Safety and effectiveness concerns of lopinavir/ritonavir in COVID-19 affected patients: a retrospective series

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\textbf{ABSTRACT}

\textbf{Context:} Originally developed for treatment of human immunodeficiency virus (HIV), the antiviral combination lopinavir/ritonavir (LPV/r) is being repurposed for treating the novel coronavirus disease (COVID-19) despite minimal experience in this markedly different population and an in-vitro derived EC50 against SARS-CoV-2 several hundred-fold greater than for HIV. We present a case series including a case of severe hyponatremia and a 32-fold overdose raising safety and effectiveness concerns in COVID-19 patients.

\textbf{Methods:} We measured LPV trough concentrations in 12 patients and reviewed their clinical charts for side effects known to occur in HIV patients.

\textbf{Findings:} Compared to established LPV trough concentrations in HIV patients, concentrations in COVID-19 patients were 3-fold greater (19.37 ± 10.12 mcg/mL versus 6.25 mcg/mL). In addition, cholestasis and dyslipidemia toxicity thresholds were exceeded in 12/12 and 11/12 patients respectively. No patients achieved the presumed therapeutic concentration. Side effects included gastrointestinal symptoms (5/12), electrolyte imbalances (4/12), liver enzyme disturbances (5/12) and triglyceride elevations (2/12).

\textbf{Conclusion:} No patients reached presumed therapeutic LPV concentrations despite experiencing side effects and exceeding cholestasis and dyslipidemia toxicity thresholds. This raises concerns for the safety and effectiveness of LPV/r. Clinicians should consider closely monitoring for side effects and not necessarily attribute them to COVID-19.

\section*{Context}

Lopinavir/ritonavir (LPV/r) is an HIV-protease inhibiting drug combination in which ritonavir’s main effect is to potentiate LPV via CYP3A4 inhibition. It has gained interest for treating severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the associated coronavirus disease (COVID-19). As of July 16, 2020, 59 studies of LPV/r in COVID-19 affected individuals were posted on ClinicalTrials.gov, with accrual goals exceeding 16,000 patients.

The LPV/r monograph \cite{1} reports on five trials, exclusively in the HIV population. Patients were young (mean age 38) and predominantly male (82%).

As of June 20, 2020, our hospital center had hospitalized over 400 COVID-19 patients with a median age of 74.0 (inter quartile range 62.\textendash 84.0) and 48\% male. Twenty individuals were treated with LPV/r. We report our clinical and laboratory findings in a subset of twelve individuals for whom measured trough drug concentrations were available and extrapolate on the kinetics and effectiveness of LPV/r in this population.

\section*{Methods}

In the context of a clinical trial (clinicaltrials.gov/ct2/show/NCT04330690), COVID-19 patients were treated with LPV/r 400/100 mg twice daily (as per HIV treatment regimen) for 14 days or until hospital discharge, whichever occurred first.

Unexpected development of severe hyponatremia in one individual initiated investigations in 12 temporally consecutive SARS-CoV-2 infected patients treated with LPV/r to determine LPV troughs and clinical and laboratory abnormalities possibly related to this therapy. Patients treated with LPV/r prior to the index case were not included as LPV measurements were unobtainable.

Plasma trough measurements of total LPV were performed with a validated liquid chromatography tandem mass spectrometry assay. Charts were comprehensively reviewed for all new adverse events known to occur in HIV patients \cite{1} with onset at least 24 h after treatment initiation.

\section*{Findings}

Trough concentrations (12 h post-dosing) were measured 2\textendash 7 days after treatment initiation in 11 of 12 patients (Table 1). In these 11 individuals, mean LPV trough concentrations were higher than concentrations expected in HIV patients (19.37 mcg/mL [standard deviation 10.12] vs 6.25 mcg/mL).

Patient 12 received 160 mL of LPV/r solution (equivalent to 12,800/3,200mg) in a single dose due to a dosing error on

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day 4. At the time, he was receiving enteral nutrition (stopped 4 h post overdose and resumed 12 h later to permit proning). The error was only discovered 24 h later and immediately reported to Poison Control, who recommended against any maneuvers to reduce delayed absorption.

LPV concentrations 34 h (t₁) and 114 h (t₂) post overdose were 34.5 mcg/mL (C₃) and 0.89 mcg/mL (C₆). The calculated half-life (t½) is 15.2 h using t½ = ln2/k = ln2/−ln(C₆/C₃)/(t₂ − t₁) where k is the elimination rate constant. We can then extrapolate a 12-hour trough (C₁₂) of 94.3 mcg/mL with C₁₂ = C₃/e−k(t₂−t₁) = 0.89/e−0.0457(114−12) (Figure 1).

Assuming that the overdose follows pharmacokinetics similar to therapeutic dosing of LPV/r in the HIV population with maximum plasma concentration (Cₘ₉₅) at 4 h [1], we extrapolate a Cₘ₉₅ of 136.0 mcg/mL.

6 of 12 patients had clinically relevant adverse events. Gastrointestinal symptoms were observed in 5/12 patients; electrolyte, liver, and triglyceride disturbances in 4/12, 5/12, and 2/12 patients respectively.

All 12 patients exceeded reported cholestasis toxicity thresholds (6.43 mcg/mL) [2]. Patient 12 had an acute acalculous cholecystitis confirmed by ultrasound performed for persistent fevers despite broad-coverage antibiotics for ventilator-associated pneumonia and central line associated bloodstream infection. 11/12 patients exceeded dyslipidemia toxicity thresholds (9.71 mcg/mL) [3]. Patients 10 and 12 were receiving propofol sedation. Patient 12’s hypertriglyceridemia necessitated cessation of propofol.

### Table 1. Clinical and Laboratory Findings in COVID-19 Affected Patients Treated with Lopinavir/Ritonavir.

| Patient ID | Age (years) | Sex | Duration of Therapy (days) | LPV 12h Trough Levels (mcg/mL) | Clinical Abnormalities | Laboratory Abnormalities (value; normal range) | Length of Stay (days) | Disposition |
|------------|-------------|-----|----------------------------|--------------------------------|-----------------------|-----------------------------------------------|---------------------|-------------|
| 1          | 82          | F   | 5                          | 20.99                          | Nausea                | ↑ total bilirubin (29.2; 1.7–18.9 μmol/L)       | 21                  | Home        |
| 2          | 28          | M   | 9                          | 10.07                          | None                  | ↑ ALT (99; 6–45 U/L)                             | 9                   | Home        |
| 3          | 89          | F   | 6                          | 8.50                           | None                  | None                                           | 11                  | Deceased    |
| 4          | 75          | F   | 9                          | 23.19                          | None                  | None                                           | 8                   | Home        |
| 5          | 82          | F   | 14                         | 40.64                          | Diarrhea              | None                                           | 29                  | Home        |
| 6          | 31          | F   | 5                          | 13.34                          | None                  | ↑ total bilirubin (33.5; 1.7–18.9 μmol/L)       | 15                  | Deceased    |
| 7          | 72          | M   | 6                          | 26.12                          | Diarrhea              | Hypophosphatemia (0.6; 0.8–1.45 mmol/L)          | 7                   | Home        |
| 8          | 59          | F   | 8                          | 12.67                          | None                  | None                                           | 10                  | Home        |
| 9          | 51          | M   | 5                          | 13.29                          | None                  | Hyponatremia (131; 133–143 mmol/L)               | 6                   | Home        |
| 10         | 81          | M   | 10                         | 31.39                          | None                  | ↑ ALT (57; 6–45 U/L)                             | 26                  | Deceased    |
| 11         | 84          | F   | 2                          | 12.92                          | None                  | ↑ TG (2.2; 0.1–1.7 mmol/L)                       | 3                   | Home        |
| 12         | 59          | M   | 4                          | 94.33*                         | Acalculous cholecystis| ↑ GGT (159; 7–50 U/L)                           | 131                 | Rehabilitation Centre |

Mean (SD) 66 (21) 7 (3) 19.37 (10.12)** 23 (35)

LPV: lopinavir; ALT: alanine aminotransferase; GGT: gamma glutamyl transferase; TG: triglycerides; SD: standard deviation.

**Extrapolated 12 h trough based on concentrations measured at 34 (34.5 mcg/mL) and 114 (0.89 mcg/mL) hours post overdose.

**Excluding the extrapolated 12 h trough from the overdose (Patient 12).

By day 3 of treatment, Patient 1 developed severe hypotension (122 mmol/L, baseline 134 mmol/L) with appropriate urinary retention (<15mmol/L), mild metabolic acidosis and hyperkalemia. In the clinical context of acute infection, morning cortisol was inappropriately normal at 285 nmol/L (<35 nmol/L). This was associated with weakness, dizziness, and new onset abdominal pain. Her hyponatremia continued to worsen, nadiring at 115 mmol/L by day 5 despite large doses of NaCl replacement. At this point, she received 50 mg of hydrocortisone before starting fludrocortisone 0.05–0.1 mg daily for 5 days. Electrolyte imbalances and symptoms quickly improved with cortico-therapy, consistent with a diagnosis of adrenal insufficiency. It was later discovered that her usual mometasone was changed to...
beclomethasone on day 1 to avoid interactions between CYP3A4 metabolized corticosteroids and LPV/r [4]. However, beclomethasone was initially unavailable and only started on day 4.

In addition to acute acalculous cholecystitis and hypertriglyceridemia, patient 12 experienced sustained bradycardia in the 48 h following the overdose of 50 (IQR 45–55) beats per minute (bpm) from a baseline of 65 (IQR 65–70) bpm. He was chronically on sotalol for atrial fibrillation which had to be discontinued. As sotalol is not metabolized [5], it is unaffected by ritonavir’s inhibition of CYP3A4. Renal function was normal at the time. The bradycardia may have been due to either of the drugs or a synergistic effect of both.

Ultimately, 3 patients died and 9 were discharged with an average hospitalization length of 29 days (standard deviation 35).

Discussion

Schoergenhofer [6] and Gregoire [7] have reported that COVID-19 patients treated with LPV/r develop higher LPV concentrations than HIV patients. In these reports, patients were also older than in the LPV/r monograph trials [1]. Older individuals typically receive more medications which may increase drug-drug interactions or lead to the accumulation of side effects. This difference may also be attributed to downregulation of hepatic CYP450 in the acute inflammatory phase [8] and possibly the lower metabolic and clearance rates in this acutely ill and elderly population. Notably, nearly all trough concentrations in our patients exceeded toxicity thresholds for cholestasis [2] and dyslipidemia [3]. High concentrations may also increase frequency and severity of other potential complications, including gastrointestinal symptoms, acute gastritis, and severe anemia as reported by Cao [9].

LPV is 98–99% protein bound. In the absence of human serum (i.e., 100% freely available drug), in-vitro data suggests a half-maximal effective concentration (EC50) for LPV against SARS-CoV-2 of 16.74 mcg/mL [10] compared to 0.006–0.017 mcg/mL for various HIV strains [1]. This concentration is unlikely to be obtained in-vivo without excessive risk, as an accidental overdose of 32 times the intended amount still resulted in sub-therapeutic levels. Even using the calculated Cmax, peak free LPV concentration is only 1.36–2.72 mcg/mL (1–2% of the total Cmax calculated above).

The reason(s) why plasma concentrations were not higher in the setting of a 32-fold overdose are unclear. Drug absorption may have been compromised by the effects of critical illness. For such large volume overdose, holding enteral feeds may also have contributed as food increases LVP/r solution absorption [1]. It is also possible that intestinal transporters were saturated [11] and that splanchic circulation was decreased [12]. Finally, co-administration of dexamethasone, a known CYP inducer, may have contributed to increased metabolism and clearance of LPV [1]. No other potential drug-drug interactions were identified.

Our report has limitations. Although comprehensive, our chart review is at risk of inconsistencies in extraction and clinical documentation. While our findings (n = 12) are consistent with those of Schoergenhofer [6] (n = 8) and Gregoire [7] (n = 12), the three studies are small, limiting external validity to the entire COVID-19 population. Finally, there is no control group, preventing conclusions on efficacy and limiting conclusions on safety as many of LPV/r’s side effects may be consequences of COVID-19 itself.

Conclusion

Our report supports previous findings that treating COVID-19 affected patients with standard HIV LPV/r dosages yields approximately 3-fold higher trough LPV concentrations which may result in adverse events [6,7,9]. Despite this risk, the in-vitro derived EC50 for LPV on SARS-CoV-2 was not reached in any of our patients, including the 32-fold overdose. This may in part explain why Cao et al. demonstrated no benefit from LPV/r in hospitalized patients [9]. Consistent with these findings and in light of issues raised by their interim analysis, on July 4, 2020, the World Health Organization reported the Solidarity Trial’s recommendation to immediately discontinue the LPV/r treatment arms due to futility and safety concerns [13].

Most importantly, this report will impact on patients enrolled in other trials or who are offered this treatment as part of their clinical care. Our findings should alert clinicians to pay attention for LPV/r side effects and complications. Closer clinical monitoring will enable early detection of toxicity and allow for therapeutic adjustment and possible discontinuation of LPV/r.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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