Chronic Inhibition of CYP3A is Temporarily Reduced by Each Hemodialysis Session in Patients With End-Stage Renal Disease

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Drug dosing is challenging in patients with end-stage renal disease. Not only is renal drug elimination reduced, but nonrenal clearance pathways are also altered. Increasing evidence suggest that uremia impacts drug metabolizing enzymes and transporters leading to changes in nonrenal clearance. However, the exact mechanisms are not yet fully understood, and the acute effects of dialysis are inadequately investigated. We prospectively phenotyped cytochrome P450 3A (CYP3A; midazolam) and P-glycoprotein (P-gp)/organic anion-transporting proteins (OATP; fexofenadine) in 12 patients on chronic intermittent hemodialysis; a day after (“clean”) and a day prior to (“dirty”) dialysis. Unbound midazolam clearance decreased with time after dialysis; median (range) reduction of 14% (−3% to 41%) from “clean” to “dirty” day ($P=0.001$). Fexofenadine clearance was not affected by time after dialysis ($P=0.68$). In conclusion, changes in uremic milieu between dialysis sessions induce a small, direct inhibitory effect on CYP3A activity, but do not alter P-gp/OATP activity.

Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

☑ Previous studies have shown that drug metabolism and transport are affected in patients with end-stage renal disease (ESRD). Reduced expression of drug metabolizing proteins and transporting proteins has been described, but there is limited knowledge on a potential acute effect of uremic toxin removal by hemodialysis on the activity of these proteins. Elucidation of the underlying mechanisms may improve drug dosing in this patient population.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

☑ This study investigated if there is a direct inhibitory effect on CYP3A-OATP and P-gp/OATP activity with increasing degree of uremia by comparing midazolam (total and unbound) and fexofenadine exposure the day before vs. the day after hemodialysis.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

☑ Increasing degree of uremia between dialysis sessions showed a small, direct inhibitory effect on CYP3A activity. The P-gp/OATP transporter activity was unaffected.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

☑ Attention should be given to dosing of CYP3A substrate drugs with narrow therapeutic range in relation to dialysis sessions in patients with ESRD.

It is well known that patients with end-stage renal disease (ESRD) require dose adjustments of drugs predominantly cleared by the kidneys. However, there is increasing awareness that nonrenal drug elimination may also be altered in ESRD, which further complicates drug dosing in this patient population.$^{1-3}$ Although the role of accumulated uremic toxins and the associated pathophysiological changes are considered to be important, the underlying mechanisms of pharmacokinetic alterations of nonrenally cleared drugs are inherently complex and still not well-characterized.

Nonrenal drug elimination is largely mediated by cytochrome P450 (CYP) metabolizing enzymes, with a major contribution of the CYP3A subfamily,$^4$ as well as by drug transporters, such as P-glycoprotein (P-gp) and organic anion-transporting polypeptides (OATPs). Increasing evidence strongly suggest that CYP-mediated drug metabolism and drug transporter function are altered in patients with ESRD.$^5,6$ Midazolam is considered to be the gold standard probe drug to assess CYP3A phenotype in vivo,$^7$ and has previously been investigated in patients with ESRD with inconclusive results.$^8-10$ Midazolam exposure was similar in patients with ESRD and healthy controls after oral dosing,$^9$ but significantly increased in ESRD after i.v. dosing.$^{10}$ One plausible explanation is that intestinal and hepatic CYP3A activity may be differentially affected by uremia, but this is unknown because the absolute bioavailability of midazolam has, to our knowledge,

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never been assessed in patients with ESRD. Fexofenadine is a substrate for P-gp and several OATPs (1A2, 1B1, 1B3, and 2B1), and has been used to phenotype transporter activity in patients with ESRD, showing consistently lower clearance compared with healthy controls. The underlying mechanisms behind these alterations are unclear. The leading hypothesis is that uremic toxins reduce nonrenal elimination either or both through altered transcription and a direct inhibitory effect on drug-metabolizing and drug-transporting proteins. The impact of uremic toxins on protein expression is supported by experimental studies in animal models of renal failure where CYP3A expression was reduced. Similarly, in renal failure rat models, the expression of P-gp was reduced in the intestine and increased in the liver, whereas OATP expression was reduced in the liver. Furthermore, the acute effect of removing uremic toxins with hemodialysis has demonstrated an enhanced expression of CYP3A in vivo. Increased concentrations of uremic toxins have also directly decreased hepatic CYP3A activity in vitro. These findings are further supported by the erythromycin breath test study of Nolin et al. where hemodialysis acutely increased CYP3A activity. However, it is unknown if the reduction in uremic toxins by hemodialysis reduced CYP3A metabolism and/or P-gp and OATP activity because erythromycin is an unsuitable substrate for these proteins. These findings do, however, support the notion that dialyzable uremic toxins may directly inhibit drug-metabolizing and drug-transporting proteins. Consequently, nonrenal clearance may fluctuate in patients receiving intermittent hemodialysis due to constant changes in the uremic milieu.

Plasma protein binding, and thus the unbound drug fraction, may be disturbed in patients with ESRD. It has been shown that drug binding capacity in blood and plasma is reduced with increasing degree of renal failure, and also that hemodialysis acutely increases protein binding capacity. Plausible explanations may be hypoalbuminemia, disturbances in acid-base balance, accumulation of endogenous substances, such as uremic toxins that competitively displace drugs from their plasma protein binding sites, and conformational change of plasma proteins. Total plasma drug concentrations will be altered by changes in protein binding, but according to the well-stirred model of hepatic clearance, the unbound drug exposure remains unaffected. Hence, investigating unbound midazolam pharmacokinetics may help elucidate the underlying mechanisms of the observed pharmacokinetic alterations with variable degrees of uremia.

The overall aim of this study was to investigate if the potential change in CYP3A- and P-gp/OATP activity between dialysis sessions is clinically relevant with regard to dosing schedules of substrate drugs in patients with ESRD receiving chronic intermittent hemodialysis. This was done by investigating if hemodialysis (i.e. reduction of uremic toxins), has an acute effect on unbound and total midazolam clearance (CYP3A activity), and total fexofenadine clearance (P-gp/OATP activity). Absolute midazolam bioavailability was determined prior to and after hemodialysis, in order to separately assess effects on intestinal and hepatic CYP3A activity.

METHODS

Study participants and study design

This study was an open-labeled, prospective, nonrandomized study. Patients with ESRD of at least 18 years of age, receiving chronic intermittent hemodialysis, not in need of excessive fluid removal, with a single-pool Kt/V ≥ 1.2 in the last 28 days were eligible for inclusion. Each patient underwent two investigation days; the “clean” day investigation was performed the day after a hemodialysis session and the “dirty” day was performed 3–4 days following a previous hemodialysis session (6 days for one subject receiving biweekly dialysis). The patients, therefore, skipped one dialysis session due to practical reasons. The investigations on the “dirty” and “clean” days were not separated by more than 2 weeks. Investigation sequence was not randomized but 7 of 12 patients performed the “clean” investigation first. Concomitant drugs were kept unchanged in the study period.

Patients were recruited from the dialysis unit at Akershus University Hospital. The study was performed in accordance to the Declaration of Helsinki and in accordance with Good Clinical Practice. All patients signed a written informed consent before inclusion. The regional ethics committee of Health Region South-East approved the study (REK number: 2017/2165) together with the local data protection officer.

Pharmacokinetic investigation

The patients fasted (food, drink, and drugs) overnight before the investigation days except for water. At the investigation days, patients received 1.5 mg oral midazolam (0.75 mL, 2 mg/mL oral syrup; Perrigo, Dublin, Ireland) and a 120 mg fexofenadine tablet (TELFAST; Sanofi-Aventis, Paris, France), followed by a 1 mg i.v. midazolam dose after 4 hours (1 mL. 1 mg/mL; B. Braun, Melsungen, Germany). A standard hospital breakfast was served 2 hours after oral drug intake. Venous blood samples (6 mL EDTA) for determination of both total midazolam and unbound midazolam and total fexofenadine concentrations were collected before (0 hours) and 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 4.25, 4.5, 5, 5.5, 6, 7, and 8 hours after oral midazolam/fexofenadine administration. The i.v. midazolam was administered via a peripheral venous catheter. Blood samples were drawn in EDTA vacutainers and centrifuged immediately after collection. Plasma was aliquoted into 3 vials and stored at −80°C until analysis: 2 for analysis of total-midazolam and unbound midazolam concentrations, and 1 for fexofenadine concentrations.

Analytical assays

Midazolam (total and unbound) and fexofenadine concentrations were determined by a validated ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. The UPLC-MS/MS system consisted of a Vanquish UPLC coupled to an Alitk triple quadrupole mass spectrometer (Thermo-Fisher, Waltham, MA). Positive electrospray ionization and selected reaction monitoring were performed with compound-tuned conditions. The mass to charge (m/z) transitions are presented in supplementary digital content (SDC) Table S1. The analytical column was an Accucore Vanquish C18, 2.1 × 50 mm reverse phase column (Thermo-Fisher). Mobile phase A consisted of 5% acetonitrile and 10 mM ammonium formate, whereas mobile phase B consisted of 90% acetonitrile and 10% methanol. Mobile phases were delivered in a gradient flow rate of 0.4 mL/min. The gradient started at 30% of mobile phase B, switched to 95% at 1.4 minutes, switched back to 30% at 2.65 minutes, and equilibrated for 1.85 minutes until the next injection. The retention time for midazolam and fexofenadine was 0.7 and 1.4 minutes, respectively. Total run time was 4.5 minutes.

For determination of total (protein bound plus unbound) midazolam and fexofenadine concentrations, 100 μL of plasma was added 200 μL of 95% acetonitrile and 5% methanol and deuterated internal standards to 5 ng/mL (midazolam-d6) and 50 ng/mL (fexofenadine-d6). The
samples were vortex-mixed briefly, followed by 1 hour of incubation at –20°C. Samples were centrifuged for 10 minutes at 2,272 g at 4°C. Supernatant (50 µL) was added 50 µL mobile phase A before injection (5 µL) into the UPLC-MS/MS system (see below). Calibrators and quality control samples were prepared in blank plasma and analyzed in each series. Ten calibrators in the range 0.25–100 ng/mL were applied for total midazolam and 2.5–1,000 ng/mL for fexofenadine. The calibration curve for midazolam was best fitted by quadratic regression without weighting or forced origin. For fexofenadine, the calibration curve was best fitted by quadratic regression with a weighting factor of 1/s and forced origin. The back-calculated values of calibrators were within 87.5–112% and 88.7–113% of nominal values for midazolam and fexofenadine, respectively. There was no detectable carryover of the analyte from high-level controls (100 ng/mL for midazolam and 1,000 ng/mL for fexofenadine) to blanks.

To determine unbound concentrations of midazolam, plasma samples were placed in a water bath holding 37°C to assure physiological equilibrium between unbound and bound midazolam. Plasma (1.0 mL) was added to a CentriFree ultrafiltration device (Merck Millipore Group, Darmstadt, Germany) and centrifuged in a fixed-angle rotor at 1,500 g at 37°C for 30 minutes yielding ~ 300 µL plasma ultrafiltrate (containing the unbound concentration). The ultrafiltrate was added 0.5 M ammonium acetate in a 1:1 ratio and deuterated internal standard to 1 ng/mL (midazolam-d6). The samples were vortex-mixed briefly before liquid-liquid extraction with 2,000 µL ethyl acetate. The ethyl acetate layer was evaporated under nitrogen and the sample was reconstituted in 50 µL mobile phase A before (10 µL) injection. Calibrators and quality control samples were prepared in Ringer’s solution as it was interchangeable with blank plasma for unbound midazolam determination (data not shown), and analyzed in each series. Seven calibrators ranging from 0.1–1 ng/mL were applied. The calibration curve for midazolam was best fitted by quadratic regression with a weighting factor of 1/s² and ignored origin. The back-calculated values of calibrators were within 87.6–113% of nominal values. Because the true unbound midazolam concentration was unknown, blank plasma was spiked to a total concentration of 5 ng/mL and was prepared in the same manner as the other samples and included in each run as control samples. These controls samples for unbound concentration determination had a coefficient of variation < 6% (SDC Table S2).²⁹

Samples with total or unbound midazolam concentrations above the upper limit of quantification were diluted in blank plasma or ultrafiltrate, respectively, and reanalyzed. Within-series and between-series performance of the total midazolam, fexofenadine, and unbound midazolam assays were assessed with resulting coefficients of variation < 9%, < 8%, and < 12%, respectively. The mean accuracy ranged from 99.9–109% for total midazolam, 93.7–111% for fexofenadine, and from 92.9–105% for unbound midazolam (SDC Table S3).

Pharmacokinetic calculations

\[
\text{AUC}_{\text{Oral}} = \text{AUC}_{0-\infty} + \frac{C_4}{k_{el}}
\]

(1)

The maximum concentrations (\(C_{\text{max}}\)) and time to \(C_{\text{max}}(T_{\text{max}})\) are the actual observed values. Pharmacokinetic variables were determined using non-compartmental analyses. The elimination rate constant (\(k_{el}\)) was estimated from the slope of the semilogarithmic concentration-time curve using at least three concentrations in the terminal elimination phase. For total midazolam and unbound midazolam, \(k_{el}\) from the i.v. midazolam injection was used for estimation of terminal half-life, volume of distribution and area under the time-concentration curve from 4 hours to infinity (\(\text{AUC}_{4→\infty}\)) after both oral and i.v. dosing. AUCs were calculated with the trapezoidal method. For total midazolam and unbound midazolam, exposure from the oral dosing (\(\text{AUC}_{\text{Oral}}\)) was calculated by Eq. 1, where the \(\text{AUC}_{4→\infty}\) was estimated by dividing concentration at 4 hours (\(C_4\)) by the \(k_{el}\). Exposure from the i.v. midazolam dose was calculated by Eq. 2, where \(\text{AUC}_{4→\infty}\) was estimated by dividing \(C_4\) by the \(k_{el}\). The theoretical initial plasma concentration at the time of i.v. administration (4 hours) was extrapolated using the method of residuals, and used in the calculation of \(\text{AUC}_{4→\infty}\). The amount remaining from the oral dose (\(\text{AUC}_{4→\infty}\)), i.e., \(C_4/k_{el}\) was subtracted from the \(\text{AUC}_{\text{Oral}}\) because the i.v. dose was administered before the oral dose was completely eliminated. This oral residual was also subtracted from the measured concentrations following the i.v. administration in i.v. figures in the manuscript.

\[
\text{AUC}_{\text{IV}} = \left( \text{AUC}_{4→\infty} + \frac{C_4}{k_{el}} \right) - \frac{C_4}{k_{el}}
\]

(2)

\[
F = \frac{\text{AUC}_{\text{Oral}}}{\text{AUC}_{\text{IV}}} \cdot \frac{\text{Dose}_{\text{IV}}}{\text{Dose}_{\text{Oral}}}
\]

(3)

The oral midazolam bioavailability (\(F\)) was calculated by Eq. 3, incorporating Eqs. 1 and 2 above and the doses used as described previously (1.5 and 1.0 mg, respectively).

Midazolam clearance (CL) was calculated by dividing the i.v. dose by \(\text{AUC}_{\text{IV}}\). Volume of distribution was calculated as CL divided by \(k_{el}\). Unbound fraction (\(f_u\)) of midazolam was calculated with unbound midazolam exposure (\(\text{AUC}_{u}\)) divided by the corresponding total exposure (\(\text{AUC}_{\text{IV}}\)) following oral and i.v. doses. The averages of i.v. and oral doses are reported unless stated otherwise.

Fexofenadine apparent oral clearance was calculated by dividing the oral dose by \(\text{AUC}_{\text{Oral}}\). Apparent oral volume of distribution was calculated by dividing apparent oral clearance by \(k_{el}\).

Exposure after an oral dose can be described by the well-stirred venous equilibrium model as presented by Benet and Hoener 28 (Eqs. 4 and 5). This relationship is general and is valid for both low extraction and high extraction ratio drugs with hepatic metabolism after oral dosing. 28

\[
\text{AUC}_{\text{oral}} = \frac{f_u \cdot F_G \cdot \text{Dose}}{f_u \cdot \text{CL}_{\text{int}}}
\]

(4)

Unbound exposure after oral dosing is independent of changes in \(f_u\) (Eq. 5). 28

\[
\text{AUC}_{u,\text{oral}} = f_u \cdot AUC_{\text{oral}} = \frac{f_u \cdot F_G \cdot \text{Dose}}{\text{CL}_{\text{int}}}
\]

(5)

Equation 5 was rearranged and was applied to estimate the oral intrinsic clearance of midazolam, \(\text{CL}_{\text{int}}/(\text{Fraction absorbed (\(F_{abs}\))} \times \text{Fraction escaping gut wall metabolism (\(F_{G}^{'})\})\), at both investigation days (Eq. 6).

\[
\frac{\text{CL}_{\text{int}}}{\text{F}_{abs} \cdot F_{G}^{'}} = \frac{\text{Dose}}{\text{AUC}_{u,\text{oral}}}
\]

(6)

The \(\text{CL}_{\text{int}}\) was not estimated after i.v. dosing because midazolam is an intermediate extraction ratio drug (\(E\) is 0.26–0.44) 29–32 and exposure after i.v. dosing is dependent on hepatic blood flow. 28,33,34

Sample size and statistics

Absolute bioavailability of midazolam in patients with ESRD has, to our knowledge, never been reported. Assuming that patients with ESRD have similar absolute bioavailability on the "clean" day as healthy controls, a total of 10 patients were needed to detect a 25% change in absolute bioavailability of 29% with SD of 10%, while having a power of 80% and a significance level of 5%. 35 Two extra patients were included, to a total of 12 patients, to account for eventual dropouts.

Nonparametric statistical tests were used. Changes in pharmacokinetic parameters and clinical chemistry from "clean" to "dirty" day were
RESULTS

Twelve patients on chronic intermittent hemodialysis were included in this study and completed both pharmacokinetic investigations between March and October 2018. Demographic data of the patients at inclusion are shown in Table 1. The “clean” and “dirty” days were initiated 18 hours (13–20 hours) and 75 hours (61–133 hours) after the end of the previous dialysis session, respectively. The blood urea nitrogen (BUN) increased by 112% (36–236%) from the “clean” to the “dirty” day (P < 0.001; Table 1). Concentrations of plasma albumin and total protein were both significantly reduced at the “dirty” day (Table 2).

The last four concentrations (from 5.5 to 8 hours) from the “dirty” investigation day were missing in one patient and \( k_d \) in this patient was estimated from concentrations between 2 and 4 hours. No adverse events were observed during the study period.

Table 1 Demographic data at the first investigational day

| Age, years | Gender, male/female | Total body weight, kg | BMI, kg/m² | Diabetes, type 1/type 2/no diabetes | Single pool Kt/V | PTH, pmol/L | Average fluid removal\( ^{a} \), mL | Time of dialysis, months | Dialysis sessions per week | Total plasma protein, g/L | P value |
|------------|---------------------|----------------------|------------|-----------------------------------|----------------|------------|-----------------|---------------------|------------------|---------------------|--------|
| 68 (47–78) | 6/6                 | 78 (60–110)          | 25 (19–38) | 1/4/7                             | 1.4 (1.1–2.0)  | 42 (0–114) | 100 (0–2400)    | 14 (3–39)          | 3 (2–5)          | 72 (61–85)         | 0.003  |

Continuous data presented as median (range). \( ^{a} \) Per dialysis session prior to study inclusion.

Table 2 Clinical data the day before (“dirty”) and the day after hemodialysis (“clean”)

| Time since previous dialysis, hours | “Clean” day | “Dirty” day | \( \Delta \) | P value |
|-------------------------------------|-------------|-------------|------------|--------|
| 18 (13–20)                          | 75 (61–133) | 59 (43–120) | 0.003     |        |
| Creatinine, µmol/L                  | 436 (272–730) | 636 (371–1144) | 238 (99–492) | 0.003 |
| BUN, mmol/L                         | 9.4 (4.5–13.0) | 19.3 (11.8–29.9) | 10.3 (3.7–21.0) | <0.001 |
| Cystatin C, mg/L                    | 5.15 (3.62–8.63) | 5.82 (3.83–10.2) | 0.43 (−0.20 to 1.52) | 0.002 |
| Uric acid, µmol/L                   | 198 (169–318) | 365 (205–631) | 165 (15–425) | 0.003 |
| Albumin, g/L                        | 42 (37–50) | 40 (32–47) | −3 (8–4) | 0.032 |
| Total plasma protein, g/L           | 72 (61–85) | 71 (60–77) | −2.5 (−14 to 2) | 0.036 |
| ASAT, IU/L                           | 21 (8–50) | 22 (11–44) | −2 (−14 to 10)\( ^{a} \) | 0.48 |
| ALAT, IU/L                           | 17 (9–36) | 19 (12–40) | 1 (−3 to 9) | 0.12 |

\( ^{a} \)\( n = 11 \).

Unbound midazolam exposure

The median unbound oral and i.v. midazolam exposure increased by 16% (−3% to 68%; P < 0.001), and 16% (−3% to 76%; P = 0.012) from “clean” to “dirty” day, respectively (Figure 1b and Table 3). Individual unbound midazolam concentration vs. time plots are presented in the SDC Figure S3. The absolute bioavailability of midazolam was not significantly different between the investigation days; median change of −4%, ranging from −30% to 9% (P = 0.18; SDC Figure S2a and Table 3). However, the absolute midazolam bioavailability showed large interindividual variability, ranging from 11−95%, with a median of 44% at the “clean” day and from 16−67% with a median of 40% at the “dirty” day (SDC Figure S2a and Table 3).

Unbound midazolam fraction

Absolute \( f_u \) of midazolam after both oral and i.v. doses was 1.5% (0.8−2.2%) on the “clean” day vs. 2.0% (1.1−3.0%; P = 0.002) on the “dirty” day, giving a relative increase in unbound fraction of 32% (−3% to 51%) with increasing uremia (Figure 2).

Fexofenadine exposure

There were no significant changes in fexofenadine AUC or any other pharmacokinetic parameters from the “clean” to the
"dirty" investigation days (Figure 3 and Table 4). Individual plots of fexofenadine exposure are presented in SDC Figure S4. Fexofenadine AUC$_{0-5}$ was used for comparison in the patient missing the last four concentrations (5.5–8 hours) at the "dirty" investigational day.

**DISCUSSION**

The main finding in this study was the acute inhibition of CYP3A in the period between intermittent hemodialysis sessions in patients with ESRD. Midazolam plasma protein binding was also similarly affected by time after a hemodialysis session.

**Table 3 Pharmacokinetic parameters from total and unbound midazolam concentrations at the day before ("dirty") and the day after hemodialysis ("clean")**

|                      | "Clean" day | "Dirty" day | Δ          | P-value |
|----------------------|-------------|-------------|------------|---------|
| **Total midazolam**  |             |             |            |         |
| AUC$_{oral,0-∞}$, ng·h/mL | 23.5 (11.4–33.7) | 20.6 (12.0–35.8) | −2.73 (−9.70 to 5.70) | 0.27    |
| AUC$_{IV,0-∞}$, ng·h/mL  | 31.5 (16.7–162.7) | 31.7 (20.8–136.8) | −0.55 (−25.9 to 11.9) | 0.11    |
| C$_{max,oral}$, ng/mL   | 9.12 (4.20–13.56) | 7.12 (3.32–13.99) | −1.02 (−4.75 to 2.82) | 0.18    |
| T$_{max,oral}$, hours | 0.50 (0.50–1.03) | 0.50 (0.25–1.50) | −0.002 (−0.50 to 0.98) | 0.72    |
| F (%)                 | 44 (11–95)  | 40 (16–67)  | −4 (−30 to 9)   | 0.18    |
| CL, L/hour           | 31.8 (6.15–59.7) | 31.7 (7.31–48.2) | −0.15 (−15.9 to 5.84) | 0.23    |
| V$_{d}$, L           | 97.2 (11.6–252) | 101 (13.9–422) | −1.5 (−25.8 to 171) | 0.62    |
| k$_{el}$, hour$^{-1}$ | 0.318 (0.204–0.529) | 0.309 (0.104–0.524) | −0.005 (−0.134 to 0.185) | 0.97    |
| t$_{1/2}$, hour      | 2.18 (1.31–3.41) | 2.24 (1.32–6.67) | 0.02 (−0.76 to 3.75) | 0.79    |

| Unbound midazolam    |             |             |            |         |
| AUC$_{oral,u,0-∞}$, ng·h/mL | 0.33 (0.16–0.76) | 0.39 (0.18–0.87) | 0.07 (−0.01 to 0.32) | <0.001 |
| AUC$_{IV,u,0-∞}$, ng·h/mL  | 0.56 (0.31–1.19) | 0.67 (0.37–1.18) | 0.07 (−0.02 to 0.32) | 0.012   |
| C$_{max,oral,u}$, ng/mL   | 0.120 (0.059–0.223) | 0.166 (0.058–0.381) | 0.028 (−0.028 to 0.171) | 0.034   |
| T$_{max,oral,u}$, hour | 0.50 (0.48–1.03) | 0.50 (0.25–2.07) | 0.0 (−0.53 to 1.55) | 0.86    |
| f$_{u}$               | 41 (17–76)  | 46 (25–66)  | 1 (−11 to 11)  | 0.91    |
| CL$_{u}$, L/hour      | 1778 (840–3212) | 1500 (845–2695) | −239 (−1018 to 52) | 0.012   |
| V$_{d,u}$, L          | 5928 (1438–12296) | 5412 (1648–16761) | −658 (−4376 to 4465) | 0.38    |
| k$_{el,u}$, hour$^{-1}$ | 0.305 (0.154–0.584) | 0.269 (0.161–0.513) | −0.010 (−0.160 to 0.177) | 0.47    |
| t$_{1/2,u}$, hour     | 2.28 (1.19–4.51) | 2.58 (1.35–4.31) | 0.09 (−1.57 to 1.66) | 0.62    |

AUC, area under the concentration-time curve; CL, clearance; C$_{max}$, maximum concentration after oral dose; F, bioavailability; f$_{u}$, unbound fraction; k$_{el}$, elimination rate constant; t$_{1/2}$, elimination half-life; T$_{max}$, time to C$_{max}$; V$_{d}$, volume of distribution.

Continuous data presented as median (range), Δ is calculated as ("dirty" day– "clean" day).

Subscript-u denotes unbound parameter.
session, underlining the importance of investigating unbound midazolam concentrations in this setting. Although it is difficult to interpret clinical pharmacokinetic data mechanistically, the most plausible explanation for our findings is that changes in the uremic milieu between consecutive dialysis sessions acutely affect the functional CYP3A protein. This is in line with the hypothesis that increased levels of uremic toxins may directly inhibit drug metabolism. In the present study, the median CYP3A activity was reduced by 14% in the period between the “clean” and “dirty” day, which for most substrate drugs will not necessitate dose adjustments. However, 5 patients showed more than 20% reduction in CYP3A activity. Thus, it might be advisable to closely monitor effect of drugs with narrow therapeutic range to minimize adverse effects in selected patients. In addition, the fivefold variability in unbound oral midazolam exposure underline the importance of careful dose individualization of CYP3A substrate drugs with narrow therapeutic range in the ESRD population.

Drug transporters are known to significantly influence the systemic exposure of many drugs. OATP substrate drugs have been shown to have reduced clearance with increasing degree of renal failure, suggesting that the OATP function is decreased in patients with kidney disease. In the current trial, we found that hemodialysis did not alter the oral clearance of fexofenadine. This indicates that reduced OATP function in patients with ESRD most likely represents either a constitutive inhibition of activity or an altered protein expression, and not an acute inhibitory effect by uremic toxins. Nolin et al. showed that hemodialysis acutely improved CYP3A activity using erythromycin as a probe drug. However, erythromycin is a less specific CYP3A substrate compared with midazolam because it also undergoes P-gp-efflux and OATP uptake, in addition to CYP3A metabolism.

In a previous study in ESRD, no significant change in midazolam pharmacokinetics was shown after oral dosing, but this study only relied on total midazolam concentrations. These findings are supported by the current study as well, showing no difference in total clearance of midazolam in the period between hemodialysis sessions. However, this is probably explained by the fact that the true acute change in CYP3A activity is counterbalanced by the increased unbound midazolam fraction (30%) on the “dirty” day as compared with the “clean” day, resulting in an unchanged total midazolam clearance. This finding clearly demonstrates the importance of measuring unbound midazolam plasma concentrations when assessing CYP3A activity in patients with ESRD.

To our knowledge, this is the first study to investigate absolute oral bioavailability of midazolam in patients with ESRD. The median midazolam absolute bioavailability was above 40% with a wide range from 11% to 95%. This is somewhat higher than the 25–30% bioavailability assessed by semi-simultaneous midazolam dosing in healthy volunteers (SDC Figure S2b), which may support the hypothesis that patients with ESRD have altered drug metabolism capacity due to reduced expression of CYP3A enzymes. A direct inhibitory effect on CYP3A enzymes by the fluctuating degree of uremia was observed in the present study. Therefore, it may be proposed that the likely combination
of the two mechanisms, direct inhibition and reduced expression, explains the alterations in CYP3A-mediated drug metabolism observed in patients with ESRD.

A major strength of this study is that CYP3A activity was assessed with midazolam, a specific probe for CYP3A, and the semi-simultaneous dosing design reduces interocassion variability. Furthermore, measuring unbound midazolam concentrations enabled estimation of oral intrinsic clearance, and, thus, the CYP3A activity in the liver. Our study is also in accordance with the European Medicines Agency (EMA) guideline on the evaluation of pharmacokinetics in patients with decreased renal function, stating that drugs with > 90% protein binding should be investigated with respect to unbound concentrations. In addition, unbound concentrations were determined at all sampling times, making the calculation of unbound midazolam exposure robust.

There are some limitations to our study. The sample size was calculated to investigate changes in absolute bioavailability and was, therefore, underpowered to assess any potential associations between degree of uremia (BUN) and CYP3A activity. Because the patients had to skip one dialysis session, it may be argued that the findings represent a “worst case scenario,” but it is still clinically relevant (e.g., comparable to situations without weekend dialysis). In addition, no patients with anuria were included in the study because they cannot easily skip sessions. Thus, the most uremic patients with no residual kidney function were excluded. It is unlikely that this has affected the results because renal elimination of midazolam is minimal. However, interdialytic fluctuation in the degree of uremia is likely higher in patients with anuria, with potentially a larger impact on the nonrenal metabolism of drugs. For practical purposes, it was not possible to include these patients. One patient received hemodialysis twice weekly and one patient received at-home dialysis five times per week, but their treatment adequacy (Kt/V) was comparable to the other patients, and all patients had comparable BUN concentrations to other clinical trials. We have not quantified free fatty acids or any specific uremic toxins, such as 3-carboxy-4-methyl-5-propyl-2-furanpropionate or indoxyl sulfate, so the perpetrator(s) of the inhibition of CYP3A or the change in \( f_u \) of midazolam was not assessable from this study.

In conclusion, the time from the last dialysis session (i.e., increased degree of uremia), induced a small reversible inhibitory effect on CYP3A activity, whereas P-gp/-OATP activity was unchanged. The magnitude of the fluctuating inhibition of CYP3A activity will probably not have clinical implications for most CYP3A substrates. However, attention should be given to dosing of CYP3A substrate drugs with narrow therapeutic range in relation to dialysis sessions in these patients. In addition, changes in protein binding of highly bound probe drugs must be taken into account in future phenotyping trials in patients with ESRD.

**SUPPLEMENTARY INFORMATION**

Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

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**CONFLICTS OF INTEREST**

The authors declared no competing interests for this work.

**AUTHOR CONTRIBUTIONS**

E.J.E., B.W., H.K.Z., H.C., A.Å., and I.R. wrote the manuscript. E.J.E., B.W., H.K.Z., and I.R. performed the research. E.J.E., B.W., H.K.Z., and I.R. designed the research. E.J.E., B.W., H.K.Z., and I.R. analyzed the data.

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