Effect of Hemodialysis on Hepatic Cytochrome P450 Functional Expression

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Abstract. Cytochrome P450 (CYP) functional expression is reduced in uremia and normalized after restoration of kidney function via transplantation. The aim of this study was to evaluate the effect of conventional hemodialysis on the functional expression of CYP1A, 2C, and 3A. We also investigated the role of nuclear factor-κB (NF-κB) in CYP regulation during uremia. Primary cultures of normal rat hepatocytes were incubated with serum obtained from end-stage renal disease patients pre- and post-hemodialysis and healthy control subjects, in the presence and absence of the NF-κB inhibitor andrographolide. Uremic pre-hemodialysis serum caused significant reductions (P<0.01) in CYP1A (44%), 2C (27%), and 3A (35%) protein expression compared to control serum, while dialyzed serum (i.e., obtained immediately post-hemodialysis) had no effect. CYP1A2, 2C11, and 3A2 mRNA expression, as well as CYP3A activity, were similarly impacted by uremic serum and were improved to >80% of control values after hemodialysis. NF-κB inhibition nearly eliminated the effect of uremic serum on CYP functional expression. This is the first study to demonstrate that conventional hemodialysis acutely improves altered CYP functional expression observed in rat hepatocytes incubated with uremic human serum.

Keywords: hemodialysis, end-stage renal disease, cytochrome P450, gene expression, drug metabolism

Introduction

Chronic kidney disease (CKD), including end-stage renal disease (ESRD), alters the renal clearance (i.e., glomerular filtration) and consequently the pharmacokinetic disposition of drugs, and recent compelling data indicate that alterations in non-renal drug clearance (i.e., metabolism and transport) also occur (1 – 3). Non-renal clearance of drugs consists largely of hepatic metabolism mediated by cytochrome P450 (CYP) enzymes, considered the most significant contributors to drug metabolism in vivo, since they are responsible for the biotransformation of up to 60% – 80% of drugs currently marketed that undergo oxidative metabolism (4).

Several studies have demonstrated modifications in the functional expression of CYP enzymes in experimental models of uremia (1, 5 – 8), and various uremic byproducts (e.g., “uremic toxins”) have been implicated, including urea, parathyroid hormone, and indoxyl sulfate. However, CYP regulation has not been well studied in chronically uremic ESRD patients undergoing hemodialysis (HD). Decreased activities of CYP2C9 (9) and CYP3A4 (10) have been reported in uremic patients, which could have significant clinical implications since numerous CYP2C9 and 3A4 substrates are frequently prescribed to these patients.

Restoration of kidney function after transplantation
leads to a sustained improvement in the uremic state and in hepatic drug metabolism (1, 11). On the other hand, HD therapy only temporarily improves uremia and does not appear to generate long-term improvements in CYP expression (i.e., pre-HD) (1); moreover, HD results in short-term improvements in the erythromycin breath test (i.e., pre- versus post-HD), which has been interpreted as reflecting hepatic CYP3A4 activity (12). The latter finding implicates one or more rapidly acting, dialyzable uremic toxins, and the acuteness of the response suggests that hemodialysis-induced improvements in erythromycin breath test (EBT) results occur independently of transcriptional or translational modification, but the acute effect of HD on CYP expression has not been assessed to date. Therefore, the purpose of this study was to evaluate the effect of conventional hemodialysis on the functional expression of CYPs by incubating primary cultures of normal rat hepatocytes with serum obtained from ESRD patients (pre- and post-HD) and healthy control subjects. Furthermore, we also evaluated the role of nuclear factor-κB in CYPs downregulation induced by uremic serum since this transcriptional factor was shown to have a key role in regulation of CYP expression in uremia and inflammation (13, 14).

Materials and Methods

Study subjects
This study was approved by the University of Montreal and Maine Medical Center Institutional Review Boards. The sera from twelve ESRD patients undergoing chronic maintenance HD were obtained after providing written informed consent, and their characteristics are presented in Table 1. Control sera were obtained from healthy volunteers. Eligibility criteria included age ≥18 years, normal hepatic function, and non-smoking. All ESRD patients were receiving HD therapy for at least one month in duration, and they underwent HD for 4 h with a high-flux polysulfone membrane and blood flow rates between 350 – 450 mL/min. Subjects taking drugs known to inhibit or induce CYPs or with evidence of an active infection were excluded. Blood samples were obtained immediately pre- and post-HD in ESRD patients, kept on ice, and centrifuged 10 min after collection at 600 × g for 10 min. Serum was harvested and stored at −80°C until experimentation.

Animals
Male Sprague-Dawley rats (Charles River, Saint-Charles, Quebec, Canada) weighing 200 – 300 g were housed in the Research Centre animal care facility and maintained on a Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, Quebec, Canada) and water ad libitum. An acclimatization period of 3 days was allowed before any experimental work was undertaken. All of the experiments were conducted according to the Canadian Council on Animal Care guidelines for care and use of laboratory animals and under the supervision of our local animal care committee.

Hepatocyte isolation and culture
Hepatocytes were isolated from normal rats according to the two-step liver perfusion method of Seglen as previously published (7). Collagenase type 4 (Worthington, Lakewood, NJ, USA) was used. After preincubating the hepatocytes for 2 h in William’s E medium containing 10% calf serum, the medium was changed for 2 mL of William’s E medium containing 10% serum obtained pre-HD, post-HD, or from control subjects. Serum from each subject was treated separately in every experiment and was counted as one replicate. Each serum was tested on at least three different hepatocytes preparations. The number of cells used for each assay is 1.5 × 10⁶ for protein assay, 3.0 × 10⁶ for mRNA, and 4.5 × 10⁶ for activity. The hepatocytes were incubated for another 24 h and then harvested by scraping in phosphate-buffered saline. For mRNA analysis, cells were harvested in RLT buffer (Qiagen, Mississauga, Ontario, Canada). Samples were stored at −80°C until analysis.

Inhibition of nuclear factor-κB (NF-κB)
To assess whether the blockade of the NF-κB signaling pathway could modify the effect of uremic serum, normal hepatocytes were incubated for 24 h in the presence of 30 μM andrographolide (Calbiochem, San Diego, CA, USA), a specific NF-κB inhibitor, with uremic or control serum.

Table 1. Demographic characteristics of ESRD patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Etiology of ESRD</th>
<th>Gender</th>
<th>Age (years)</th>
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<tbody>
<tr>
<td>1</td>
<td>HTN</td>
<td>M</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>DM</td>
<td>M</td>
<td>74</td>
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<td>3</td>
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<td>F</td>
<td>69</td>
</tr>
<tr>
<td>4</td>
<td>DM</td>
<td>M</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>PCKD</td>
<td>F</td>
<td>54</td>
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<tr>
<td>6</td>
<td>DM</td>
<td>F</td>
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<tr>
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<td>M</td>
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<tr>
<td>12</td>
<td>HTN</td>
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ESRD, end-stage renal disease; HTN, hypertension; DM, diabetes mellitus; I, ischemic; PCKD, polycystic kidney disease.
Western blot analysis
Although several CYP isoforms are implicated in the metabolism of drugs, only CYP1A2, 2C11, and 3A2, the more abundant isoforms in the rat liver and most affected by uremia (6), were assessed via Western blotting as described previously (6). Briefly, CYP was detected using polyclonal goat anti-rat 1A1, goat anti-rat 2C11, and rabbit anti-rat 3A2 (Gentest Corporation, Woburn, MA, USA). β-Actin was detected using a mouse anti-chicken β-Actin (Neo-Markers, Fremont, CA, USA). Immune complexes were revealed by secondary antibodies (swine anti-goat IgG or swine anti-rabbit IgG coupled to peroxidase from Biosource International or Goat anti-mouse IgG coupled to peroxidase from Sigma) and the Luminol derivative of Lumilight Western blotting substrate (Roche Diagnostics, Laval, QC, Canada). Immune reaction intensity was determined by computer-assisted densitometry on a LAS-3000 LCD camera (Fuji, Stamford, CT, USA) coupled to MultiGauge software (Fuji).

RNA isolation and real-time quantitative polymerase chain reaction (PCR) analysis
RNA was extracted from hepatocytes with the RNeasy Mini Kit from Qiagen. One microgram of total RNA was used to prepare cDNA by reverse transcription using the Omniscript RT kit from Qiagen and random primer from Invitrogen (Burlington, Ontario, Canada). Quantitative PCR analysis was performed using Platinum SYBR green qPCR (Invitrogen) on an iCycler real-time detection system (Bio-Rad Laboratories, Mississauga, Ontario, Canada). Specific primer sets were designed for each of the four tested mRNA (1A2, 2C11, 3A2, and GAPDH) on the basis of published cDNA sequences with the aid of the Jellyfish computer program (LabVelocity Inc., Los Angeles, CA, USA). All primers were obtained from Sigma and their specificity was confirmed by sequencing the resulting PCR product on an ABI Prism 3100 analyzer (Applied Biosystems, Foster City, CA, USA). Used PCR conditions were optimized to 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The differences in specific mRNA expression were calculated using the delta Ct method.

Evaluation of CYP3A activity
In order to evaluate the metabolic activity of CYP3A in hepatocytes incubated with serum from ESRD patients or controls, a selective fluorescent probe, 3-[3,4-difluorobenzyl)oxy]-5,5-dimethyl-4-[4-methylsulfonyl]phenyl)furan-2(5H)-one (DFH), that is specifically metabolized by rat CYP3A was used as previously reported (15). The substrate was added directly to the culture after removal of the culture media and replacement by Krebs buffer containing 12.5 mM HEPES for 30 min. The fluorescence of the metabolite 3-hydroxy-5,5-dimethyl-4-[4-(methylsulfonyl)phenyl)furan-2(5H)-one (DFH) was read on a cytofluorometer (Cytofluor 4000/TR; Perspective Biosystems, Framingham, MA, USA) using appropriate wavelengths (excitation filter: 360/40 nm and emission filter: 460/40 nm). The standard curve was prepared with known dilutions of DFH. DFB and DFH were provided by Merck Frost (Kirkland, Quebec, Canada).

Blood chemistries
Blood chemistries (urea, creatinine) were determined with a Hitachi 717 autoanalyser (Roche Diagnostics).

Statistical analyses
The results are each expressed as the mean ± S.E.M. Differences between groups were assessed by using an unpaired two-sided Student’s t-test or an ANOVA test. Significant ANOVA was followed by a post-hoc Scheffe analysis. A P value of <0.05 was considered statistically significant for all comparisons.

Results
CYP expression
As shown in Fig. 1, there was a significant decrease in CYP1A2 (44%), 2C (27%), and 3A (35%) protein expression in hepatocytes incubated with uremic pre-HD serum compared to control serum, while serum obtained immediately post-HD had no effect on CYP protein expression. CYP mRNA expression was similarly impacted by uremic serum (Fig. 2). Specifically, mRNA expression of CYP1A2, 2C11, and 3A2 was reduced by 50%, 34%, and 40%, respectively, in hepatocytes incubated with pre-HD serum and was improved but not completely normalized in hepatocytes incubated with post-HD serum.

CYP3A activity
CYP3A activity was decreased by 40% in hepatocytes incubated with uremic pre-HD serum compared to control serum (Fig. 3), and similar to mRNA expression, it was improved but not completely normalized by hemodialysis.

Effect of NF-κB inhibition on CYP3A and CYP2C
As depicted in Fig. 4, the decrease in CYP3A protein (A) and gene expression (B) by pre-HD serum was prevented by the addition of andrographolide to hepatocyte culture media. Similar results were obtained with CYP2C protein (C) and gene expression (D).
This study demonstrates that incubation of primary cultures of normal rat hepatocytes with serum from ESRD patients results in altered functional expression of CYP1A, 2C, and 3A, which is obviated by conventional hemodialysis. CYP protein expression was completely normalized, and mRNA expression was significantly improved to more than 80% of control values after incubation with post-HD serum. We have previously reported similar modifications in the functional expression of CYP enzymes in experimental models of uremia (5–7), as well as in ESRD patients, by incubating rat hepatocytes with serum obtained immediately pre-HD (1). However, this is the first study to demonstrate the near normalization of CYP functional expression in hepatocytes when serum is obtained after a hemodialysis session. Although restoration of kidney function after transplantation leads to a sustained improvement in the uremic state and in hepatic drug metabolism (11), HD therapy only temporarily improves uremia and does not appear to generate long-term improvements in CYP expression, that is, the inhibitory effect of pre-HD serum persists despite undergoing chronic maintenance HD (1).

The underlying cause(s) of altered CYP functional expression observed in kidney disease is unclear, but numerous studies indicate that uremic toxins (e.g., urea, parathyroid hormone, indoxyl sulfate, cytokines) may play a role via transcriptional or translational modifications in CYP enzymes (1, 7, 8, 13). For example, we have shown that in normal hepatocytes incubated with uremic serum from rats or patients, the total level and protein expression of several CYP isoforms decreased up to 45% compared with serum from control animals secondary to reduced mRNA expression (1, 7). Moreover, we recently reported that the uremic toxin parathyroid hormone (PTH) was a major factor in the...
The downregulation of liver CYPs observed in experimental models of ESRD (13). In the present study, we demonstrated that NF-κB is a key transcriptional factor in the downregulation of CYPs caused by uremic serum since NF-κB inhibition nearly eliminated the effect of pre-HD serum on CYP expression. These results implicate potential uremic mediators such as PTH and cytokines since these proteins act via NF-κB signaling pathways (13, 16).

Interestingly, changes in the metabolic clearance of CYP substrates that cannot be explained by transcriptional or translational modifications have also been reported. Specifically, the abilities of uremic blood and, similarly, improvement in the uremic milieu to induce acute, post-translational modifications in CYP function have been demonstrated. In a classic experiment conducted over 20 years ago, Terao and Shen used an isolated rat liver perfusion model to assess changes in the intrinsic hepatic clearance of the CYP2D6 substrate propranolol (17). Perfusion of normal liver with uremic blood obtained from uremic rats (induced by uranyl nitrate) resulted in more than a 50% decrease in the clearance of propranolol compared to the control (normal liver perfused with normal blood); the reduced clearance was nearly identical to that observed when uremic liver (i.e., liver from uremic rats) was perfused with uremic blood. Furthermore, perfusion of uremic liver with normal blood restored the clearance of propranolol to values nearly identical to that observed in controls. This suggests that CYP2D6-mediated metabolism of propranolol is not downregulated in uremia. Similarly, the metabolism of losartan, an angiotensin II–receptor antagonist and substrate of CYP2C9 and CYP3A4, in hepatic microsomes prepared from rats in two different experimental models of uremia (ureteral ligation or uranyl nitrate) was not different than in normal microsomes (18). However, losartan metabolism in normal microsomes was reduced by nearly 50% in the
presence of uremic serum obtained from uremic rats. Losartan metabolism was decreased by about 30% in the presence of the uremic toxin indoxyl sulfate alone (18). In another experiment, incubation of normal human hepatic microsomes with the CYP3A4 substrate midazolam in the presence of uremic human plasma resulted in an 80% reduction in CYP3A4 activity compared to the control (19). Lastly, hepatic intrinsic clearance of the CYP3A4 substrate erythromycin has been shown to be acutely improved by hemodialysis in patients with ESRD (i.e., pre- versus post-HD) (12). Collectively, these data suggest that modifications in CYP activity may occur independently of transcriptional or translational modification. That is, rapid fluctuations in the concentrations of uremic toxins may acutely alter CYP metabolic activity in the setting of ESRD and hemodialysis.

One explanation for these findings may be that chronic exposure to elevated concentrations of dialyzable uremic toxins may result in transcriptionally or translationally mediated alterations in CYP functional expression in vivo, despite brief reductions in the concentrations post-HD. If HD led to sustained reductions in uremic toxin concentrations similar to transplantation, it is possible that sufficient time would elapse to allow normalization of CYP functional expression via transcriptional or translational mechanisms. Moreover, conventional hemodialysis rapidly and efficiently removes low molecular weight solutes, many of which are known uremic toxins. It is plausible that isolation of post-HD serum, which has been transiently depleted of uremic toxins, for hepatocyte incubation leads to an improvement in the functional expression of CYPs in vitro that does not translate to a corresponding improvement in vivo. The invasiveness and technical difficulty associated with obtaining human hepatic tissue precluded our ability to directly assess hepatic CYP expression in our patients.

Recently, Sun et al. reported decreased erythromycin uptake by hepatocytes incubated with the uremic toxin 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid (CMPF) via a decrease in organic anion-transporting polypeptide 2 (OATP2) protein expression and activity (8). Similarly, we recently demonstrated decreased OATP2 protein expression in uremic rats as well as in hepatocytes incubated with uremic serum (2). OATP uptake, the first step in the elimination of many xenobiotics, exposes compounds to detoxification enzymes (3, 20). Therefore, OATP downregulation may explain part of the decrease in drug elimination by the liver, as suggested previously (3, 8). These findings may explain why some studies have reported decreased drug metabolism in experimental models of kidney disease without transcriptional or translational modification of CYPs.

In conclusion, this is the first study to demonstrate that conventional hemodialysis acutely improves the altered functional expression of CYP1A, 2C, and 3A observed in rat hepatocytes incubated with uremic human serum. The data presented here corroborates previous work indicating that HD results in short-term improvements in the intrinsic clearance of the CYP3A4 substrate erythromycin (i.e., pre- versus post-HD) (12); further implicates one or more rapidly acting, dialyzable uremic toxins; and provides mechanistic insight into reports of altered CYP function in humans with ESRD.

Acknowledgments

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References

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