Hemodialysis Acutely Improves Hepatic CYP3A4 Metabolic Activity

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The uremic syndrome remains poorly understood despite the widespread availability of dialysis for almost four decades. To date, assessment of the biologic activity of uremic toxins has focused primarily on in vitro effects, rather than on specific biochemical pathways or enzymatic activity in vivo. The activity of cytochrome P450 (CYP) 3A4, the most important enzyme in human drug metabolism, is decreased in uremia. The purpose of this study was to assess the effect of hemodialysis and hence varying concentrations of uremic toxins on CYP3A4 activity using the 14C-erythromycin breath test and the traditional phenotypic trait measure, 20-min 14CO2 flux. CYP3A4 activity increased by 27% postdialysis (P = 0.002 compared with predialysis) and was significantly inversely related to plasma blood urea nitrogen concentration (rP = −0.50, P = 0.012), but not to several middle molecules. This is the first study in humans characterizing uremia as a state in which hepatic CYP3A4 activity is acutely improved by hemodialysis.

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ter Institutional Review Board and the Radiation Safety Committee. ESRD patients were studied on a regularly scheduled hemodialysis day. Hepatic CYP3A4 activity was assessed via the erythromycin breath test as described previously (Figure 1) (17,18). Briefly, the test involves a single 0.074 mmol (0.04 mg, 3 μCi) intravenous dose of [14C-N-methyl] erythromycin (Metabolic Solutions Inc., Nashua, NH), followed by breath collections at timed intervals. Breath samples were collected immediately before receiving the dose and at 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min after receiving the dose. Upon completion of breath collection, patients underwent hemodialysis for 4 h with a high-flux polysulfone membrane and blood and dialysate flow rates of 400 ml/min and 800 ml/min, respectively. The erythromycin breath test was repeated beginning 2 h postdialysis as described above.

Sample Analyses
The amount of 14C exhaled in breath samples was quantified by liquid scintillation counting, and the rate of excretion of the administered 14C dose (3 μCi), expressed as percent administered dose exhaled per minute, was estimated at each time point (18). The primary endpoint was the traditional 20-min flux phenotypic trait measure (14CO2 flux), expressed as the percent of the administered dose exhaled per hour (18). The mean area under the 14C excretion rate-time curve was also determined (18). Plasma β2-microglobulin (β2-M) concentrations were measured using a commercially available ELISA kit from Orgentec Diagnostika (Mainz, Germany). Intact parathyroid hormone (iPTH) concentrations were determined using the Immulite 1000 ELISA assay ( Diagnostic Products Corp., Los Angeles, CA). Concentrations of TNF-α, plasma protein thiols, and protein-associated carbonyl groups were determined as we have described previously (19).

Statistical Analyses
The primary aim of this study was to assess whether hepatic CYP3A4 activity in subjects with ESRD was acutely altered by hemodialysis. Determination of the target sample size was based on the 4.9% intra-individual coefficient of variation of erythromycin breath test results previously reported (20). A sample size of 12 subjects per group with a two-sided type I error of 0.05 was calculated to have >80% power for detecting a 10% difference in results within subjects (e.g., pre- versus postdialysis).

Pre- versus postdialysis comparisons of erythromycin breath test results, TNF-α, β2-M, iPTH, thiols, and carbonyls were made by the paired two-sided t test. Relationships between breath test results and concentrations of each were evaluated by Spearman’s rho correlation coefficient (rS). All statistical calculations were performed with GraphPad Prism 4.02 (GraphPad Software, San Diego, CA). Data are presented as mean ± SD. A P value <0.05 was considered statistically significant for all comparisons.

Results
CYP3A4 Activity
A total of 12 ESRD patients (7 male, 11 white, 1 black) participated in this study without complications. The subjects were 44.2 ± 10.4 yr of age, with body mass indexes (BMI) of

Figure 1. CYP3A4 mediated metabolism of 14C-erythromycin and subsequent production of 14CO2. Adapted from Rivory et al. (27).
26.1 ± 5.5 kg/m², and Kt/V values of 1.52 ± 0.24. As depicted in Figure 2A, mean ¹⁴C excretion rate values were higher after dialysis at each time point up to 120 min, resulting in a significantly larger mean area under the ¹⁴C excretion rate–time curve (3.88 ± 1.43 predialysis versus 4.80 ± 1.66 postdialysis; P = 0.004). The 20-min ¹⁴CO₂ flux increased by 27% after dialysis, from 2.34 ± 0.80 predialysis to 2.98 ± 1.04 postdialysis (P = 0.002; Figure 2B).

**Relationship between CYP3A4 and Markers of Uremia, Inflammation, and Oxidant Stress**

We quantified several uremic toxins of varying molecular weights (Table 1), including blood urea nitrogen (BUN) (low molecular weight solute), TNF-α, β₂-M, iPTH (middle molecular weight solutes), and biomarkers of oxidant stress (plasma protein carbonyl groups and plasma protein reduced thiol groups), and examined the relationship between each toxin and CYP3A4 activity in an effort to identify possible causes of altered activity. A significant inverse relationship was observed between ¹⁴CO₂ flux and BUN (rₛ = −0.50, P = 0.012; Figure 3). No significant relationships were observed between ¹⁴CO₂ flux and TNF-α, β₂-M, iPTH, thiols, or carbonyls.

**Discussion**

Loss of kidney function leads to the retention of a multitude of solutes normally excreted by the kidney, which in turn mediates diverse cellular and organ system dysfunction (21). To date, assessment of the biologic activity of uremic retention solutes has focused primarily on *in vitro* effects, rather than on specific biochemical pathways or enzymatic activity *in vivo* (22). Despite more than a century of careful study, only a limited number of dysfunctional cell biologic processes have been able to be directly attributed *in vivo* to uremic solute retention. In this study, we demonstrate that hemodialysis acutely improves altered hepatic CYP3A4 activity in uremia. Specifically, CYP3A4 activity was increased by 27% (P = 0.002) 2 h postdialysis. To our knowledge, this is the first study in humans to characterize uremia as a state in which hepatic CYP3A4 activity is modifiable by conventional hemodialysis therapy. The acuity of the response suggests that improvements in CYP activity occur independent of transcriptional or translational modification, and therefore that a rapidly acting, dialyzable byproduct of uremia acutely inhibits hepatic intrinsic clearance mediated by CYP3A4.

Our finding of an inverse relationship between hepatic CYP3A4 activity and the concentration of plasma BUN but not of several middle molecules suggests that low molecular weight solutes may be primarily responsible for reduced CYP3A4 activity in uremia. The association of pre- and postdialysis plasma BUN concentrations with CYP3A4 activity does not decrease as rapidly as the concentration of low molecular weight solutes, suggesting that the decrease in CYP activity is due to solutes with a higher molecular weight. These results support the hypothesis that low molecular weight solutes are primarily responsible for reduced CYP activity in uremia. Further research is needed to identify the specific low molecular weight solutes responsible for this effect.

**Table 1. Measures of uremia, inflammation, and oxidant stress**

<table>
<thead>
<tr>
<th></th>
<th>Predialysis</th>
<th>Postdialysis</th>
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</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>57.6 ± 18.0b</td>
<td>22.9 ± 13.3</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>57.8 ± 10.8b</td>
<td>51.2 ± 6.9</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>412 ± 417</td>
<td>439 ± 378</td>
</tr>
<tr>
<td>β₂-M (ng/ml)</td>
<td>11.8 ± 2.8</td>
<td>10.6 ± 1.6</td>
</tr>
<tr>
<td>Carbonyls (nmol/mg)</td>
<td>0.13 ± 0.05</td>
<td>0.13 ± 0.07</td>
</tr>
<tr>
<td>Thiols (µM)</td>
<td>315.5 ± 63.9b</td>
<td>413.9 ± 50.0</td>
</tr>
</tbody>
</table>

*P < 0.05 versus postdialysis.

**Figure 2. Pre- versus post-HD CYP3A4 activity.** (A) Depicts mean (±SD) ¹⁴C excretion rate–time curves. (B) Depicts changes in 20-min ¹⁴CO₂ flux values within individuals pre- and postdialysis. Mean values are indicated by dashed lines. HD, hemodialysis.
not prove causality, but indicates that BUN can be used as a surrogate for dialyzable toxins that contribute to alterations in CYP3A4 function. These results are supported by earlier published data from experimental models of kidney disease and various in vitro methods (2,9,10). For example, metabolism of the CYP3A4 substrate losartan in rat hepatic microsomes was reduced by nearly 50% in the presence of uremic serum obtained from animals in two different renal failure models (ureteral ligation or uranyl nitrate) (9). Similarly, 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 with control (10).

A recent pharmacokinetic study of the ketolide antibiotic agent and CYP3A4 substrate telithromycin in patients with varying levels of kidney disease illustrates the clinical importance of our findings (23). Telithromycin area under the plasma concentration-time curves were nearly 50% greater in nondialyzed patients with creatinine clearances of 11 to 40 ml/min than in healthy subjects (creatinine clearance > 80 ml/min). Of note, when telithromycin was administered 2 h postdialysis in ESRD patients (when BUN concentrations and the level of uremia were low), telithromycin clearance was normalized. Indeed, the investigators speculated that the normalization of telithromycin exposure postdialysis was due the removal of “uremic substances that might have the ability to decrease the drug’s intrinsic clearance by inhibiting metabolic enzymes” (23), a concept that now is clearly validated.

It is now well understood that uremic patients are subjected to increased exposure to oxidative stress and inflammatory stimuli, either or both of which could contribute to altered hepatic CYP activity (24,25). We did not observe a relationship between oxidative stress biomarkers and CYP3A4 activity. Also, we were unable to demonstrate a relationship between TNF-α, iPTH, or β2-M and CYP3A4 activity despite previous reports of associations (excluding β2-M) with the expression or activities of various CYP (2,26). This may reflect either a different pathophysiology or the relatively small sample size.

We assessed CYP3A4 activity in vivo via the erythromycin breath test, which is based on the principle that radiolabeled erythromycin undergoes N-demethylation by CYP3A4 and the demethylated carbon (14C) rapidly appears in breath as 14CO2 (Figure 1). The erythromycin breath test estimates the rate at which 14CO2 is exhaled after the dose and thus estimates the metabolic activity of hepatic CYP3A4. It has been validated as a model probe for assessing CYP3A4 activity and has been used extensively for this purpose (17,27). A single 20-min breath sample has been shown to correlate with hepatic CYP3A4 activity and is the standard approach to using the test (17,28). Notably, Dowling and colleagues utilized the erythromycin breath test to demonstrate that hepatic CYP3A4 activity is decreased in patients with ESRD compared to healthy subjects (14). Recently, however, Sun and colleagues observed in vitro alterations in the hepatic uptake of erythromycin in addition to changes in metabolism by various uremic toxins (13), suggesting that changes in hepatic drug transport may affect the standard interpretation of erythromycin breath test results. Thus, it is conceivable that modified hepatic uptake of erythromycin in addition to improved CYP3A4 activity could have contributed to the improvement in erythromycin breath test results that we observed postdialysis. In addition, a potential weakness of our study is that we did not include a control group in which two consecutive breath tests were administered without a hemodialysis session in between. Thus, we cannot rule out factors related to the dialysis procedure itself (i.e., other than uremic toxin removal, such as release of CYP3A4 inducing substances from the dialyzer, blood cell activation through shear stress, or ultrafiltration resulting in deswelling of hepatocytes) as potential mechanisms for the postdialysis improvement in CYP3A4 activity.

In conclusion, this is the first study in humans to characterize uremia as a state in which hepatic CYP3A4 activity is acutely improved by conventional hemodialysis therapy. These results may have important clinical implications; ultimately, better understanding of the effects of uremia and dialysis on CYP3A4 activity may help guide drug dosing in ESRD, a patient population known to require multiple medications and to have a disproportionately high rate of adverse drug events. Further study will be required before any definitive recommendations on drug dosing can be established. In addition, this study demonstrates an inverse relationship between CYP3A4 activity and the concentration of plasma BUN but not of several middle molecules, suggesting that urea and other dialyzable low molecular weight solutes for which urea is a surrogate may be primarily responsible. This provides one of the first precise in vivo descriptions of uremic toxicity characterized at a cellular and biochemical level. In future studies, this novel approach may be helpful in facilitating identification of the uremic toxin(s) responsible, which in turn may ultimately provide a target for therapeutic interventions.
Acknowledgments

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