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 ^{14}C-erythromycin breath test and the traditional phenotypic trait measure, 20-min $^{14}CO_2$ flux. CYP3A4 activity increased by 27% postdialysis ($P = 0.002$ compared with predialysis) and was significantly inversely related to plasma blood urea nitrogen concentration ($r_s = -0.50$, $P = 0.012$), 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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Chronic kidney disease alters the renal clearance (*i.e.*, glomerular filtration) and hence the pharmacokinetic disposition of drugs, and accumulating evidence indicates that modifications in nonrenal drug clearance also occur (1–3). Nonrenal clearance of drugs consists largely of hepatic metabolism mediated by cytochrome P450 (CYP) enzymes, the most significant contributors to drug metabolism *in vivo*. In particular, the CYP3A4 isoform is responsible for the metabolism of >50% of drugs currently marketed that undergo oxidative metabolism, many of which are used in the management of patients with kidney disease (4,5). Alterations in CYP3A4 function in uremia have significant clinical implications *via* drug accumulation, exaggerated pharmacologic responses and toxicity, and contribution to the apparent increase in the frequency of adverse drug events in patients with kidney disease (6,7).

The regulation of CYP3A4 in ESRD patients undergoing hemodialysis has not been well studied. Alterations in CYP3A4 expression and/or activity have been observed in experimental models of uremia (2,3,8 to 13) and a recent report using the ^{14}C-erythromycin breath test demonstrates that CYP3A4 activity is reduced in ESRD patients compared to healthy subjects (14). Although restoration of kidney function after transplantation leads to a sustained improvement in the uremic state and in hepatic drug metabolism (2,15), hemodialysis therapy only temporarily improves uremia and does not appear to generate long-term improvements in CYP3A function (2). However, the acute effect of hemodialysis on CYP3A4 activity *in vivo* has not been studied to date. We hypothesized that hepatic CYP3A4 activity would be inversely related to the level of uremic toxins, and that removal of uremic toxins *via* hemodialysis would lead to acute changes in CYP3A4 activity. Thus, the purpose of this study was to assess the effect of conventional hemodialysis on hepatic CYP3A4 metabolic activity in ESRD patients using the erythromycin breath test and the phenotypic trait measure 20-min $^{14}CO_2$ flux, and to evaluate the relationship between CYP3A4 activity and the concentrations of several uremic toxins.

Materials and Methods

Study Subjects

Twelve patients with ESRD and undergoing chronic hemodialysis participated in this study after providing written informed consent. All subjects underwent a screening evaluation that was based on a complete medical history, physical examination, medication history, and conventional biochemical tests. Eligibility criteria included normal hepatic function, body weight within 40% of ideal weight for height, body frame size, and sex according to the 1983 Metropolitan Life Insurance Company weight tables (16), documented compliance with dialysis prescriptions as determined by a Kt/V ≥1.20 within the 28-d period before the study day, and a negative pregnancy test for women of child-bearing potential. Subjects taking drugs known to inhibit or induce CYP3A4 or with a known sensitivity or previous adverse reaction to erythromycin were excluded. All participants were instructed to abstain from grapefruit products and herbal supplements/teas for at least 72 h before and during the study day.

Study Design

This was a prospective cohort study. The study adhered to the Declaration of Helsinki and was approved by the Maine Medical Cen-

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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 [^{14}C-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 ^{14}C exhaled in breath samples was quantified by liquid scintillation counting, and the rate of excretion of the administered ^{14}C 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 ($^{14}CO_2$ flux), expressed as the percent of the administered dose exhaled per hour (18). The mean area under the ^{14}C excretion rate-time curve was also determined (18). Plasma β_2-microglobulin (β_2-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% intraindividual 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, β_2-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 (r_S). 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

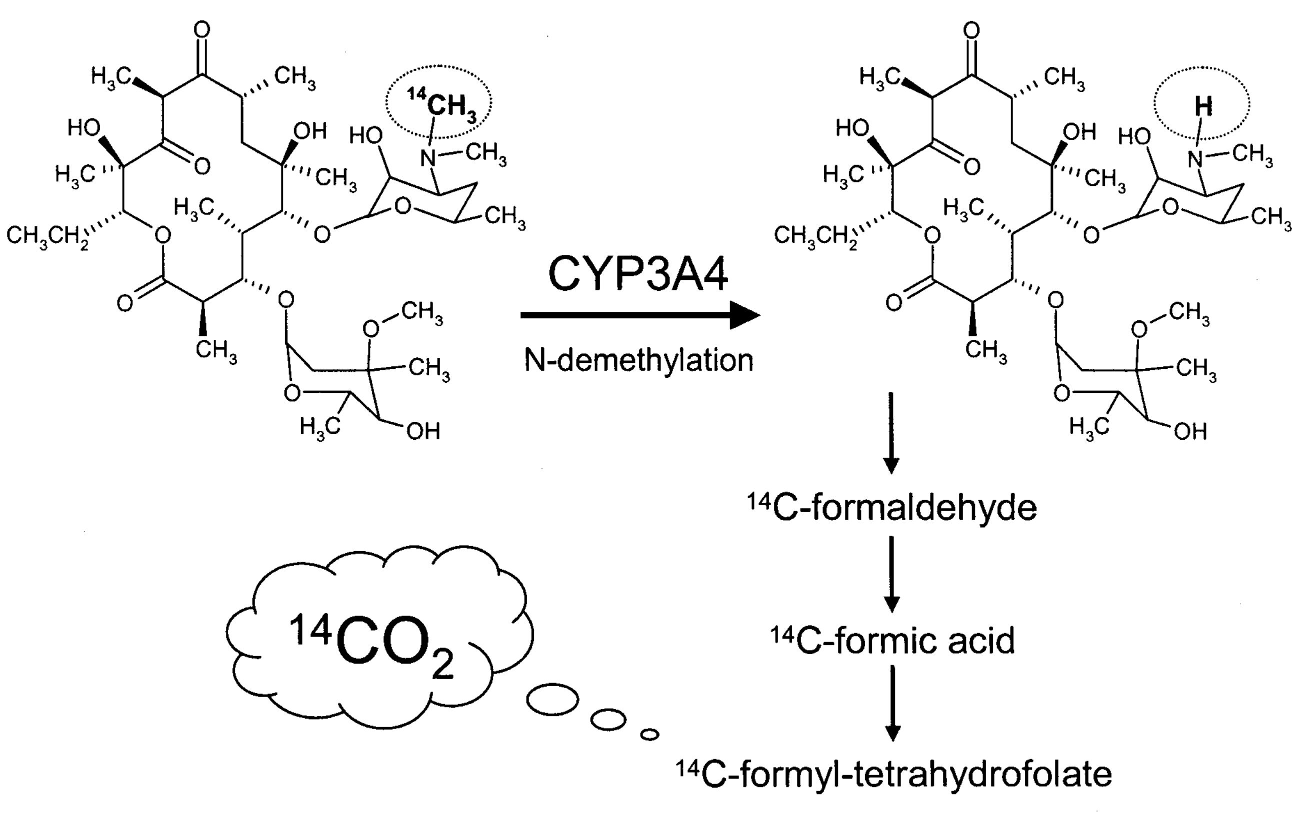

Figure 1. CYP3A4 mediated metabolism of ^{14}C-erythromycin and subsequent production of $^{14}CO_2$. Adapted from Rivory *et al.* (27).

26.1 ± 5.5 kg/m^2, and Kt/V values of 1.52 ± 0.24. As depicted in Figure 2A, mean ^{14}C excretion rate values were higher after dialysis at each time point up to 120 min, resulting in a significantly larger mean area under the ^{14}C excretion rate-time curve (3.88 ± 1.43 predialysis *versus* 4.80 ± 1.66 postdialysis; P = 0.004). The 20-min $^{14}CO_2$ 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, β_2-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 $^{14}CO_2$ flux and BUN ($r_s = -0.50$, $P = 0.012$; Figure 3). No significant relationships were observed between $^{14}CO_2$ flux and TNF-α, β_2-M, iPTH, thiols, or carbonyls.

Table 1. Measures of uremia, inflammation, and oxidant stress[a]

	Predialysis	Postdialysis
BUN (mg/dl)	57.6 ± 18.0[b]	22.9 ± 13.3
TNF-α (pg/ml)	57.8 ± 10.8[b]	51.2 ± 6.9
iPTH (pg/ml)	412 ± 417	439 ± 378
β_2-M (ng/ml)	11.8 ± 2.8	10.6 ± 1.6
Carbonyls (nmol/mg)	0.13 ± 0.05	0.13 ± 0.07
Thiols (μM)	315.5 ± 63.9[b]	413.9 ± 50.0

[a]BUN, blood urea nitrogen; iPTH, intact parathyroid hormone; β_2-M, β_2-microglobulin.
[b]$P < 0.05$ *versus* postdialysis.

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

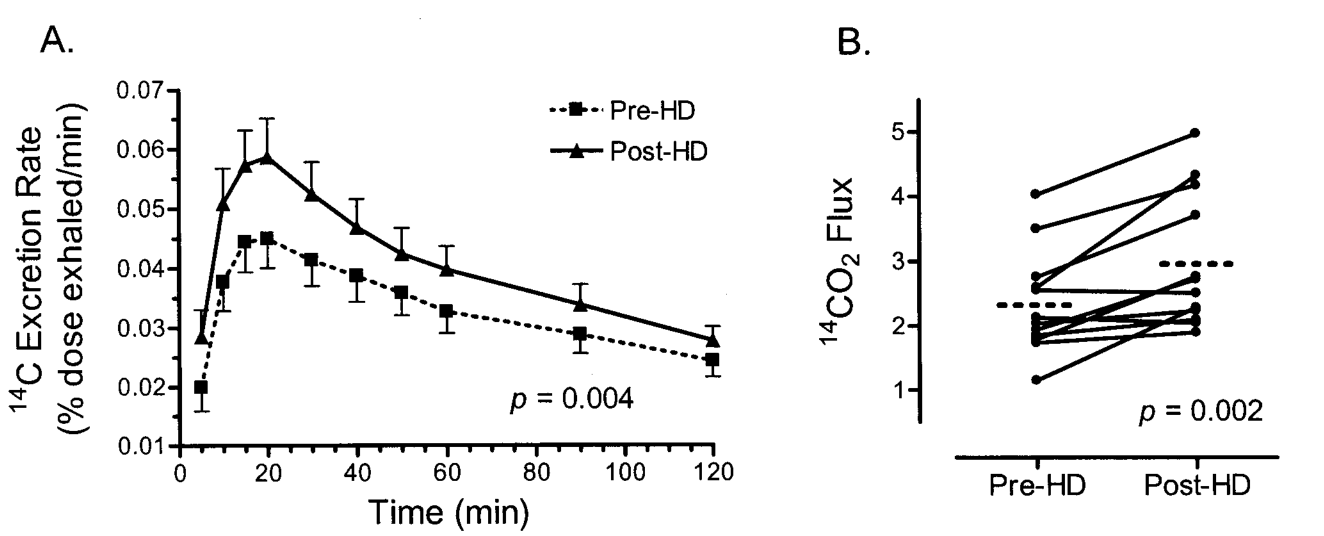

Figure 2. Pre- *versus* post-HD CYP3A4 activity. (A) Depicts mean (±SD) ^{14}C excretion rate-time curves. (B) Depicts changes in 20-min $^{14}CO_2$ flux values within individuals pre- and postdialysis. Mean values are indicated by dashed lines. HD, hemodialysis.

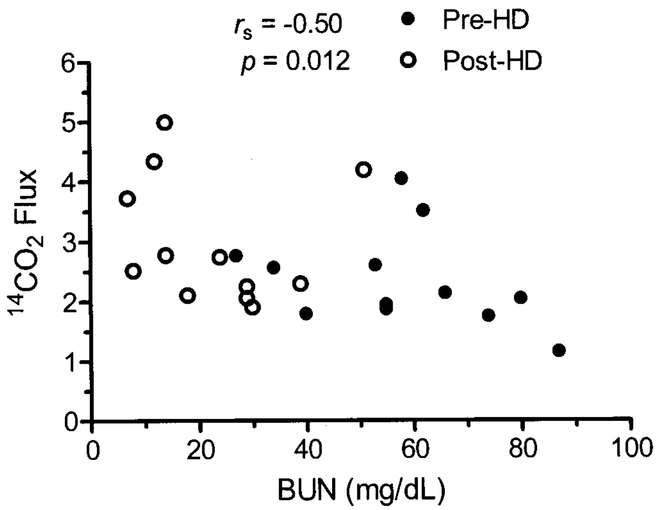

Figure 3. Plot of the relationship between individual 20-min $^{14}CO_2$ flux values and blood urea nitrogen (BUN).

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 β_2-M and CYP3A4 activity despite previous reports of associations (excluding β_2-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 (^{14}C) rapidly appears in breath as $^{14}CO_2$ (Figure 1). The erythromycin breath test estimates the rate at which $^{14}CO_2$ 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.

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