Plasma Concentrations and Safety of Lopinavir/Ritonavir in COVID-19 Patients

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Background: Although the efficacy of lopinavir/ritonavir has not been proven, it has been proposed as an off-label treatment for COVID-19. Previously, it has been reported that the plasma concentrations of lopinavir significantly increase in inflammatory settings. As COVID-19 may be associated with major inflammation, assessing the plasma concentrations and safety of lopinavir in COVID-19 patients is essential.

Methods: Real-world COVID-19 data based on a retrospective study.

Results: Among the 31 COVID-19 patients treated with lopinavir/ritonavir between March 18, 2020 and April 1, 2020, higher lopinavir plasma concentrations were observed, which increased by 4.6-fold (interquartile range: 3.6–6.2), compared with the average plasma concentrations in HIV. Lopinavir concentrations in all except one patient were above the upper limit of the concentration range of HIV treatment. Approximately one to 5 patients prematurely stopped treatment mainly because of an ADR related to hepatic or gastrointestinal disorders.

Conclusions: Lopinavir plasma concentrations in patients with moderate-to-severe COVID-19 were higher than expected, and they were associated with the occurrence of hepatic or gastrointestinal adverse drug reactions. However, a high plasma concentration may be required for in vivo antiviral activity against SARS-CoV-2, as suggested by previous studies. Therefore, in the absence of adverse drug reaction, lopinavir dosage should not be reduced. Caution is essential because off-label use can be associated with a new drug safety profile.

Key Words: COVID-19, SARS-CoV-2, plasma concentration, lopinavir, therapeutic drug monitoring, drug safety

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INTRODUCTION

Since early December 2019, a pandemic infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been spreading globally. To date, no therapeutic agent has demonstrated significant clinical efficacy against this novel coronavirus. However, because of the urgent need for treatment, several antiviral drugs, including lopinavir/ritonavir (LPV/r), are being used off-label.[1] Since many years, LPV has been approved as a protease inhibitor in HIV infection. LPV is prescribed in HIV infection along with another protease inhibitor, ritonavir (RTV), which is a potent P450 cytochrome CYP3A4 inhibitor that significantly increases LPV plasma exposure.[2] LPV/r has been proposed in previous SARS-CoV-1 and Middle East respiratory syndrome coronavirus outbreaks in 2003 and 2012, respectively.[3,4] LPV/r inhibits the replication of SARS-CoV-2 in vitro.[5] Therefore, the drug is considered to be potentially useful in patients with SARS-CoV-2 infection. To date, LPV has been assessed only in one randomized clinical trial for COVID-19, showing no benefit beyond standard care in critical patients.[6] No therapeutic drug monitoring (TDM) has been performed for LPV/r to assess an ideal drug exposure.

This retrospective cohort study was conducted to assess the plasma concentrations and safety of LPV/r in COVID-19 patients.

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MATERIALS AND METHODS

Data were collected from a COVID-19 care center (Cochin Hospital, Paris). According to our study protocol, patients were eligible to receive a 7-to-10-day LPV/r treatment as a specific anti-COVID-19 therapy if: (1) pneumonitis evocating a SARS-CoV-2 infection was observed by CT scan and (2) they required oxygen (minimal flow rate 2–3 L per minute). Patients with acute respiratory distress syndrome at the time of admission and those requiring intubation were excluded from the study. COVID-19 was considered as confirmed if the nasopharyngeal polymerase chain reaction (PCR) test result was positive, or as suspected if the PCR test result was negative but in the presence of typical CT abnormalities. LPV/r tablets were swallowed whole, and the administration time was reported by nurses. TDM was performed in a routine care setting within the first 3 days of therapy. Plasma concentrations were considered as peak at 4 ± 2 hours after drug intake or trough at 12 ± 3 hours after drug intake. The plasma concentrations of LPV and RTV were quantified using high-performance liquid chromatography-tandem mass spectrometry (Xevo TQD; Waters, Saint-Quentin, France) using a BEH C18 analytical column (1.7, 1.7 μm, 50 × 2.1 mm; Waters, Saint-Quentin, France) and a mobile phase composed of 60% water and 40% methanol (Sigma-Aldrich, St. Louis, MO) with 0.05% (vol/vol) formic acid (Sigma-Aldrich, St. Louis, MO). The safety of LPV/r treatment was assessed in a routine care setting. Spontaneous reports of suspected adverse drug reactions (ADRs) were notified to the regional center of pharmacovigilance. After a case-by-case assessment by a senior pharmacologist, cases were reported to the French pharmacovigilance system. The study was performed in accordance with the Declaration of Helsinki guidelines, and patients were informed that their data could be used for research. This study was approved by the Cochin Hospital Institutional Review Board (number 2020–08019).

RESULTS

We included 31 consecutive COVID-19 patients who received LPV/r treatment between March 18, 2020 and April 1, 2020. The median age of patients was 63 years [interquartile range (IQR): 51–78 years], and 71% of them were men (Table 1). Pulmonary injury, revealed by CT scan, was mostly moderate-to-extensive. All patients had a positive SARS-CoV-2 PCR for nasopharyngeal swabs except 4 (12%), who had a doubtful or negative result. Median C-reactive protein (CRP) and interleukin (IL)-6 levels at admission were 94.1 mg/L (45.4–176.0 mg/L) and 60.4 ng/mL (29.7–164.5 ng/mL), respectively. LPV/r 400/100 mg tablets were administered twice a day starting from the first 48 hours of hospitalization. Median time for LPV/r administration after the onset of symptoms was 8 days (IQR: 7–10 days). None of the patients had any drug–drug interaction involving RTV, and 13 (42%) patients did not receive concomitant therapy. LPV/r treatment duration was 7 days (IQR: 3–8 days). At the end of LPV/r treatment, 17 patients were still hospitalized with oxygen dependency, 8 patients were transferred to the intensive care unit or died, 5 patients recovered, and clinical outcome of one patient, who had been transferred to another hospital during treatment, was unknown.

A total of 24 patients were assessed for TDM. Plasma assays of 3 patients were unavailable because of technical issues, and 4 patients were excluded from the analysis because of sample collection outside peak or trough timings. Median time intervals for collecting peak and trough blood samples after tablet administration were 4.6 hours (IQR: 3.1–5.5 hours) and 14.0 hours (IQR: 14.0–14.6 hours), respectively. LPV plasma concentrations ranged from 8317 to 35,012 ng/mL. Median levels for peak (Cmax) and trough (Cmin) were 26,475 ng/mL (IQR: 11,952-33,868 ng/mL) and 21,857 ng/mL (IQR: 16,991-26,435 ng/mL), respectively (Fig. 1A). LPV plasma concentrations for all samples except one were far above the upper limit of the concentration ranges observed in HIV patients. The extent of increase in LPV plasma concentrations was between 2- and 8-fold in most treated patients (Fig. 1B). The magnitude of increase was not associated with CRP or IL-6 levels (Figs. 1C, D). At the LPV assay, the alanine aminotransferase (ALT) levels were below 2 times than the upper limit of normal (ULN) in 20 patients (83%), and they were between 2 and 8 times ULN in 4 patients (17%).

LPV/r was discontinued before the end of the scheduled course for 14 patients (45%) (Table 1) because of occurrence of ADRs (n = 7, 22%), therapeutic limitations or death of patients (n = 4, 13%), and poor efficacy (n = 3, 10%). Suspected ADRs were assessed as probably related to LPV/r therapy. They consisted of 4 cases of liver injuries (3 cases of moderate cytolytic hepatitis with ALT level 3 to 6 times ULN and one case of isolated hyperbilirubinemia), 2 cases of gastrointestinal disorders (nausea/vomiting and diarrhea), and one case of psychiatric disorders (agitation/anxiety). The ADRs were mild, and all patients recovered after drug withdrawal.

DISCUSSION

This study showed that the LPV plasma concentrations in COVID-19 patients were much higher than expected. The median plasma concentrations were approximately 4.6- to 8-fold higher (IQR: 3.6–6.2) than the therapeutic levels observed in HIV patients. They were in all samples except one significantly higher than the upper limit of the concentration ranges observed in HIV patients.

In severe COVID-19, major inflammation is associated with elevated blood IL-6 and NF-κB levels.[7] Besides, it is long-standing known that inflammatory responses and infections impair drug metabolism.[8] In animals and humans, inflammation and IL-6 downregulate CYP450 through NF-κB activation, resulting in reduced drug metabolism.[9,10] Hence, the metabolism of voriconazole or tacrolimus, which is highly dependent on CYP3A4, is altered in inflammatory settings. However, LPV is already associated with a highly efficient cytochrome inhibitor, RTV. The further blockage of metabolism and increase in LPV concentrations through this mechanism is unknown. A prospective pharmacokinetic study revealed that the total LPV concentrations in HIV patients

TABLE 1. Demographic and Clinical Characteristics of Patients

Patient Characteristics	
Age, yr	63 (51–78)
Sex, male:female	22:9
Comorbidities, n (%)	
Hypertension	11 (35)
Diabetes	8 (26)
Cardiovascular diseases (others)	7 (23)
Malignancy or immunosuppression	6 (19)
Chronic respiratory disease (including asthma)	3 (10)
Hepatitis or liver cirrhosis (Child-Pugh B or more)	2 (6)
Rheumatic disease	2 (6)
Chronic kidney failure	1 (3)
None	6 (19)
SARS-CoV-2 PCR, n (%)	
Positive	25 (80)
Doubtful*	2 (6)
Negative*	2 (6)
N/A	2 (6)
Extent of pneumonia on CT scan at admission, n (%)	
Minor (less than 10%)	1 (3)
Moderate (between 10% and 25%)	9 (29)
Extensive (between 25% and 50%)	12 (39)
Severe (more than 50%)	7 (23)
N/A	2 (6)
Oxygen saturation at admission (without oxygen), n (%)	92.5 (90–96)
C-reactive protein at admission, mg/L	94.1 (45.4–176.0)
Interleukin (IL)-6 at admission, ng/mL	60.4 (29.7–164.5)
Time interval between onset of symptoms and start of lopinavir/ritonavir treatment, d	8 (7–10)
Type of COVID-19-related drug or antibiotics associated with lopinavir/ritonavir,† n (%)	
None	13 (42)
Cephalosporin or penicillin	6 (19)
Cephalosporin and macrolide	3 (10)
Macrolide	3 (10)
Corticosteroids	2 (6)
Corticosteroids and antibiotics	2 (6)
Sarilumab (anti-IL-6)	2 (6)
Duration of lopinavir/ritonavir therapy, d	7 (3–8)
Serum ALT levels at the time of lopinavir assay,‡ n (%)	
<ULN	13 (54)
1–2 ULN	7 (29)
2–4 ULN	3 (13)
4–8 ULN	1 (4)
Reasons for lopinavir/ritonavir therapy termination, n (%)	
Scheduled end of treatment	17 (52)
Adverse drug reaction	7 (22)
Therapeutic limitation or death	4 (13)
Poor efficacy	3 (10)
Types of adverse drug reaction accountable to lopinavir/ritonavir	
Cytolytic hepatitis	3
Isolated hyperbilirubinemia	1
Nausea and vomiting	1
Diarrhea	1
Agitation/anxiety	1

Data are presented as median (IQR) or in numbers (%).

*PCR resulting in doubtful or negative results was repeated twice for each patient.

†Penicillin: piperacillin/tazobactam, cephalosporin: cefotaxime, macrolide: azithromycin or rovamycin

‡Serum ALT levels at the time of lopinavir assay ±1 d expressed in fold-changes above the ULN range. Data are presented for the 24 patients included in lopinavir therapeutic drug monitoring.

vary with inflammation, and they are correlated with circulating α1-acid glycoprotein levels, a type 1 acute phase protein.[11] Interestingly, unbound LPV concentration was minimally altered during inflammation, suggesting a change in drug distribution rather than metabolism. In our analysis, we did not find a correlation between LPV and CRP levels, measured at the time of admission. Hence, it is possible that major inflammation in COVID-19 patients led to the significant increase observed in LPV plasma concentrations. However, the exact underlying mechanism for this change is unknown.

The main ADRs observed in our study and attributed to LPV/r therapy were moderate hepatobiliary disorders. Moderate-to-severe elevations in serum aminotransferase levels (>5 times ULN) were observed in 3%–10% of HIV patients treated by LPV/r.[12] These elevations are usually asymptomatic, and can resolve with drug continuation. For liver injuries, drug causality is challenging to assess in the case of COVID-19 as up to 20% of the patients show increased transaminases.[13] However, in our study, transaminases quickly decreased after discontinuing LPV administration, suggesting a probable causality of this drug. Furthermore, hepatobiliary disorders were mainly cytolytic with a moderate increase in serum transaminases. Hepatic failure or dysfunction was not observed in any patient, and it is unlikely that the transient abnormal liver tests were associated with a decrease in hepatic metabolic ability. Overall, the ADRs reported in this study are in accordance with the LPV/r safety profile.[2] However, because of the relatively limited number of treated patients, conclusions were difficult to draw. Physicians should be aware that the off-label use of drugs can be associated with an altered drug safety profile.[14] The drug is used in a setting, where it has not been correctly assessed or experienced, which can lead to an increase in ADRs and even an unfavorable benefit–risk ratio.[15]

Antiviral activity assays on cultured Vero-E6 cells revealed that 50% effective concentration (EC_{50}) of LPV

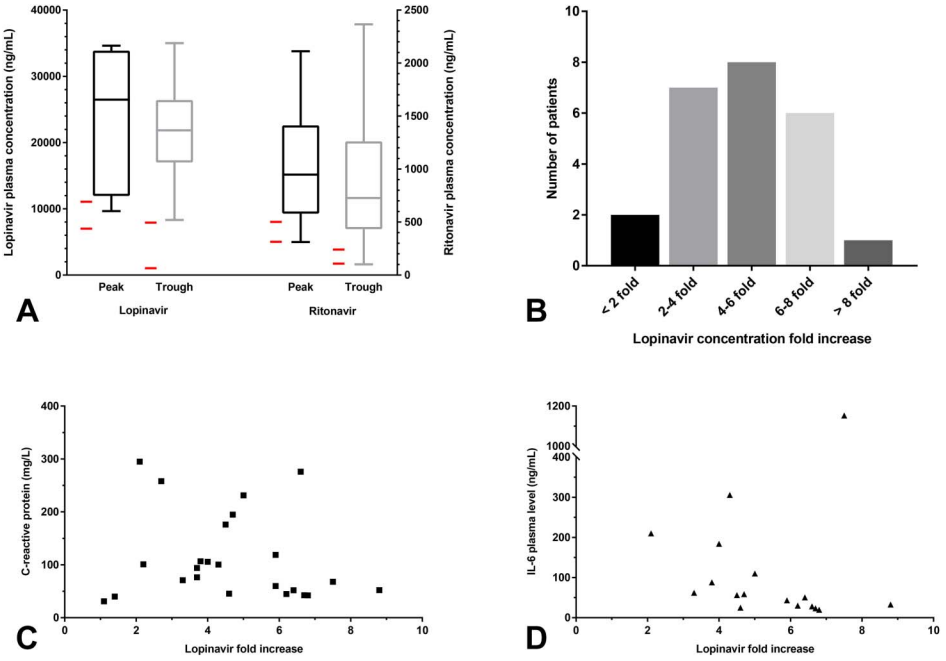

FIGURE 1. Lopinavir/ritonavir plasma concentrations and magnitude of increase in lopinavir plasma concentrations of COVID-19 patients as compared to those of HIV patients. A, Plasma concentrations analyzed at peak and trough in 6 and 18 patients with COVID-19, respectively. Boxes represent interquartile range and median, whiskers represent min and max values. Horizontal red lines represent the peak and trough concentrations observed in HIV patients after 400 mg/100 mg lopinavir/ritonavir twice daily (ie, for lopinavir at peak 7000–11,000 ng/mL and at trough 1000–8000 ng/mL; for ritonavir at peak 300–500 ng/mL and at trough 100–250 ng/mL).[18,19] B, Magnitude of increase in lopinavir plasma concentrations compared with the average plasma concentrations observed in HIV patients (ie, 9000 ng/mL at peak and 4000 ng/mL at trough). C and D, C-reactive protein ($r^2 = 0.03$) and interleukin-6 plasma levels ($r^2 = 0.02$) according to the magnitude of increase in lopinavir plasma concentrations.

on SARS-CoV and SARS-CoV-2 is 17.1 μM (ie, 10,800 ng/mL) and 26.6 μM (ie, 16,800 ng/mL), respectively.[5,16] In our study, the median trough plasma level was 20,153 ng/mL (IQR: 16,633–26,505 ng/mL), slightly the above-mentioned concentrations. Whether these plasma concentrations can effectively inhibit in vivo SARS-CoV-2 replication, especially in the lungs, is still unknown. Furthermore, COVID-19-associated vasculopathy or thrombosis can limit pulmonary diffusion. Altogether, these findings suggest that maintaining high LPV plasma concentration is essential for the possible clinical efficacy of LPV in COVID-19.

Finally, similar to most HIV protease inhibitors, LPV demonstrates high protein binding.[17] Therefore, to determine an appropriate LPV dose for SARS-CoV-2 infection, it is essential to consider free LPV concentrations. For standard HIV-1, free Cmin is 75 ng/mL, which is significantly lower than the EC_{50} reported for coronaviruses.[17] Therefore, targeting the antiviral activity in COVID-19 patients is challenging. In the absence of ADRs, LPV/r dosage should not be reduced on the basis of TDM.

Our study has several limitations. First, because of the retrospective use of routine care data, plasma assays were performed at different times, which prevented an accurate pharmacokinetic estimation. Second, because of different practices in different wards, the COVID-19 severity at baseline was heterogeneous between patients. However, in all patients, the LPV plasma concentrations, regardless of assay timing, were unexpectedly higher than those of HIV patients. Third, unbound concentrations, which are the active concentrations, were not estimated.

CONCLUSIONS

In conclusion, we found that LPV/r treatment in COVID-19 was associated with unexpectedly high plasma concentrations. However, in the absence of ADRs, LPV dosage should not be reduced because a high plasma concentration may be required for in vivo antiviral activity, as suggested by previous studies. Approximately one to 5 patients withdrew LPV/r therapy because of moderate ADRs. Caution is needed in this context of off-label drug use, which can be associated with a new drug safety profile.

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