggest that adverse drug events occur as a result of altered pharmacokinetics in patients with CKD (3–5, 7, 8, 15). Although the mechanisms remain controversial, several reports indicate that altered drug pharmacokinetics in CKD is mediated by accumulation of uremic waste products (16–19). These uremic waste products become toxins at high levels and include amino acid metabolites, hormones, cytokines, and other metabolic waste products. Many of these toxins are cleared in dialysis; however, some circulate bound to plasma proteins and are poorly cleared (20).

Altered transcriptional regulation of several genes has also been associated with epigenetic modifications in the setting of CKD (21, 22). Previous studies have suggested that the uremic environment of CKD produces epimutations including DNA methylation and histone posttranslational modifications, which result in altered gene expression (23). In the context of regulating CYP enzymes, transcriptional activation occurs in tandem with the binding of PXR and HNF-4α as well as posttranslational histone modifications, leading to a permissive chromatin environment (24–26). Therefore, it is possible that alterations in histone modifications play an important role in the mechanism of CKD-induced changes in hepatic CYP2C and CYP3A expression.

The objective of this study was to identify possible mechanisms by which hepatic drug-metabolizing enzyme function and expression are altered in CKD. We hypothesize that altered drug metabolism in CKD is secondary to decreased nuclear receptor binding to the promoters of CYP2C and CYP3A as a result of histone modulation. In this study, we investigate the nuclear receptor and epigenetic regulation of hepatic drug-metabolizing enzymes in a well-characterized rat model of CKD.

**MATERIALS AND METHODS**

**Experimental rat model**

Male Sprague-Dawley rats, weighing 150 g, were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA). Twelve rats were randomly separated into 2 groups, which simulated normal kidney function and CKD. CKD was surgically induced by a 2-stage 5/6 subtotal nephrectomy (5). Control rats were pair-fed the same amount of standard rat chow that was ingested by CKD rats on the previous day. Six weeks after the initial surgery, rats were euthanized by isoflurane anesthesia followed by decapitation. Liver tissue was harvested, snap-frozen in liquid nitrogen, and stored at −80°C before analysis. Serum creatinine and urea were determined by the London Laboratory Services Group (London, ON, Canada) using standard methods. The animal protocols were approved by the University of Western Ontario Animal Care Committee.

**Testosterone metabolism and analyte quantification**

Lever microsomal fractions were isolated by differential centrifugation as published previously (11). Testosterone was selected as a probe substrate to determine hepatic CYP2C11 and CYP3A enzyme function as described previously (11).

**RNA extraction and real-time PCR analysis**

RNA was extracted from rat livers using TRIzol (Life Technologies, Waltham, MA, USA) according to the manufacturer’s protocol. cDNA was synthesized from 1 μg of total RNA according to the manufacturer’s protocol with qScript cDNA Supermix (Quanta BioSciences, Gaithersburg, MD, USA), and relative mRNA expression was quantified by real-time PCR with PerfeCta SYBR Green Fastmix (Quanta BioSciences). Primer sets for CYP1A1, CYP1A2, CYP2C11, CYP3A2, PXR, HNF-4α, constitutive androstane receptor (CAR), and RXR were generated using U.S. National Center for Biotechnology Information (NCBI) Primer-BLAST (NCBI, Bethesda, MD, USA; Table 1). Gene expression was normalized to β-actin using the ΔΔCt method.

**Western blot analysis**

Protein expression of CYP2C11 and CYP3A2 was assessed in hepatic microsomal fractions using Western blot analysis as described previously (11). HNF-4α and PXR Western blots were performed in hepatic lysates. Immunoblots were performed according to the antibody manufacturer’s recommendation for CYP2C11 (Detroit R&D Inc., Detroit, MI, USA), CYP3A2 (Millipore, Billerica, MA, USA), HNF-4α (GeneTex, Irvine, CA, USA), and PXR (Abcam, Cambridge, MA, USA). Secondary horseradish peroxidase (HRP)-linked antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Immune complexes were revealed by HRP (Millipore), and band intensity was determined by densitometry (Quantity One 1-D Analysis Software on a VersaDoc Imaging System; Bio-Rad, Hercules, CA, USA).

**Chromatin immunoprecipitation (ChiP)**

ChiP was performed on rat liver tissue using a previously published method with slight modification (27). In brief, DNA was cross-linked to protein with 1% formaldehyde and subsequently sheared by sonication. Chromatin samples were precleared with protein A/G agarose and then were incubated with antibodies to RNA polymerase II (RNA Pol II; 1 μg/μl aliquot, 17–620; Millipore), PXR (5 μg/μl aliquot, sc-25381; Santa Cruz Biotechnology), HNF-4α (5 μg/μl aliquot, sc-8087; Santa Cruz Biotechnology), acetyl histone H3 (5 μg/μl aliquot, K9 and K14, 06–559; Millipore), acetyl histone H4 (5 μg/ μl aliquot, K5, K8, K12, and K16, 06–866; Millipore), histone 3 lysine 27 trimethylation (H3K27me3; 5 μg/μl aliquot, 07–449; Millipore), histone 3 lysine 9 trimethylation (H3K9me3; Millipore), histone 4 lysine 3 asymmetric dimethylation (H4R3me2; 5 μg/μl aliquot, 39705; ActivMotif, Carlsbad, CA, USA), histone 3 lysine 4 dimethylation (H3K4me2; 5 μg/μl aliquot, 07–030; Millipore) overnight at 4°C. Separate aliquots were treated with the same amount of nonimmune IgG/μl aliquot, 17–620, Millipore) and incubated with nonimmune IgG (sc-2020; Santa Cruz Biotechnology) instead of specific antibody.
IgG from the same host species to determine nonspecific binding (Santa Cruz Biotechnology or Millipore). The binding of RNA Pol II, PXR (−217 to −208), and HNF-4α (−190 to −180) to the rat CYP2A2 promoter (28) was quantified by real-time PCR using primers to amplify −292 to −172 (Table 1). This primer set was also used to determine histone modifications in the CYP2A2 promoter. A site upstream of the binding sites known not to bind these transcription factors was also amplified as a control. GeneInspector (Textco Biotechnology Software, Inc., West Lebanon, NH, USA) was used to identify putative rat HNF-4α binding sites in the CYP2C11 promoter (28) was quantified by real-time PCR using primers to amplify −793 to −692 bp (Table 1). To determine histone H4 acetylation and H4R3me2 in the CYP2C11 promoter, −793 to −692 bp was also amplified. PCR primers were also used to amplify −145 to −22 bp to assess CYP2C11 promoter binding of RNA Pol II as well as H3K4me2, H3K9me3, and H3K27me3 epigenetic markers (Table 1).

**Statistical analysis**

Statistical differences between control and CKD rats were assessed using the unpaired Student’s t test. Results are expressed as means ± sem, and a value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Serum biochemistry and body weight**

Serum levels of creatinine and urea in rats with surgically induced CKD were 2.74- and 1.97-fold higher, respectively, than those in controls ($P<0.05$; Table 2). There was no difference in body weight between CKD and control rats.

**Hepatic CYP3A- and CYP2C-mediated drug metabolism**

To confirm decreased CYP2C- and CYP3A-mediated metabolism in CKD, we evaluated metabolism of testosterone, using rat liver microsomes. A 68% decrease in the CYP2C11-mediated production of 16α-OH-testosterone was demonstrated in rats with CKD compared with that in control rats ($P<0.05$; Fig. 1A). CYP3A activity assessed by measuring 6β-OH-testosterone formation was also significantly decreased by 78% in CKD rats compared with that in controls ($P<0.05$; Fig. 1B).

**Protein and mRNA expression of drug-metabolizing enzymes**

Microsomal protein expression of CYP2C11 and CYP3A2 was decreased by 65.6 and 88.8%, respectively, in CKD rats compared with that in controls ($P<0.05$; Table 2).

**TABLE 1. Real-time PCR primers**

<table>
<thead>
<tr>
<th>Gene/promoter</th>
<th>Direction</th>
<th>Primer, 5′–3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-metabolizing enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat CYP1A1</td>
<td>Forward</td>
<td>ATGTCAGCTCTGACATGGTACTCT</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ATGCCGCTCACTGGCTCTGTAAC</td>
</tr>
<tr>
<td>Rat CYP1A2</td>
<td>Forward</td>
<td>AACCAGCGCCCTGACAGGATTAG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GCCGCTGAGGTTTGGAGGGGCA</td>
</tr>
<tr>
<td>Rat CYP2C11</td>
<td>Forward</td>
<td>CCGTTGAGCTGGITTGGAGG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CAGGTCAGCTCTGACATGGTACTCT</td>
</tr>
<tr>
<td>Rat CYP3A2</td>
<td>Forward</td>
<td>GGCAGCTGAGGTTTGGAGGGGCA</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GCCGCTGAGGTTTGGAGGGGCA</td>
</tr>
<tr>
<td>Nuclear receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat PXR</td>
<td>Forward</td>
<td>TGGACAGAGGCTCTGCTCTGTA</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GGCAGCTGAGGTTTGGAGGGGCA</td>
</tr>
<tr>
<td>Rat HNF-4α</td>
<td>Forward</td>
<td>GGGAGCTGAGGTTTGGAGGGGCA</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GCCGCTGAGGTTTGGAGGGGCA</td>
</tr>
<tr>
<td>Rat CAR</td>
<td>Forward</td>
<td>CTTCTGCTGGGCAATTCAAG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGGGACAGTGGTGTGGAGGTA</td>
</tr>
<tr>
<td>Rat RXR</td>
<td>Forward</td>
<td>AACCCCCCCTAGGCTCTCAT</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TATGTTTTGGCCGAGGAGG</td>
</tr>
<tr>
<td>Nuclear receptor and RNA Pol II binding sites</td>
<td>Forward</td>
<td>GGGTCACAGGCTCTGCTGTAAC</td>
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<tr>
<td>−292 to −172 rat CYP3A2 promoter</td>
<td>Reverse</td>
<td>GCCATGTCAGCTCTGCTGTAAC</td>
</tr>
<tr>
<td>−793 to −692 rat CYP2C11 promoter</td>
<td>Forward</td>
<td>CCGTCTGCTGATTTGGACTG</td>
</tr>
<tr>
<td>−145 to −22 rat CYP2C11 promoter</td>
<td>Reverse</td>
<td>CCCAGAGGCTGAGGTTTGGAG</td>
</tr>
<tr>
<td>Rat RNA Pol II GAPDH binding site</td>
<td>Forward</td>
<td>CTTAGTTGGCCGAGGAGG</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTAGTTGGCCGAGGAGG</td>
</tr>
</tbody>
</table>

**TABLE 2. Characteristics of control and CKD rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CKD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>478 ± 20</td>
<td>451 ± 12</td>
</tr>
<tr>
<td>Serum creatinine (μM)</td>
<td>23 ± 1</td>
<td>63 ± 12*</td>
</tr>
<tr>
<td>Serum urea (μM)</td>
<td>6.1 ± 0.5</td>
<td>12.0 ± 0.3*</td>
</tr>
</tbody>
</table>

Data are means ± sem; $n = 6$. *$P < 0.05$ vs. control.
Real-time PCR indicated 70.5 and 97.8% decreases in hepatic CYP2C11 and CYP3A2 mRNA expression in CKD rats, respectively, compared with that in controls (P<0.05; Fig. 1D). RNA Pol II recruitment to the CYP3A2 and CYP2C11 promoters

A potential transcriptional mechanism of CYP2C11 and CYP3A2 down-regulation was investigated by measuring the binding of RNA Pol II to the promoters of CYP2C11 and CYP3A2 using ChIP. Rats with CKD had 76 and 71% decreases in recruitment of RNA Pol II to the CYP2C11 and CYP3A2 promoters, respectively (P<0.05; Fig. 2). There was no difference between RNA Pol II recruitment in the uremic group compared to the IgG control ChIP. Nonimmune IgG ChIP resulted in negligible binding to the CYP2C11 and CYP3A2 promoters.

Hepatic nuclear receptor expression and recruitment to the CYP3A2 and CYP2C11 promoters in CKD

To determine the effect of nuclear receptors on transcriptional regulation of CYP2C11 and CYP3A2 in CKD, we assessed the steady-state levels of nuclear receptor mRNA and protein along with promoter recruitment. CKD did not significantly alter the hepatic mRNA expression of PXR, HNF-4α, CAR, or RXRα (Fig. 3A). Protein expression of PXR and HNF-4α was also unchanged (Fig. 3B, C). Although a previously described binding site for HNF-4α in the CYP3A2 promoter had been established (28), we used GeneInspector to identify a novel putative binding site at −757 of the CYP2C11 promoter. ChIP was used to assess the binding of HNF-4α to its binding sites in the CYP2C11 and CYP3A2 promoters. Binding of HNF-4α to its binding sites in the CYP2C11 and CYP3A2 promoters was significantly decreased by 57 and 77%, respectively, in CKD rats compared with that in controls (P<0.05; Fig. 4A, B). Recruitment of PXR to the CYP3A2 promoter was also significantly decreased by >57% in CKD rats than in controls (P<0.05; Fig. 4C).

Decreased histone acetylation in the CYP2C11 and CYP3A2 promoters of rats with CKD

Epigenetic modulation can cause gene silencing through decreased histone acetylation (29). To assess the possibility that altered histone acetylation in the promoters of CYP2C11 and CYP3A2 occurs in tandem...
with decreased nuclear receptor binding in CKD, ChIP was used with antibodies against acetyl histone H3 and acetyl histone H4 in control and CKD liver tissue. Notably, histone H3 acetylation was decreased in both the CYP2C11 (−145 to −22) and CYP3A2 (−292 to −172) promoter regions by 77% (P<0.05; Fig. 5A, B). Histone H4 acetylation was also decreased in the CYP3A2 promoter (P<0.05; Fig. 5D); however, there was no change in H4 acetylation of the putative HNF-4α binding site in the CYP2C11 promoter (−793 to −751; Fig. 5C). Given that alterations in histone methylation can also influence gene expression (30), we next assessed whether activating and/or inhibiting histone methylation modifications occurred in hepatic CYP2C11 and CYP3A2 promoters. H3K4me2 and H4R3me2 activating modifications were examined by ChIP in the CYP2C11 and CYP3A2 promoters of control and CKD rat livers. ChIP revealed no significant change in H3K4me2 and H4R3me2 in promoters of these enzymes for rats with CKD (Fig. 6A, C). The histone methylation silencing modifications assessed included H3K9me3 and H3K27me3. No significant differences in silencing methylation modifications were found in the promoters of hepatic CYP2C11 and CYP3A2 for rats with CKD compared with those in controls (Fig. 6B, D).

**DISCUSSION**

In this study, we explored the possible underlying transcriptional factor (e.g., nuclear receptor activity) and epigenetic (e.g., posttranslational histone modifications) alterations that accompany down-regulation of the important drug-metabolizing enzymes CYP2C and CYP3A in CKD. Hepatic CYP2C11 and CYP3A2 function and expression decreased in CKD similar to levels observed in previous studies. Decreased protein expression and function of these drug-metabolizing enzymes have been consistently supported as secondary to the decrease in mRNA expression (3, 11, 31).

The activity of PXR and HNF-4α nuclear receptors commonly occurs through posttranslational modifications and protein interactions; however, some studies have indicated that changes in the expression of these nuclear receptors can alter CYP3A mRNA expression (32, 33). For example, rats exposed to depleted uranium exhibited increased hepatic PXR expression, which occurred concomitantly with increased CYP3A2 expression (32). Moreover, inflammatory animal models, such as intraperitoneal lipopolysaccharide injection in mice, have demonstrated decreased PXR and RXRα expression, which leads to impaired CYP3A11 expression (34). CKD can be considered an inflammation-associated pathological state, and although there was a slight trend toward decreased nuclear receptor expression, there was no significant change in the expression of nuclear receptors that regulate CYP2C11 and CYP3A2 in CKD compared with that in controls.

Although the expression of these nuclear receptors was not altered in our study, we demonstrated that the recruitment of hepatic PXR and HNF-4α to the CYP3A promoter was substantially decreased in CKD. To our knowledge, this is the first study in an in vivo disease model to implicate alterations in PXR and HNF-4α binding along with changes in the chromatin environment associated with recruitment of these nuclear receptors. Previous in vitro studies suggested that decreased PXR binding may be a result of protein-protein interactions, epigenetic changes, reduced recruitment of coactivators, or a combination of these mechanisms (35). Protein-protein interactions with nuclear factor κB (NF-κB) and PXR have been demonstrated through direct binding with the RXRα DNA-binding domain to inhibit transcriptional activity (36). Moreover, NF-κB has been shown to play a role in mediating uremic toxin-induced down-regulation of CYP2C and CYP3A.

![Figure 2](image_url)
expression. In a previous study, NF-κB inhibitors mitigated the down-regulation of these enzymes in rat hepatocytes treated with predialysis serum. However, postdialysis serum had no effect on drug-metabolizing enzyme expression, suggesting that the toxins involved may be cleared in dialysis (10).

Basal expression of rat CYP2C11 is regulated by growth hormone in a complex interaction between signal transducer and activator of transcription-5b

Figure 3. A) mRNA expression of nuclear receptors regulating drug-metabolizing enzymes in control and CKD rats. B, C) HNF-4α (B) and PXR (C) protein expression in control and CKD rats. Protein is standardized to β-actin, and controls were arbitrarily defined as 100%. Results are means ± SEM; n = 6. Representative Western blots are shown.

Figure 4. A, B) HNF-4α nuclear receptor binding to the promoter regions of CYP2C11 (A) and CYP3A2 (B) drug-metabolizing enzymes in control and CKD rats. C) PXR binding to the initiation site of hepatic CYP3A2 in control and CKD rats. ChIP was performed on rat liver tissue using a PXR- or HNF-4α-specific antibody. The relative level of immunoprecipitated genomic DNA was normalized to total genomic DNA. Relative binding of PXR and HNF-4α is expressed as a percentage of control binding. Results are means ± SEM; n = 6. *P < 0.05 vs. control.
(STAT5b), HNF-1α, HNF-3β, HNF-4α, and HNF-6 (37, 38). In humans, the major regulators of CYP2C9 are PXR, CAR, and HNF-4α (6). Therefore, although this study demonstrates a decrease in binding of a common nuclear receptor regulating both CYP2C11 and CYP2C9, these enzymes display distinct species differences in regulation.

The focus of our study was also to examine the associated epigenetic changes in the promoter region of these enzymes as a result of CKD. In vitro studies have shown that activation of both PXR and HNF-4α occurs through the activity of protein arginine N-methyltransferase 1 (PRMT1), which asymmetrically dimethylates histone H4R3, causing subsequent H4 acetylation and transcriptional activation (24, 26). This pathway is thought to be unidirectional because acetylation of histone H4 has been shown to inhibit H4R3me2 (35, 39). Our study demonstrated that histone H4R3me2 was not significantly altered in the promoters of hepatic CYP2C11 and CYP2C9, these enzymes display distinct species differences in regulation.

Figure 5. A, B) Histone 3 acetylation at the initiation sites of hepatic CYP2C11 (A) and CYP3A2 (B) in control and CKD rats. C, D) Histone 4 acetylation at the nuclear receptor binding sites of CYP2C11 (C) and CYP3A2 (D) in control vs. CKD rats. ChIP was performed on rat liver tissue using an acetyl-H3 (AcH3)- or acetyl-H4 (AcH4)-specific antibody. The relative level of immunoprecipitated genomic DNA was normalized to total genomic DNA. Relative levels of AcH3 and AcH4 are expressed as percentages of control. Results are means ± sem; n = 6. *P < 0.05 vs. control.

“transcriptionally silent” with no chromatin markers; “ready state,” which occurs after asymmetric dimethylation of H4R3 by PRMT1; and “active state,” which occurs during H4 acetylation and the removal of H4R3me2 markers. Therefore, it is possible that the percentage of CYP3A2 promoter in H4R3me2 ready state (Fig. 6C) is unaffected by CKD, whereas the percentage of histone H4 acetylation is significantly decreased (Fig. 5D), resulting in a higher percentage in the transcriptionally silent state. The mechanism by which H4 acetylation is decreased may be a result of NF-κB activation and histone deacetylases (HDACs) causing deacetylation to the transcriptionally silent state (35, 39). A previous study demonstrated reduced HDAC1 binding to the CYP3A4 promoter in the presence of the CYP3A4 inducer, carbamazepine (40). However, HDAC1 was not enriched in the rat CYP3A2 promoter for control or CKD rats (data not shown).

Decreased recruitment of RNA Pol II is associated with increased H3K9 methylation silencing modifications and decreased H3K9 acetylation activating modifications (27). A maternal protein restriction model of intrauterine growth restriction has shown long-term gene silencing of hepatic CYP7A1. CYP7A1 gene silencing occurred as a result of decreased RNA Pol II binding in rats (27). This study also showed decreased H3 acetylation and increased H3K9me3 in the CYP7A1 promoter. Other studies have associated these histone
modifications with decreased RNA Pol II recruitment (30, 41). In our study, H3K9me3 was unaltered in rat hepatic CYP2C11 and CYP3A2 promoters; however, H3 acetylation was decreased further, suggesting the possibility of increased HDAC activity in the promoter of these enzymes. Studies examining the epigenetic changes associated with phenobarbital-induced CAR induction of CYP2B1 have also noted increases in H3 acetylation as well as methylation modifications including increased H3K4me2 and decreased H3K27me3 (42). H3K4me2 is associated with activation of transcription, whereas H3K27me3 is a marker of silencing of heterochromatin (30, 41). In our study, no significant changes in H3K4me2 and H3K27me3 were found in the promoters of CYP2C11 or CYP3A2 in CKD. Another potential silencing mechanism that could explain our results is silencing via direct DNA methylation. Although direct DNA methylation silencing modifications have been associated with PXR binding sites in mice (25), our in silico screen did not identify any CpG islands in the rat CYP2C11 or CYP3A2 promoters as predicted by CpG island searcher software (43).

Emerging evidence strongly suggests that alterations in nonrenal clearance pathways in CKD affect the pharmacokinetics of drugs (44, 45). Our study describes a possible mechanism by which these enzymes are down-regulated. We, therefore, propose a mechanistic hypothesis to explain how uremia induces altered nuclear receptor binding and epigenetic modifications to down-regulate drug disposition genes (Fig. 7). We show for the first time that hepatic CYP2C11 and CYP3A2 down-regulation in CKD is associated with both decreased nuclear receptor binding and decreased acetylation in the promoter regions of these enzymes. Future studies should address the temporal and severity relationships between changes in nuclear receptor binding and histone acetylation in CKD. Given the complex nature of uremia and the several putative uremic toxins

Figure 6. A, B, D) H3K4me2 (A), H3K9me3 (B), and H3K27me3 (D) at the initiation site of hepatic CYP2C11 and CYP3A2 in control and CKD rats. C) H4R3me2 at the nuclear receptor binding sites of CYP2C11 and CYP3A2 in control and CKD rats. ChIP was performed on rat liver tissue using an H3K4me2-, H3K9me3-, H3K27me3-, or H4R3me2-specific antibody. The relative level of immunoprecipitated genomic DNA was normalized to total genomic DNA. Relative levels of H3K4me2, H3K9me3, H3K27me3, and H4R3me2 are expressed as percentages of control. Results are means ± SEM; n = 6. *P < 0.05 vs. control.
that likely contribute to this effect, future studies are also needed to elucidate the role of specific uremic toxins in regulation of drug metabolism.

In summary, CYP2C and CYP3A enzymes are involved in the oxidative metabolism of almost half of all prescribed medications (6). They also play essential roles in cholesterol, steroid, and lipid homeostasis (46). Our study provides evidence for an association between decreased transcription factor binding and histone acetylation and down-regulation of these important genes in the setting of CKD.

This work was supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grants Program (grant 386569-2010), a Canada Graduate Scholarship Doctoral award, and the University of Western Ontario Academic Development Fund. Images used to generate the figure were from Servier Medical Art (http://www.servier.co.uk/medical-art-gallery). The authors declare no conflicts of interest.

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Received for publication July 9, 2014. Accepted for publication August 26, 2014.
Decreased nuclear receptor activity and epigenetic modulation associates with down-regulation of hepatic drug-metabolizing enzymes in chronic kidney disease

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FASEB J 2014 28: 5388-5397 originally published online September 10, 2014
Access the most recent version at doi:10.1096/fj.14-258780

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