Impact of Inflammation on Midazolam Metabolism in Severe COVID-19 Patients

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Midazolam is a benzodiazepine frequently used for sedation in patients hospitalized in the intensive care unit (ICU) for coronavirus disease 2019 (COVID-19). This drug is primarily metabolized by cytochrome P450 3A (CYP3A) isoenzymes. Several studies have suggested that inflammation, frequently observed in these patients, could modulate CYP3A activity. The objective of this work was to study the impact of inflammation on midazolam pharmacokinetics in patients with COVID-19. Forty-eight patients hospitalized in the ICU for COVID-19 and treated with midazolam administered by continuous infusion were included in this study. Midazolam and α-hydroxymidazolam concentrations were measured and patient data, including the use of CYP3A inhibitors, were collected. Total and unbound concentrations of midazolam and α-hydroxymidazolam were measured in plasma using a validated liquid-chromatography coupled with mass spectrometry method. Inflammatory condition was evaluated by C-reactive protein (CRP) level measurement. Both drug concentrations and CRP measurements were performed on 354 plasma samples. CRP elevation was significantly associated with the α-hydroxymidazolam/midazolam plasma ratio decrease, whether for the unbound fraction or for the total fraction. Conversely, inflammation was not associated with protein binding modifications. Logically, α-hydroxymidazolam/midazolam plasma ratio was significantly reduced when patients were treated with CYP3A inhibitors. In this study, we showed that inflammation probably reduces the metabolism of midazolam by CYP3A. These results suggest that molecules with narrow therapeutic margins and metabolized by CYP3A should be administrated with care in case of massive inflammatory situations.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ It is known that inflammation could impact drug pharmacokinetics. It was studied on different drug inflammation models and also in patients with coronavirus disease 2019 (COVID-19).

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Does inflammation caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) impact the pharmacokinetics of midazolam, in critical care unit patients?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ An inhibition of CYP3A isoenzyme activity is caused by inflammation, that impacts midazolam metabolism.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This work shows that particular attention should be paid on CRP levels during hospitalization, especially for patients treated with drugs with narrow therapeutic margins and using the same metabolic pathway than midazolam.

Midazolam is an imidazobenzodiazepine mainly used for anesthesia and sedation because of a short half-life and an easy use.[1] Its metabolism exclusively depends on cytochrome P450 3A (CYP3A) isoenzymes.[2] The two metabolites formed are α-hydroxymidazolam and 4-hydroxymidazolam, which are pharmacologically active.[3] The α-hydroxymidazolam is at least as potent as midazolam but only contributes to a little extent (10%) to clinical effects after intravenous administration of midazolam. The 4-hydroxymidazolam appears to be quantitatively not significant.[4] Then, the hydroxymetabolites are metabolized to glucuronide conjugates by UGTs and are pharmacologically inactive. Glucuronide conjugates are then excreted in the urine.[5]

Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is associated with severe inflammatory syndrome.[6] Indeed,

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Received November 29, 2021; accepted June 12, 2022. doi:10.1002/cpt.2698

patients hospitalized for coronavirus disease 2019 (COVID-19) display high levels of inflammatory cytokines, such as IL-6 and TNF-alpha, and a high level of serum C-reactive protein (CRP).[7] Several studies have suggested that inflammation can modulate drug-metabolizing enzymes and transporters activity.[8] For example, a previous study described very high lopinavir concentrations in patients with COVID-19 compared with patients with regular HIV.[9]

Patients hospitalized in the intensive care unit (ICU) for COVID-19 usually require sedation, analgesia, and respiratory assistance. In Nantes University Hospital, midazolam was used for patient sedation. Herein, we have evaluated the impact of inflammation (estimated using CRP levels) on midazolam metabolism in patients with COVID-19.

METHODS

Patient population and data collection

Data from patients with COVID-19 hospitalized in the ICU in the Nantes University Hospital and treated by continuous infusion midazolam for sedation between April 2020 and February 2021 were retrospectively collected. For this noninterventional monocentric retrospective study, sample data have been recorded during the medical care of patients by professionals who are following them. All data collected for this study from the patients' medical records have been filled in a board under an anonymous code. According to the French and European legislation, the use of data in a retrospective monocentric study does not need an approval of the ethics committee. This study has been recorded in Nantes Hospital by the local's data privacy officer under reference: TS005.BIO.AP.2019_15.

For all patients and at each midazolam concentration measurement, the following data were collected: age, sex, weight, height, starting date of midazolam treatment, daily midazolam posology, plasma creatinine, albumin and CRP concentrations, date of symptoms onset, date of positive SARS-CoV-2 reverse transcriptase polymerase chain reaction, COVID-19 outcome, comedications including SARS-CoV-2 repositioned drug trials (lopinavir, hydroxychloroquine, remdesivir, interferon, tocilizumab, and sarilumab), and Richmond Agitation-Sedation Scale. Then, several parameters were calculated: body mass index, body surface area (BSA) according to the Du Bois formula,[10] estimated glomerular filtration rate (eGFR) indexed by BSA (eGFR, mL/min/1.73 m^2) according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula,[11] and absolute value of the GFR (aGFR, mL/min) calculated from patient eGFR and BSA.

Midazolam and α-hydroxymidazolam quantification

Midazolam and α-hydroxymidazolam total plasma concentrations were determined using a validated liquid-chromatography coupled with the mass-spectrometry method.

Blood samples were collected in EDTA tubes and centrifuged (1,500 × *g*, 10 minutes, 4°C) upon reception at the laboratory. Then, 400 mL of deuterated internal standards solution (25 ng/mL of D4-midazolam and D4-hydroxymidazolam in acetonitrile) were added to 100 µL of plasma for protein precipitation. The mixture was vortexed for 10 minutes using a VXR basic Vibrax shaker (IKA, Staufen, Germany) and centrifuged for 10 minutes (15,000 × *g*, 8°C). One hundred microliters of supernatant were diluted in 700 µL of a water and methanol mixture (75:25 v/v). Three microliters were injected into the HPLC LC-20 AD XR (Shimatzu, Marne-la-Vallée, France). The separation was performed on a ACQUITY UPLC BEH C18 column (50 × 2.1 mm ID, 1.7 µm particles; Waters, Guyancourt, France). The tandem mass spectrometry system used was a 5,500 QTRAP (SCIEX, Villebon-sur-Yvette, France). Analytes were quantified using multiple reaction-monitoring mode. The flow rate was set to 0.4 mL/min. The column temperature was set to 30°C. For each analyte, two transitions were monitored: one for quantification (midazolam m/z = 291.1; α-hydroxymidazolam m/z = 323.9) and the other to confirm identification (midazolam m/z = 223; α-hydroxymidazolam m/z = 203). The ratio between the peak areas of the two transitions should not deviate more than 20% from the initial fixed value. All analytes were measured using positive-ion electrospray ionization. Data were acquired and processed using Analyst 1.6.3 software (SCIEX, Villebon-sur-Yvette, France).

The lower limit of quantitation (LLOQ) for midazolam and α-hydroxymidazolam was 0.025 mg/L and the upper limit was 10 mg/L. Accuracy was satisfactory, with intra-day and inter-day coefficients of variation being < 15% (20% for the LLOQ). Imprecision was also < 15% (20% for the LLOQ).

Unbound midazolam and α-hydroxymidazolam quantification

Ultrafiltration (UF) was used to separate the protein-bound midazolam and α-hydroxymidazolam from the unbound drug in plasma using Amicon UltraCentrifugal Filter Units (molecular weight cutoff 30 kDa, UFC5030BK). The driving force for UF was provided by centrifugation (Thermo Scientific, Heraeus FRESCO 21 centrifuge, Villebon-sur-Yvette, France).

UF was performed after a filter-membrane pretreatment, adapted from the protocol used by Illamola *et al.*[12] The UF units were incubated with 0.5 mL of 5% Tween 20 at room temperature for 24 hours to limit the nonspecific binding of free drug on the filter membrane. Then filters were washed with deionized water (0.5 mL) and centrifuged (1,500 × *g* and 37°C for 30 minutes). After this step, filters were inverted and centrifuged (1,000 × *g* and 37°C for 3 minutes) to remove excess water. Patient plasma (0.5 mL), previously incubated at 37°C for 30 minutes, was then added and centrifuged (14,000 × *g* and 37°C for 30 minutes). Fifty microliters of deuterated internal standards solution (D4-midazolam and D4-hydroxymidazolam 5 ng/mL in a water and methanol mixture (75:25 v/v)) were added to 50 mL of ultrafiltrate. Three microliters were injected into the HPLC system. The LLOQ for midazolam and α-hydroxymidazolam was 0.0005 mg/L and the upper limit was 0.5 mg/L.

During the preparation of the protein-free samples, the analyte might be partially lost during the centrifugation and filtration steps. The percent loss was assessed and was below 10% (we targeted a degree of loss below 15%).

C-reactive protein measurement

Blood samples were collected in EDTA tubes and centrifuged (1,500 × *g*, 10 minutes, 4°C) upon reception at the laboratory. Plasma CRP measurement was performed by the immunoturbidimetric method (CRP4 Cobas) on c701 module of a Cobas 8000 analyzer (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's instructions. The lower detection limit for the assay was 0.6 mg/L and the inter-assay coefficient of variation was between 0.7 and 2.7% on plasma.

Albumin measurement

Blood samples were collected in EDTA tubes and centrifuged (1,500 × *g*, 10 minutes, 4°C) upon reception at the laboratory. Plasma albumin measurement was performed by immunoturbidimetric method (Albumin DiAgam) on c501 module of a Cobas 8000 analyzer (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's instructions. The lower detection limit for the assay was 0.147 g/L and the inter-assay coefficient of variation was between 3.53 and 4.17% on plasma.

Statistical analysis

Generalized linear mixed models using a random effect (the patient) were performed to establish correlation between α-hydroxymidazolam/midazolam concentration ratios and CRP levels (identity link), or comparison between patients treated by CYP3A inhibitors and those without (logit link). The data were analyzed using GraphpadPrism

and R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Population pharmacokinetics analysis

A population pharmacokinetics model for midazolam and α-hydroxymidazolam total plasma levels was developed using the non-parametric adaptive grid algorithm from the Pmetrics package (version 1.9.7; Laboratory of Applied Pharmacokinetics, Los Angeles, CA) for R (version 4.1.3).[13,14] Different structural models with one or two compartments (central and peripheral) were tested to determine the best fit for the observed data. To compare the different candidate models, the Akaike Information Criterion, which is an estimator of the likelihood of the model penalized by the number of parameters in the model, was calculated. The observed vs. population predicted concentrations plots were also evaluated. Bias (mean weighted error of predicted concentrations minus observed concentrations) and imprecision (bias-adjusted mean weighted squared error of predicted concentrations minus observed concentrations) were calculated.

Additive (lambda) and multiplicative (gamma) error models were assessed. Measurement uncertainty from the midazolam and α-hydroxymidazolam assay was described by a polynomial Eq. C0 + C1*[obs] + C2*[obs]^2 + C3*[obs]3, where [obs] is the analyte plasma concentration and C0/C1/C2/C3 coefficients reflecting the variability of the assay.

Once a structural model was chosen, the influence on the pharmacokinetic parameters of inflammation-related covariates (CRP and albumin) and CYP3A inhibitors administration was tested. If a candidate covariate increased the model fit (Akaike Information Criterion, bias, imprecision and predicted vs. observed concentrations), it was included in the final model.

RESULTS

Patient characteristics

A total of 48 patients were included in the study, corresponding to 354 analyzed samples. CRP, midazolam, and α-hydroxymidazolam plasma concentrations were measured in each of them, all at steady-state (at least 18 hours after midazolam onset, from 18 to 346 hours). The median (interquartile range (IQR)) midazolam dose was 0.16 mg/kg/hour (0.14). Patient characteristics are summarized in **Table 1**.

Table 1 Patient characteristics and outcomes (*n* = 48 patients)

Patients' characteristics	
Gender, female/men (%)	12/36 (25/75)
Age, years, median (IQR)	62 (10.5)
Weight, kg, median (IQR)	87.5 (19.9)
BMI, kg/m^2, median (IQR)	29.2 (6.9)
Glomerular filtration (CKD-EPI), mL/min/1.73 m^2, median (IQR)	78 (85)
Plasma CRP concentration, mg/L, median (IQR)	113.6 (133.9)
Plasma albumin concentration, g/L, median (IQR)	17.95 (5.2)
CYP3A inhibitors, *n* (%)	23 (47.9)
Azole antifungal, *n* (%)	6 (12.5)
Erythromycin, *n* (%	14 (29.2)
Amiodarone, *n* (%)	7 (14.6)
Others, *n* (%)	1 (2.1)
Dexamethasone, *n* (%)	22 (45.8)
Tocilizumab, *n* (%)	1 (2.08)
RASS, median (IQR)	−5 (0)
In-hospital mortality, *n* (%)	21 (43.7)
All samples, *n*	354
Samples with dexamethasone, *n* (%)	88 (24.9)
Samples without dexamethasone, *n* (%)	266 (75.1)
Samples with identified CYP3A inhibitors, *n* (%)	76 (21.5)
Samples with no identified CYP3A inhibitors, *n* (%)	278 (78.5)
Samples without dexamethasone or identified CYP3A inhibitors	200 (56.5)

BMI, body mass index; CKD-EPI, chronic kidney disease epidemiology collaboration; CRP, C-reactive protein; CYP3A, cytochrome isoenzymes 3A; IQR, interquartile range; RASS, Richmond Agitation-Sedation Scale.

Midazolam pharmacokinetics

Median (IQR) midazolam and α-hydroxymidazolam total plasma concentrations were 1,135 ng/mL (1,408) and 219 ng/mL (222), respectively. Median (IQR) α-hydroxymidazolam/midazolam total plasma concentration ratio was 0.20 (0.18).

Median (IQR) midazolam and α-hydroxymidazolam unbound plasma concentrations were 31.5 ng/mL (52.1) and 12.9 ng/mL (20.4), respectively. Median (IQR) α-hydroxymidazolam/midazolam unbound plasma concentration ratio was 0.4 (0.3).

Median (IQR) midazolam and α-hydroxymidazolam unbound fraction were 1.8% (2.8) and 3.8% (6), respectively. All midazolam pharmacokinetics results are summarized in **Table 2**.

Impact of inflammation on midazolam pharmacokinetics

Total α-hydroxymidazolam/midazolam ratio decreased when the CRP concentrations increased (**Figure 1**; regression coefficient: −6.84E−4 (−7.88E−4; −5.79E−4), *P* value < 0.001). Tendency is described by a straight line ($y = -0.0006024^*X + 0.3093$; $R^2 = 0.2675$).

The same observation was done for unbound α-hydroxymidazolam/midazolam ratio (**Figure 1b**); regression coefficient: −1.44E−3 (−1.67E−3; −1.21E−3), (*P* value < 0.001). Tendency is described by a straight line: $y = -0.001271^*X + 0.6410$; $R^2 = 0.2575$).

We tried to estimate the activity of CYP3A in case of inflammation. We arbitrarily defined four levels of inflammation from low to high. We assumed that CYP3A activity was maximal and unaltered when CRP < 50 mg/L. From this supposition, we established that the median of total α-hydroxymidazolam/midazolam ratio was corresponding to a CYP3A activity of 100%. Subsequently, we estimated the CYP3A activity relative to the CRP levels: when CRP was between 50 and 150 mg/L; 150 and 250 mg/L, and upper or equal to 250 mg/L CYP3A activity was respectively estimated at 66, 53, and 33% (**Figure 2**). Patients treated by identified CYP3A inhibitors were excluded from this analysis.

Albumin levels were reduced when the CRP concentrations increased (**Figure 3a**; regression coefficient: −0.01126 (−0.0143; −0.0082), *P* value < 0.0001). Tendency is described by a straight line ($y = -0.01126^*X + 20.22$; $R^2 = 0.0987$). However, there was no correlation between the unbound fraction of midazolam and the CRP concentration (**Figure 3b**).

Table 2 Midazolam pharmacokinetics

Pharmacokinetic parameters	
Dosage, mg/kg/hour, median (IQR)	0.16 (0.14)
Delay from first symptoms to concentration measurement, day, median (IQR)	20 (12)
Delay from positive RT-PCR to concentration measurement, day, median (IQR)	15 (12.7)
Total midazolam plasma concentration, ng/mL, median (IQR)	1,135 (1408.5)
Unbound midazolam plasma concentration, ng/mL, median (IQR)	31.5 (52.1)
Unbound fraction of midazolam, %, median (IQR)	1.8 (2.8)
Total α-hydroxymidazolam plasma concentration, ng/mL, median (IQR)	219 (222)
Unbound α-hydroxymidazolam plasma concentration, ng/mL, median (IQR)	12.9 (20.4)
Unbound fraction of α-hydroxymidazolam, %, median (IQR)	3.8 (6)
Total α-hydroxymidazolam/midazolam plasma ratio, median (IQR)	0.2 (0.2)
Unbound α-hydroxymidazolam/midazolam plasma ratio, median (IQR)	0.4 (0.3)

IQR, interquartile range; RT-PCR, reverse transcriptase polymerase chain reaction.

Population pharmacokinetics analysis

The observed data was best described with a bicompartmental model. Three patients were excluded from the analysis due to inaccurate or missing midazolam dosing data. The α-hydroxymidazolam concentrations were derived from midazolam concentrations using a Michaelis–Menten equation [AHMZ] = [MZ]*B_{max}/(Kd + [MZ]). The coefficient values for the error polynomial were C0 = 50.5, C1 = 0.15, C2 = 0, C3 = 0 for midazolam, and C0 = 13.1, C1 = 0.15, C2 = 0, and C3 = 0 for α-hydroxymidazolam. A gamma error model was used, with a final cycle value of 1.743 which indicates medium to good quality data. The inclusion of median-normalized CRP in the estimation of elimination constant (K_e) using a power equation increased the model fit to the data. For midazolam, bias and imprecision were, respectively, −0.0991 and 5.71 for the population predicted concentrations and −0.341 and 1.15 for the individual predicted concentrations. For α-hydroxymidazolam, bias and imprecision were, respectively, −0.886 and 1.29 for the population predicted concentrations and −0.181 and 0.888 for the individual predicted concentrations. Parameters and diagnostic plots are shown in **Table S1** and **Figure S1**.

Impact of comedications on midazolam pharmacokinetics

Figure 4 shows that total and unbound α-hydroxymidazolam/midazolam ratios were significantly lower when patients were treated with identified CYP3A inhibitors according to GLMM (*P* value: 0.006 and 0.003, respectively).

Other comedications do not seem to have significant impact on midazolam pharmacokinetics data.

DISCUSSION

The aim of this study was to investigate the impact of inflammatory conditions induced by COVID-19 on midazolam pharmacokinetics in critical care patients.

SARS-CoV-2 induces not only acute respiratory distress syndrome but also a hyperinflammatory syndrome.[6] Clinical studies have shown an important cytokine storm in critical patients with COVID-19.[15] Indeed, patients with severe SARS-CoV-2 infection present high levels of pro-inflammatory cytokines and chemokines compared with other patients.[16–18]

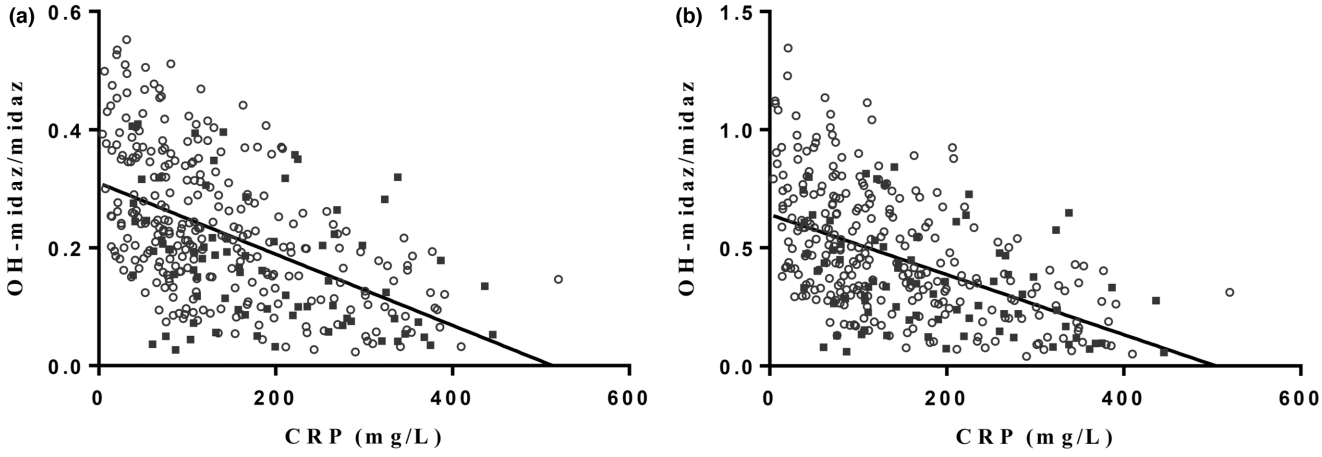

Figure 1 Relation between CRP level on midazolam metabolism. (**a**) Relation between CRP plasma concentrations and total α-hydroxymidazolam/midazolam (OH-midaz/midaz) plasma concentration ratios. Rings represent concentration ratios for patients without CYP3A inhibitors, full squares represent concentration ratio for patients treated with CYP3A inhibitors. The straight line represents the tendency of all samples ($y = -0.0006024*X + 0.3093$, $R^2 = 0.2675$). (**b**) Relation between CRP plasma concentrations and unbound α-hydroxymidazolam/midazolam (OH-midaz/midaz) plasma concentration ratios. Rings represent concentration ratios for patients without CYP3A inhibitors, full squares represent concentration ratios for patients treated with CYP3A inhibitors. The straight line represents the tendency of all samples ($y = -0.001271*X + 0.6410$, $R^2 = 0.2575$).

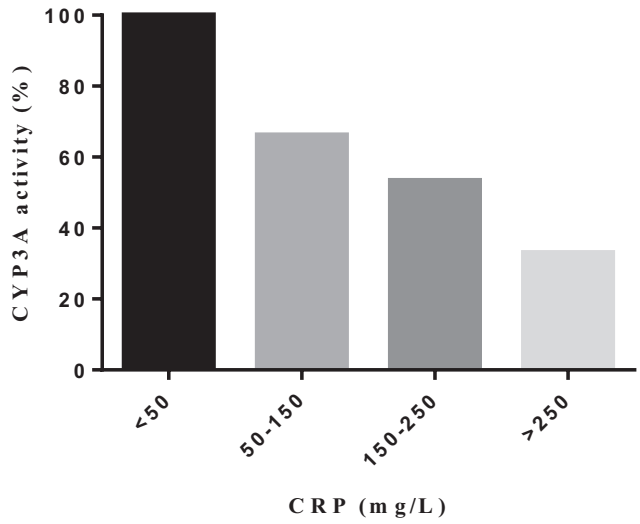

Figure 2 CYP3A activity at different level of inflammation. CYP3A activity was calculated based on total α-hydroxymidazolam/midazolam plasma concentration ratios assuming that median ratio when CRP < 50 mg/L correspond to 100% activity. Activities were represented by median.

Previous studies showed that inflammation is a major factor of pharmacokinetic variability.[19] The impact of pro-inflammatory cytokines on the activity of enzymes and transporters involved in drug resorption and metabolism has been recently described.[19] It can be partly explained by a transcriptional inhibitory effect of inflammation. An increase in plasma CRP concentrations may precede an increase in CYP substrate plasma/blood concentration. The increase of CRP should alert to the increased risk of overdose. For example, increased CRP levels were associated with increased voriconazole residual concentrations in immunocompromised patients.[20–24] Inhibition of CYP3A4 and CYP2C19 activities was evoked to explain this phenomenon.[25] Many drugs were repositioned in COVID-19 and pharmacokinetic changes have been observed for several of them. For example, patients with COVID-19 treated with lopinavir had concentrations 3–5 times higher than patients with HIV usually treated with this drug.[9,26] Similar observations have been made with clozapine.[27] A downregulation of CYP450 isoenzymes by COVID-19 are also evoked in these studies.

In our study, we demonstrated that α-hydroxymidazolam/midazolam ratio is reduced in severe inflammation situations. The population pharmacokinetics analysis also showed a significant impact of inflammation on the elimination of midazolam, described by an inverse relationship between CRP and K_e. These results clearly show that high levels of plasma CRP are associated with slower metabolization of midazolam by CYP3A. This corroborates previous studies and adds proof that the metabolic capacities of CYP are altered by the high levels of inflammation.

However, we observe a very high interindividual variability that cannot be explained by inflammation alone. It is likely that in our ICU patient population, other factors, such as age, overweight, renal function, or genetic polymorphism, may have an important effect on the metabolism of this molecule. Moreover, many of the patients included were overweight (median body mass index of 29 kg/m^2) and obesity is also associated with inflammation, which could interfere with the metabolism of midazolam. We found no evidence of a time lag between CRP elevation and altered midazolam metabolism, probably because the patients were all already in the ICU with inflammatory levels at the time of hospitalization.

In this work, we also showed that there are no significant modifications on plasma protein binding of midazolam in these conditions. The unbound fraction of midazolam was consistent with previous published studies (about 95–98%).[28] Even if the level of plasma albumin was reduced because of inflammation, the level of free fraction of midazolam was unaffected. We could suppose that midazolam bound to another plasma protein as it has been reported with other drugs that could bind to orosomucoid, an inflammatory protein.[29]

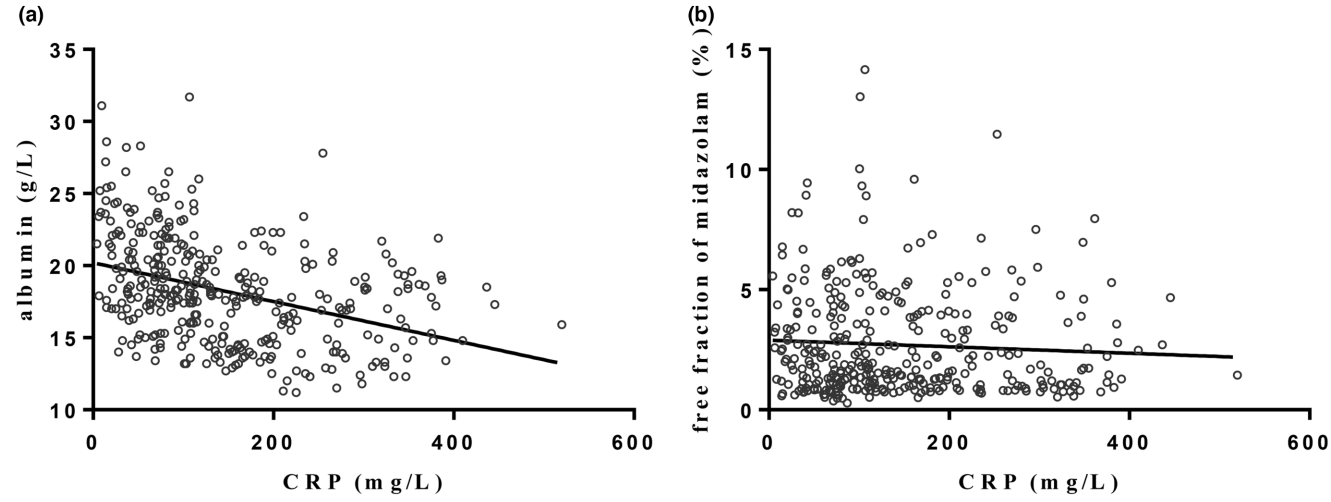

Figure 3 Relation between albumin or unbound midazolam fraction and CRP plasma concentration. (**a**) Relation between CRP and albumin plasma concentrations. The straight line represents the tendency of all samples ($y = -0.01126*X+20.22$; $R^2 = 0.0987$). (**b**) Relation between CRP plasma concentrations and the unbound fraction of midazolam. The straight line represents the tendency of all samples ($y = -0.001355*X+2.895$; $R^2 = 0.004$).

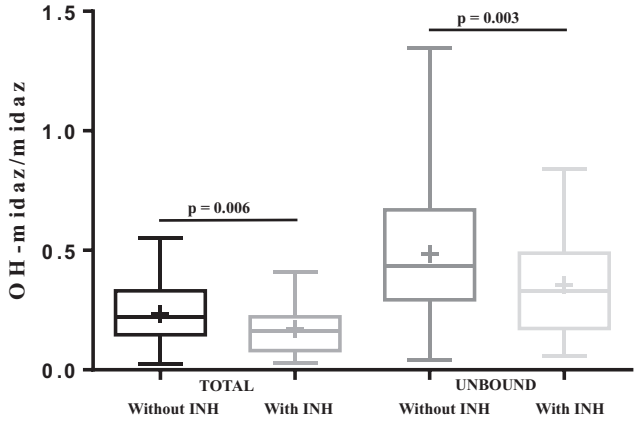

Figure 4 Comparison of total and unbound plasma α-hydroxymidazolam/midazolam (OH-midaz/midaz) plasma concentration ratios in patients with or without CYP3A inhibitors (INH). Boxes show 25th and 75th percentiles, median value, minimum and maximum (whiskers), and mean (plus sign).

These results are clinically important and may explain potential delayed awakening sometimes observed in patients with COVID-19 suggesting a slowed metabolism of anesthetic drugs in these patients. In addition, we also showed the impact of CYP3A inhibiting molecules (erythromycin, azole antifungal, and amiodarone) even population analysis did not retain this as covariate, probably due to the difference in potency and in the onset delay of CYP3A inhibition of these drugs.

This work had some limitations. First of all, CRP was the only inflammatory marker measured. It would have been interesting to measure other inflammatory markers or cytokines, such as orosomucoid, IL-6, or TNFα to determine whether a similar correlation existed and whether certain parameters were more predictive of CYP3A inhibition. Second, all patients in this study had low levels of albumin,[30] probably due to COVID-19 inflammatory conditions.[31] Their liver function was not evaluated, therefore, we could not confirm that this parameter has no influence on CYP3A activity. Third, it would have been interesting to study the hepatic drug-metabolizing activity for midazolam before and after anti-IL-6 drugs, but, unfortunately, only one patient was treated with tocilizumab before the first midazolam sample collected. Finally, we could not truly study the effectiveness of the SARS-CoV-2 repositioned drug trials as lopinavir/ritonavir and their impact on midazolam pharmacokinetic because they were not administrated at the same time as we collected samples.

This work showed the impact of inflammation on the midazolam pharmacokinetics in ICU patients with COVID-19. These results suggest that great care should be taken with narrow therapeutic margin drugs (e.g., colchicine) using the same metabolic pathway as midazolam and that particular attention to CRP level should be paid during hospitalization for better medical care and drug monitoring.

SUPPORTING INFORMATION

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

ACKNOWLEDGMENTS

The authors warmly thank the intensive care and laboratory teams as well as the patients who contributed to this work. We all hope for a better future. We thank Thierry Bompoil for his precious help.

FUNDING

No funding was received for this work.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

E.C.L.C., E.C., R.B., M.M., G.D., A.G., E.D., D.M., and M.G. wrote the manuscript. E.C.L.C. and M.G. designed the research. E.C.L.C, R.B., and M.G. performed the research. E.C.L.C., R.B., A.G., and M.G. analyzed the data.

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