BRIEF REPORT

Neglecting Plasma Protein Binding in COVID-19 Patients Leads to a Wrong Interpretation of Lopinavir Overexposure

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Boffito *et al.* recalled the critical importance to correctly interpret protein binding. Changes of lopinavir pharmacokinetics in coronavirus disease 2019 (COVID-19) are a perfect illustration. Indeed, several studies described that total lopinavir plasma concentrations were considerably higher in patients with severe COVID-19 than those reported in patients with HIV. These findings have led to a reduction of the dose of lopinavir in some patients, hypothesizing an inhibitory effect of inflammation on lopinavir metabolism. Unfortunately, changes in plasma protein binding were never investigated. We performed a retrospective cohort study. Data were collected from the medical records of patients hospitalized for COVID-19 treated with lopinavir/ritonavir in intensive care units or infectious disease departments of Toulouse University Hospital (France). Total and unbound concentrations of lopinavir, C reactive protein, albumin, and alpha-1-acid glycoprotein (AAG) levels were measured during routine care on the same samples. In patients with COVID-19, increased total lopinavir concentration is the result of an increased AAG-bound lopinavir concentration, whereas the unbound concentration remains constant, and insufficient to reduce the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) viral load. Although international guidelines have recently recommended against using lopinavir/ritonavir to treat severe COVID-19, the description of lopinavir pharmacokinetics changes in COVID-19 is a textbook case of the high risk of misinterpretation of a total drug exposure when changes in protein binding are not taken into consideration.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Consideration of protein binding is of critical importance to correctly interpret drug exposure, notably for drugs presenting both high plasma protein binding and low hepatic extraction ratio.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ In patients with coronavirus disease 2019 (COVID-19), the dose adjustment of lopinavir according to its total plasma concentration is a textbook case of misinterpretation of lopinavir exposure when changes in protein binding are not taken into consideration.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ In patients with COVID-19, increased total concentration of lopinavir is the result of an increased AAG-bound concentration whereas the unbound concentration of lopinavir remains constant, and insufficient to reduce the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) viral load.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ In a context of acute inflammation, as in patients with COVID-19, the unbound plasma concentration of a highly protein-bound drug, and not the total concentration, is the accurate marker of drug exposure.

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Brief Report on "Towards consensus on correct interpretation of protein binding in plasma and other biological matrices for COVID-19 therapeutic development".

Boffito *et al.*[1] recalled the critical importance to correctly interpret protein binding. Changes of lopinavir pharmacokinetics in coronavirus disease 2019 (COVID-19) are a perfect illustration. Indeed, several studies[2–4] described that total lopinavir plasma concentrations were considerably higher in patients with severe COVID-19 than those reported in patients with HIV. These findings have led to a reduction of the dose of lopinavir in some patients,[5] hypothesizing an inhibitory effect of inflammation on lopinavir metabolism.[3,5] Unfortunately, changes in plasma protein binding were never investigated.

METHODS

We performed a retrospective cohort study that was approved by the Toulouse University Hospital review board (registration number: RnIPH 2020-29; CNIL number: 2206723 v 0). Data were collected from the medical records of patients hospitalized for COVID-19 treated with lopinavir/ritonavir in intensive care units or infectious disease departments of Toulouse University Hospital. T-lopinavir (total concentration), U-lopinavir (unbound concentration), C reactive protein (CRP), albumin (ALB) and alpha-1-acid glycoprotein (AAG) levels were measured during routine care on the same sample.

Both total and unbound lopinavir concentrations were measured on samples handled 8 to 14 hours post-last dose and at least 3 days after treatment initiation. Ultrafiltration was used to separate bound and unbound forms of lopinavir, as previously applied by Illamola *et al.*[6] and more recently by Gregoire *et al.* for patients with COVID-19 plasma samples.[2] Because many factors (e.g., no pretreatment of ultrafiltration device, filtration rate, temperature, and duration of the centrifugation) can lead to aberrant results, as previously demonstrated for other highly bound drugs,[7] ultrafiltration was compared with equilibrium dialysis (i.e., gold standard) using quality controls (i.e., 20 plasma samples containing lopinavir at 5 mg/L (total concentration) and a CRP level ranging from 6 to 253 mg/L). Both U-lopinavir and T-lopinavir were quantified by a validated liquid chromatography-tandem mass spectrometry method (ISO 15189). No significant difference was observed between equilibrium dialysis and ultrafiltration (data not shown).

As the protein binding of lopinavir is characterized by a saturable binding to AAG and a nonsaturable binding to ALB at physiologic protein concentrations,[8] whereas AAG and ALB concentrations were, respectively, much higher and lower than their physiological ranges in our patients with COVID-19, the following model was used to analyze the variations in T-lopinavir as a function of the U-lopinavir:

$$Y_i = x_i + \frac{B_{max_i} x_i}{Kd + x_i} + \varepsilon_i$$

where Y_i is the T-lopinavir measured on the ith patient, x_i is the corresponding unbound concentration, B_{max_i} the maximum binding capacity (proportional to the binding protein concentration), Kd was assumed to be the same for all individuals to ensure identifiability, and ε_i is the residual term normally distributed $N(0, \sigma^2)$.

Several models were used to describe the B_{max} interpatient variability:
Model 1: $B_{max_i} = a$
Model 2: $B_{max_i} = a + b \times CRP_i$
Model 3: $B_{max_i} = a + c \times AAG_i$
Model 4: $B_{max_i} = a + b \times CRP_i + c \times AAG_i$

with CRP_i and AAG_i being, respectively, the CRP and AAG concentrations measured in the ith patient. Standard goodness of fit plots were performed for the model validation. An effect was considered to be significant when both the Wald test of nullity of the corresponding parameter and the change in log-likelihood were significant (*P*value < 0.05). All analyses were performed in R.

RESULTS

Thirty-six couples of T-lopinavir and U-lopinavir from 19 patients with laboratory-confirmed severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection were measured. Median (25th–75th percentiles) age was 64.0 (60.0–69.5) years, and 77% were men. Median weight was 86.0 (60.0–105.4) kg. The median daily dose was 400 (400–800) mg/day, and the formulation was tablet form for 68% of the samples. Median CRP, ALB, and AAG concentrations were 193.0 (71.3–305.1) mg/L, 21.8 (17–25) g/L, and 2.01 (1.77–2.41) g/L, respectively. Median T-lopinavir and ritonavir plasma concentrations were 14.2 (7.9–20.9) mg/L and 0.36 (0.2–0.6) mg/L, respectively. The median U-lopinavir was 0.14 (0.07–0.25) mg/L.

Figure 1 shows how total T-lopinavir changes with U-lopinavir. The third model involving only AAG concentration was the final model and showed that T-lopinavir increased as a function of AAG. The parameter estimates are indicated in **Figure 1** (top left). The final model can be rewritten as:

$$Y_i = x_i + \frac{AAG_i x_i}{Kd + x_i} + \varepsilon_i$$

with *Kd* 0.090 mg/L.

Because inflammation is known to inhibit the expression of many enzymes and transporters[9] and because ritonavir is systematically combined with lopinavir to induce a competitive inhibition of CYP3A4 and P-glycoprotein (i.e., ritonavir is a pharmacokinetic booster),[10] the impact of ritonavir, CRP, and AAG concentrations on U-lopinavir was investigated. U-lopinavir were not related to CRP and AAG levels, but significantly increased with ritonavir concentration ($r = 0.59$ $P < 8. \, 10^{-5}$), suggesting that ritonavir might have blunted the inhibitory effect of inflammation on lopinavir clearance.

DISCUSSION

In patients with COVID-19, increased T-lopinavir is the result of an increased AAG-bound lopinavir plasma concentration whereas U-lopinavir remains constant, and insufficient to reduce the SARS-CoV-2 viral load.[11] Such a link between the inflammatory state (increased AAG) and changes in T-lopinavir was previously described in pregnant women.[12,13] Yet, studies published so far describing the pharmacokinetics of lopinavir in patients with COVID-19 have overlooked the importance of changes in plasma protein binding, which could lead to a wrong interpretation of lopinavir overexposure.

Link between total and unbound lopinavir concentration according to AAG level
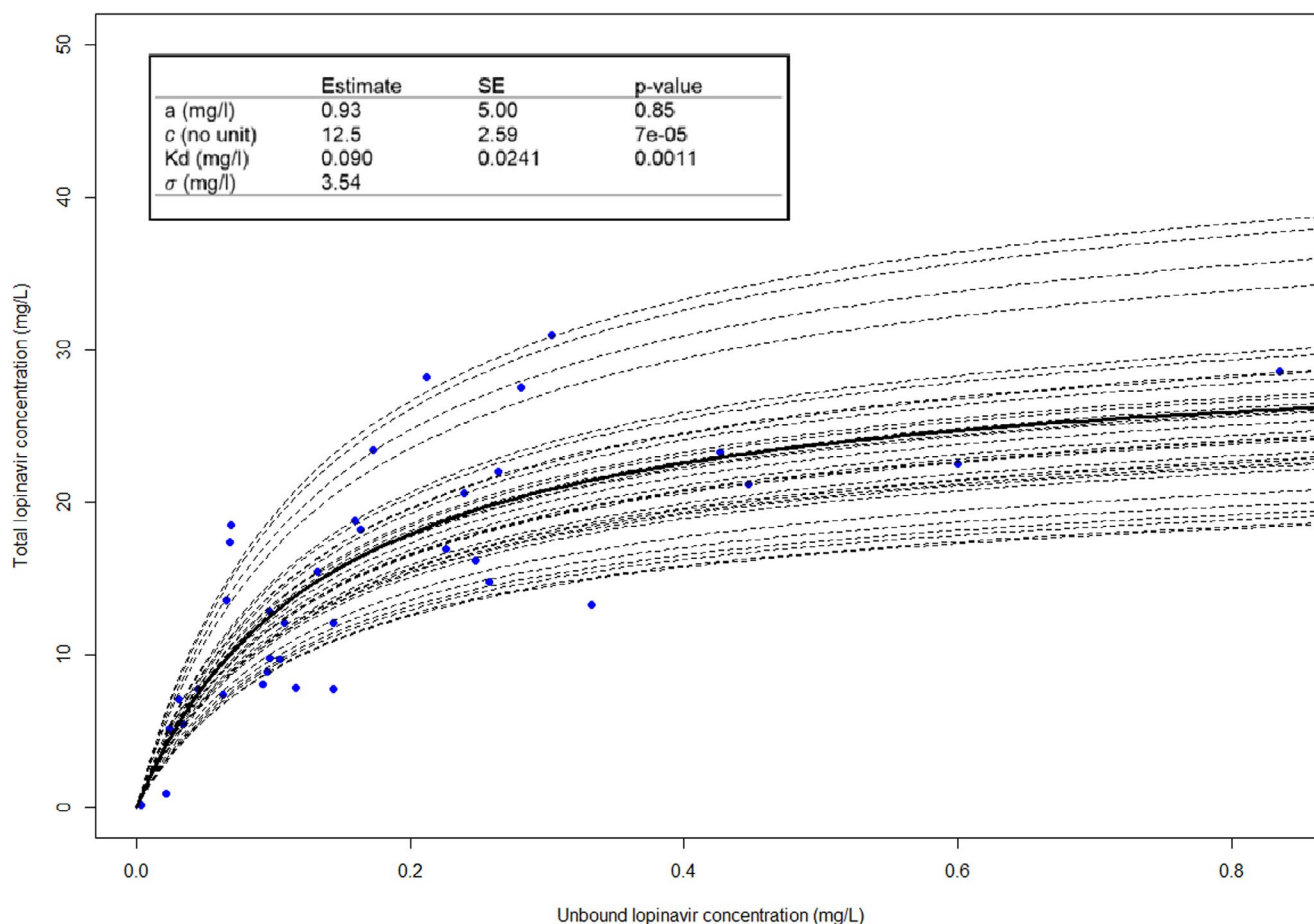
Figure 1 Link between total and unbound lopinavir concentration according to α1-acid glycoprotein (AAG) concentrations. This figure shows how the total lopinavir plasma concentration (*y*-axis) changes with the unbound lopinavir plasma concentration (*x*-axis). The plain curve describes the variations in average total concentration as a function of the unbound concentration. The dashed curves are the total concentrations given by model 3 for the observed values of AAG concentrations. The departures from the average curve (solid black line) are mainly explained by patient having different AAG concentrations (maximum binding capacity (B_{max})). Parameter estimates for model 3 are indicated at the top left of the figure.

Although international guidelines have recently recommended against using lopinavir/ritonavir (https://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/) to treat severe COVID-19, the description of lopinavir pharmacokinetics changes in COVID-19 is a textbook case of the high risk of misinterpretation of a drug total exposure when changes in protein binding are not taken into consideration. This paper highlights the need to evaluate unbound and bound plasma concentrations for future evaluation of the pharmacokinetics of new drugs with high binding affinity to plasma proteins and low extraction hepatic ratio tested in patients with COVID-19 as recently recalled by Boffito *et al*.[1]

CONFLICTS OF INTEREST
The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
F.S.L., P.G., and D.C. wrote the manuscript. F.S.L., P.G., D.C., and E.G. designed the research. E.M., F.B., B.M., S.R., G.M.B., O.E., and C.Sc. performed the research. D.C., Z.D., S.B., C.So., and EG. analyzed the data.

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