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Safety of hydroxychloroquine and darunavir or lopinavir in COVID-19 infection

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Background: Combination therapy with hydroxychloroquine and darunavir/ritonavir or lopinavir/ritonavir has been suggested as an approach to improve the outcome of patients with moderate/severe COVID-19 infection.

Objectives: To examine the safety of combination therapy with hydroxychloroquine and darunavir/ritonavir or lopinavir/ritonavir.

Methods: This was an observational cohort study of patients hospitalized for COVID-19 pneumonia treated with hydroxychloroquine and darunavir/ritonavir or lopinavir/ritonavir. Clinical evaluations, electrocardiograms and the pharmacokinetics of hydroxychloroquine, darunavir and lopinavir were examined according to clinical practice and guidelines.

Results: Twenty-one patients received hydroxychloroquine with lopinavir/ritonavir (median age 68 years; 10 males) and 25 received hydroxychloroquine with darunavir/ritonavir (median age 71 years; 15 males). During treatment, eight patients (17.4%) developed ECG abnormalities. Ten patients discontinued treatment, including seven for ECG abnormalities a median of 5 (range 2–6) days after starting treatment. All ECG abnormalities reversed 1–2 days after interrupting treatment. Four patients died within 14 days. ECG abnormalities were significantly associated with age over 70 years, coexisting conditions (such as hypertension, chronic cardiovascular disease and kidney failure) and initial potential drug interactions, but not with the hydroxychloroquine concentration.

Conclusions: Of the patients with COVID-19 who received hydroxychloroquine with lopinavir or darunavir, 17% had ECG abnormalities, mainly related to age or in those with a history of cardiovascular disease.

Introduction

Many trials have evaluated potential treatments for COVID-19. Such studies need to consider the efficacy and tolerance of the treatment in patients infected with SARS-CoV-2.

Reports have suggested that hydroxychloroquine reduces the SARS-CoV-2 viral load *in vitro*.[1,2] However, hydroxychloroquine was not associated with reductions in mortality *in vivo*.[3–5] The safety profile of hydroxychloroquine is well established for the approved indications (lupus, rheumatoid polyarthritis). However, hydroxychloroquine can cause significant electrocardiograph QT prolongation and increase the risk of torsade de pointes, even at a therapeutic dose.[6] A 2018 study of patients with lupus or rheumatoid arthritis on long-term hydroxychloroquine found conduction abnormalities in almost 16% of cases and QT prolongation in 7%–15% of cases.[7]

Lopinavir and darunavir are two PIs boosted by ritonavir and long used to treat HIV infection.

The lopinavir/ritonavir combination remains a treatment for COVID-19 in many countries, although no clinical benefit was observed in a small study.[8,9] It has been hypothesized that lopinavir/ritonavir could improve the prognosis of COVID-19 when treatment is given early. Darunavir may be an alternative to lopinavir, as molecular modelling studies show that it is effective in SARS-CoV-2.[10] Lopinavir can cause QT prolongation, while darunavir has a safe cardiac profile.[6] Lopinavir and darunavir plus ritonavir

combinations are CYP450 3A4 inhibitors, which can significantly increase serum hydroxychloroquine levels.[11]

Combination therapy has been suggested as an approach to improve the outcome of patients with moderate/severe COVID-19 infection.[12]

Therefore, this work examined the safety, particularly the cardiologic toxicity on the QT interval, of the therapeutic combinations hydroxychloroquine/lopinavir/ritonavir and hydroxychloroquine/darunavir/ritonavir in COVID-19 infection.

Methods

According to the guidelines of Saint-André Hospital, CHU de Bordeaux, France, patients with confirmed COVID-19 infection (at least one positive COVID-19 nasopharyngeal PCR test result or typical CT findings) hospitalized with the following conditions may benefit from a specific therapy: patients with pneumonia and a need for oxygen support; or patients with at least one of the following risk factors for developing severe COVID-19 according to the French Haut Conseil de Santé Publique rules: age >70 years, BMI >25 kg/m^2, diabetes, chronic respiratory disease, any cardiovascular history, chronic kidney failure, Child cirrhosis ≥B, HIV infection with CD4 <200 cells/mm^3 and immunosuppressive treatment.

Patients were given hydroxychloroquine 200 mg, two tablets twice a day on day 1, then two tablets in the morning and one tablet in the evening if weight ≥60 kg from day 2 to 7; one tablet in the morning and in the evening if weight <60 kg; together with lopinavir/ritonavir 200 mg/50 mg (two capsules in the morning and evening) or darunavir/ritonavir (darunavir 800 mg one capsule per day and ritonavir 100 mg one capsule per day) from day 1 to 7 if lopinavir/ritonavir was not available.

A standard ECG was obtained before starting treatment and then once a day from day 1 to 7. The ECG was reviewed by cardiologists. Conduction disorders were defined as PR prolongation >200 ms, QRS duration >120 ms or second/third degree atrioventricular block. Repolarization disorders were defined as QTc prolongation >500 ms or >60 ms compared with baseline and negative or double hump T waves. In patients with a QRS duration >120 ms or paced rhythm, the adjusted QTc = QTc − QRS duration + 90 ms.[13] Clinical evaluations and laboratory testing were performed according to the standard of care between the start of treatment and the patient's discharge. The residual plasma concentrations of hydroxychloroquine, lopinavir and darunavir were determined on days 3 and 7 after starting the treatment, as recommended by the French National Agency against HIV/AIDS in its guidelines published on 6 March 2020.

Plasma lopinavir, ritonavir, darunavir and hydroxychloroquine concentrations were measured by validated LC–MS/MS methods (Waters® Alliance 2690/Quattro Micro). The Laboratory of Pharmacology conducted external quality-control surveys or inter-laboratory comparisons of these molecules.

Data were extracted from the electronic medical records and de-identified. Concomitant treatments were reviewed by a pharmacist specializing in infection. Potential drug interactions were defined as drugs that can affect hydroxychloroquine and/or PI/ritonavir concentrations and/or drugs that can cause ECG changes. Drugs potentially interacting with hydroxychloroquine and/or PI/ritonavir were discontinued or their doses modified during treatment.

Data are reported as the median and IQR. Differences between groups were compared using the χ^2 test and Student's *t*-test as appropriate.

Ethics

Patients were informed about the off-label use of hydroxychloroquine and lopinavir/ritonavir or darunavir/ritonavir. According to French law and the French Data Protection Authority, the handling of these data for retrospective research purposes was declared to the Data Protection Officer of the University Hospital of Bordeaux who acknowledge the use of anonymized data. Patients were notified about the anonymized use of their healthcare data via the department's booklet.

Results

Between 15 March and 30 April 2020, 46 patients received combination therapy with PI/ritonavir with hydroxychloroquine, including 21 patients who received hydroxychloroquine with lopinavir/ritonavir and 25 patients who received hydroxychloroquine with darunavir/ritonavir.

The patients included 25 men (54%) and the median age was 68 (IQR 57–81) years. Thirty-seven patients (80%) had at least one risk factor for developing severe COVID-19. Twenty patients (46%) had an initial potential drug–drug interaction (Table 1).

Twenty-four patients (52%) reported adverse events during follow-up (Table 1). The most common adverse events were diarrhoea grade I and II ($n = 13$). Ten patients discontinued treatment: seven for ECG abnormalities (six with repolarization disorders and one with a repolarization and conduction disorder) and one each for hepatic enzyme elevation, severe nausea and a medical decision (palliative care).

Eight patients (17.4%) developed ECG abnormalities during treatment: six repolarization disorders, one conduction disorder and one repolarization and conduction disorder. Three patients in the lopinavir/ritonavir group (14%) discontinued the treatment because of a repolarization disorder: treatment was stopped after 3 days of treatment in one case and after 6 days in two cases. Four patients in the darunavir/ritonavir group (16%) discontinued the treatment: three for repolarization disorders and one with a repolarization and conduction disorder; treatment was stopped between days 2 and 5 of treatment. All ECG abnormalities were reversed 1 or 2 days after treatment interruption. Table 2 shows the demographic and clinical characteristics of the eight patients who developed ECG abnormalities.

In the patients with ECG abnormalities, the median hydroxychloroquine trough concentrations were 170 (IQR 127–204) ng/mL at day 3 and 222 (IQR 51–314) ng/mL at day 7; the median lopinavir concentrations were 23 613 (IQR 22 168–26 063) and 20 402 (IQR 7824–29 771) ng/mL, respectively, and the median darunavir concentrations were 17 600 (IQR 8034–24 664) and 5576 (IQR 1534–9618) ng/mL.

In patients without ECG abnormalities, the median hydroxychloroquine trough concentrations at days 3 and 7 were 111 (IQR 80–176) and 199 (IQR 157–258) ng/mL, respectively; the median lopinavir concentrations were 17 419 (IQR 13 980–23 459) and 9707 (IQR 4843–16 590) ng/mL, respectively, and the median darunavir concentrations were 5670 (IQR 3759–12 968) and 3775 (IQR 2140–4409) ng/mL, respectively (Table 2).

There was no significant difference between the trough plasma hydroxychloroquine concentrations when in combination with lopinavir or darunavir; the median hydroxychloroquine trough concentration was 122 (IQR 83–178) ng/mL with lopinavir and 114 (IQR 84–165) ng/mL with darunavir at day 3 ($P = 0.995$) and 199 (IQR 155–272) ng/mL with lopinavir and 199 (IQR 130–254) ng/mL with darunavir at day 7 ($P = 0.919$).

In the univariate analyses, ECG abnormalities were significantly associated with age ≥70 years ($P = 0.021$), coexisting conditions [such as hypertension ($P < 0.001$) and chronic cardiovascular disease ($P = 0.047$)], initial potential drug interaction ($P = 0.009$),

Table 1. Baseline demographic and clinical characteristics of the patients and adverse events

	Patients treated with hydroxychloroquine and lopinavir/ritonavir (*N* = 21)	Patients treated with hydroxychloroquine and darunavir/ritonavir (*N* = 25)
Age (years), median (IQR)	68 (54–81)	71 (61–80)
Sex, *n* (%)		
female	11 (52)	10 (40)
male	10 (48)	15 (60)
BMI (kg/m^2), *n* (%)		
<18.5	0 (0)	2 (8)
≥18.5 to <25	14 (67)	9 (36)
≥25 to <30	5 (24)	10 (40)
≥30	2 (9)	4 (16)
Quick SOFA score, *n* (%)		
0	11 (52)	10 (40)
1	10 (48)	12 (48)
2	0 (0)	3 (12)
3	0 (0)	0 (0)
Coexisting conditions, *n* (%)		
diabetes	5 (24)	7 (28)
chronic respiratory disease	4 (19)	8 (32)
chronic obstructive pulmonary disease	1	4
asthma	3	2
advanced lung fibrosis	0	2
chronic cardiovascular disease	3 (14)	12 (48)
cardiac arrhythmia	2	5
ischaemic cardiopathy	2	8
hypertensive cardiopathy	0	2
pacemaker	0	1
hypertension	7 (33)	11 (44)
cancer	4 (19)	5 (20)
autoimmune disease	2 (9)	2 (8)
immunosuppressive treatment	4 (19)	4 (16)
Child cirrhosis ≥B	0 (0)	0 (0)
HIV infection with CD4 <200 cells/mm^3	0 (0)	0 (0)
Potential drug interaction, *n* (%)	12 (57)	9 (36)
Clearance (CKD-EPI) (mL/min), *n* (%)		
>60	16 (76)	18 (72)
30 to ≤60	5 (24)	5 (20)
<30	0 (0)	2 (8)
Adverse events, *n*		
ECG abnormalities (*N* = 8)	4	4
repolarization disorder	3	3
conduction disorder	1	0
repolarization and conduction disorder	0	1
bradycardia	0	0
other		
diarrhoea grade I/II	5	8
diarrhoea grade III/IV	0	0
nausea	2	0
hypoglycaemia	0	0
rash	0	0
headache	0	0
hepatic enzyme increased	0	1
Discontinued treatment (*N* = 10), *n*		
ECG abnormalities	3	4
hepatic enzyme elevated	0	1
nausea	1	0
palliative care	1	0

CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration.

Table 2. Comparison of characteristics of patients with and without ECG abnormalities

	Patients with ECG abnormalities ($N = 8$)	Patients without ECG abnormalities ($N = 36$)	P
Age (years), median (IQR)	81 (76–88)	63 (55–76)	0.021
Sex, *n* (%)			
female	5 (62.5)	16 (42)	0.293
male	3 (37.5)	22 (58)	
BMI (kg/m^2), *n* (%)			
<18.5	0 (0)	2 (5)	0.4
≥18.5 to <25	5 (62.5)	17 (45)	
≥25 to <30	3 (37.5)	13 (34)	
≥30	0	6 (16)	
Quick SOFA score, *n* (%)			
0	2 (25)	19 (50)	0.052
1	4 (50)	18 (47)	
2	2 (25)	1 (3)	
3	0 (0)	0 (0)	
Coexisting conditions, *n* (%)			
diabetes	3 (37.5)	9 (23.7)	0.419
chronic respiratory disease	2 (25)	10 (26.3)	0.939
chronic cardiovascular disease	5 (62.5)	10 (26.3)	0.047
hypertension	8 (100)	10 (26.3)	<0.001
cancer	2 (25)	6 (15.8)	0.871
autoimmune disease	0 (0)	4 (10.5)	0.337
immunosuppressive treatment	1 (12.5)	9 (23.7)	0.688
Potential drug interaction, *n* (%)	8 (100)	13 (34)	0.009
Clearance (CKD-EPI) (mL/min), *n* (%)			
>60	4 (50)	30 (79)	0.003
30 to ≤60	2 (25)	8 (21)	
<30	2 (25)	0 (0)	
Residual plasma concentration (ng/mL), median (IQR)			
hydroxychloroquine day 3	170 (127–204)	111 (80–176)	0.779
hydroxychloroquine day 7	222 (51–314)	199 (157–258)	0.846
lopinavir day 3	23 613 (22 168–26 063)	17 419 (13 980–23 459)	0.376
lopinavir day 7	20 402 (7824–29 771)	9707 (4843–16 590)	0.162
darunavir day 3	17 600 (8034–24 664)	5670 (3759–12 968)	0.011
darunavir day 7	5576 (1534–9618)	3775 (2140–4409)	0.702

CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration.

creatinine clearance ($P = 0.003$) and the residual plasma darunavir concentration on day 3 ($P = 0.011$).

Four patients died within 14 days, including one during the treatment. The causes of death were closely related to COVID-19 infection and these patients had severe comorbidities.

Discussion

Hydroxychloroquine is structurally and mechanistically similar to the class IA anti-arrhythmic quinidine, which can prolong the QT interval and increase the risk of torsade de pointes.

The cardiologic toxicity on the QT interval depends on the plasma concentration and cumulative dose of hydroxychloroquine in patients with systemic lupus erythematosus.[13] At a hydroxychloroquine dosage of 200 mg × 3/day, the expected steady-state plasma concentration (>15 days of treatment) ranges from 200 to 800 ng/mL. In the early period, we expected lower concentrations, but a treatment target of at least 100 ng/mL was necessary, as demonstrated in an *in vitro* study.[1]

The median trough plasma hydroxychloroquine level at day 3 was 122 ng/mL when given with lopinavir and 114 ng/mL when given with darunavir, although about one-third of the patients did not reach the target of 100 ng/mL at day 3. At day 7, the 36 patients who completed the treatment (10/46 patients discontinued treatment) had hydroxychloroquine plasma levels above this threshold. This finding raises some concerns regarding the short-term efficacy of hydroxychloroquine against SARS-Cov-2 *in vivo*.

The plasma concentrations of lopinavir and darunavir were elevated after 3 days of treatment, compared with those usually observed in HIV-infected patients.[14] These concentrations remained above the target on day 7. The PI concentration varies with C-reactive protein level and we hypothesized that, in the case

of marked inflammatory syndrome, the total PI concentration might be increased, whereas the unbound fraction might be similar to that in HIV patients.[15] These pharmacological results are consistent with the rapid activity of PIs against SARS-CoV-2 in humans.

Regarding toxicity, we identified no hydroxychloroquine overdose (>800 ng/mL) with the associated PI/ritonavir combinations. However, eight patients (17.4%) showed ECG abnormalities not associated with the plasma hydroxychloroquine level. None of the patients with ECG abnormalities had COVID-19-associated stress cardiomyopathy or myocarditis (based on the troponin level or trans-thoracic echocardiography). The proportion of ECG abnormalities was roughly the same as in patients receiving long-term hydroxychloroquine in systemic lupus erythematous, but the population characteristics were very different.[12] As previously described, the occurrence of ECG abnormalities is easily anticipated since we observed these abnormalities mainly in older people with existing cardiovascular disease and all patients had potential drug–drug interactions or concomitant use of medications that prolong the QT/QTc interval. Despite therapeutic adaptation at the start of combination therapy, we could not prevent drug accumulation/synergic effect resulting in QT interval prolongation. However, all ECG abnormalities recovered 1–2 days after stopping all medications involved in this toxicity and we observed no torsade de pointes or ventricular rhythm disorders during follow-up.

Our study has limitations related to its observational and retrospective design and the patients were not randomized to receive either lopinavir/ritonavir or darunavir/ritonavir. However, the standardization of care based on national and local guidelines resulted in very little missing data in this cohort.

Conclusions

The combination of hydroxychloroquine and a PI with ritonavir is associated with a risk of ECG abnormalities, especially in patients aged 70 years or older, with initial potential drug interactions or with a coexisting condition (such as hypertension, chronic cardiovascular disease and kidney failure). The residual hydroxychloroquine concentrations were not significantly associated with ECG abnormalities. The ECG abnormalities were reversed 1 or 2 days after stopping treatment.

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This study was carried out as part of our routine work.

Transparency declarations

None to declare.

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