Inflammation Research (2023) 72:895–899
https://doi.org/10.1007/s00011-023-01718-y

Inflammation Research

BRIEF REPORT

Colchicine reduces the activation of NLRP3 inflammasome in COVID-19 patients

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Received: 29 November 2022 / Revised: 8 February 2023 / Accepted: 6 March 2023 / Published online: 14 March 2023

Abstract

Objective To evaluate whether colchicine treatment was associated with the inhibition of NLRP3 inflammasome activation in patients with COVID-19.

Methods We present a post hoc analysis from a double-blinded placebo-controlled randomized clinical trial (RCT) on the effect of colchicine for the treatment of COVID-19. Serum levels of NOD-like receptor protein 3 (NLRP3) inflammasome products—active caspase-1 (Casp1p20), IL-1β, and IL-18—were assessed at enrollment and after 48–72 h of treatment in patients receiving standard-of-care (SOC) plus placebo vs. those receiving SOC plus colchicine. The colchicine regimen was 0.5 mg tid for 5 days, followed by 0.5 mg bid for another 5 days.

Results Thirty-six patients received SOC plus colchicine, and thirty-six received SOC plus placebo. Colchicine reduced the need for supplemental oxygen and the length of hospitalization. On Days 2–3, colchicine lowered the serum levels of Casp1p20 and IL-18, but not IL-1β.

Conclusion Treatment with colchicine inhibited the activation of the NLRP3 inflammasome, an event triggering the 'cytokine storm' in COVID-19.

Trial registration numbers RBR-8jyhxh

Keywords Colchicine · COVID-19 · Inflammasome · Cytokines

Responsible Editor: John Di Battista.

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Introduction

Pneumonia is the main complication of coronavirus disease 2019 (COVID-19), which may evolve to respiratory distress syndrome in some patients [1]. Pulmonary aggression by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) results in alveolar macrophage activation followed by immune cell recruitment and activation with consequent tissue inflammation [2]. For a few patients, inflammation breaks the boundaries of the lungs, becomes systemic, and increases the chance of hospitalization.

In severe COVID-19, a cascade of inflammatory mediators (e.g., TNF, IL-6) known as 'cytokine storm' contributes to high serum levels of C-reactive protein (CRP) and a poor prognosis [3]. By evaluating serum levels of NLRP3 inflammasome products, we found elevated serum levels of IL-18 and Casp1p20, demonstrating that this protein platform is

active in hospitalized COVID-19 patients and that its activation correlated with serum IL-6 and CRP and a worse outcome [4]. For those with fatal outcomes, we demonstrated elevated expression of the NLRP3 proteins in lung specimens, as also reported by Toldo et al. [5].

Colchicine is an anti-inflammatory drug used for gout and systemic inflammatory diseases such as Behçet's disease and familial Mediterranean fever [6]. For these indications, the drug is considered safe and efficacious. Many possible mechanisms of action of colchicine have been suggested (e.g., inhibition of microtubule polymerization, inhibition of neutrophil extracellular traps release, and inhibition of platelet activation), including inhibition of the NLRP3 inflammasome [7]. In an RCT with 72 moderate-to-severe COVID-19 patients, 36 receiving colchicine plus SOC and 36 receiving placebo plus SOC, colchicine administration was associated with a reduction in the need for supplemental oxygen and the length of hospitalization [8]. The size effect for both outcomes, calculated using Cohen's d, was 0.76 and 0.70, respectively. The beneficial effects seemed to occur within the first 72 h, concurrently with a significant reduction in serum CRP. Although our and other groups have found beneficial effects of colchicine in COVID-19 patients, a greater number of studies failed to demonstrate a reduction in hospitalization, intensive care unit admission, or mortality, as reviewed in meta-analyses [9–11], which concluded that colchicine is not beneficial for treatment in outpatient and inpatient settings. Hence, colchicine is currently not indicated for COVID-19 [12–14].

Aiming to investigate possible mechanisms by which patients in our RCT had better outcomes when receiving colchicine, we evaluated the inhibition of NLRP3 inflammasome activation by comparing serum Casp1p20, IL-1β, and IL-18 levels at Day 0 and Day 2 or 3 (48–72 h of treatment) of patients receiving SOC plus placebo and those receiving SOC plus colchicine.

Methods

Trial design

In this post hoc analysis of the RCT, we quantified serum NLRP3 inflammasome products from 72 enrolled patients. It was a double-blinded placebo-controlled RCT, with 1:1 randomization for placebo or colchicine arms, from 11 April to 31 August 2020 [8]. The study was registered on the Brazilian National Registry (http://www.ensaiosclinicos.gov.br/rg/RBR-8jyhxh/).

Intervention and SOC

The intervention was colchicine 0.5 mg tid for 5 days, followed by 0.5 mg bid for another 5 days; if body weight was > 80 kg, the first dose was 1 mg. SOC was azithromycin 500 mg/day for 7 days; methylprednisolone 0.5 mg/kg/day for 5 days, if the patient's need for supplemental oxygen was ≥ 6 L/min at enrollment or after; hydroxychloroquine 400 mg/day for 10 days; and unfractionated heparin 5000 UI tid, until the discharge.

Study population

COVID-19 was diagnosed by positive RT-PCR in nasopharyngeal swabs and findings of pneumonia in computed tomography scans. The inclusion criteria were: moderate (fever, dyspnea, and imaging findings of pneumonia) or severe (the same as moderate plus respiratory rate ≥ 30 times/minute or oxygen saturation ≤ 92%) COVID-19; age ≥ 18 years; body weight > 50 kg; and negative serum β-HCG for women. The exclusion criteria were: mild or critical (respiratory failure or shock) form of COVID-19 [15]; diarrhea resulting in dehydration; pregnancy or lactation; metastatic cancer or immunosuppressive chemotherapy; use of protease inhibitors; chronic liver disease; and renal failure.

Experimental procedures

Serum samples were obtained at Day 0 (enrollment) and Day 2 or 3 and stored at − 80 °C until the experiments were performed. Casp1p20 and IL-18 levels were evaluated by ELISA (R&D Systems, Minneapolis, MN, USA), and IL-1β levels by multiplex assay, using the Milliplex® MAP Human cytokine/chemokine Magnetic Bead Panel kit (Merck, Darmstadt, Germany), on the Luminex® 200™ FLEXMAP 3D™ (Austin, TX, USA), following manufacturers' recommendations.

Statistical analysis

Statistical analysis was performed using GraphPad Prism® 7 (GraphPad Software Inc., USA). For clinical and laboratory parameters comparisons, Fisher's exact test, Student's *t* test, or Kruskal–Wallis test were used, followed by Dunn's multiple comparisons test. For all tests, $p < 0.05$ was considered statistically significant.

Results

Thirty-six patients received SOC plus colchicine, and thirty-six received SOC plus placebo. Demographic characteristics, comorbidities, severity of COVID-19, laboratory findings,

and treatments are presented in Supplementary Table. 46% of patients were males, and the median age was 55 years. Patients needing supplemental oxygen comprised 93%, and median serum CRP was equal to 9.2 mg/dL, suggestive of the presence of systemic inflammation. On Day 0, both groups were comparable regarding gender, age, comorbidities, time of COVID-19 symptoms, biochemical and hemogram parameters, and serum CRP. The frequencies of drugs received as SOC were comparable on Day 3.

On Day 3, the percentage of patients without oxygen therapy differed between the groups, with an advantage for the group receiving SOC plus colchicine (log rank test; $p < 0.05$). Serum CRP was lower for the SOC plus colchicine group (4.3, 2.1–6.4 vs. 8.4, 3.7–12.8 mg/dL, $p < 0.01$) on Day 2. Figure 1 shows the evaluation of inflammasome activity. After 48–72 h of treatment, serum levels of Casp1p20 were reduced in the SOC plus colchicine group, with no effect on serum levels in the SOC plus placebo group. Serum levels of IL-18 decreased and were comparable in both groups after treatment, but a difference from Day 0 was found only for patients receiving colchicine. No effect was found for serum IL-1β.

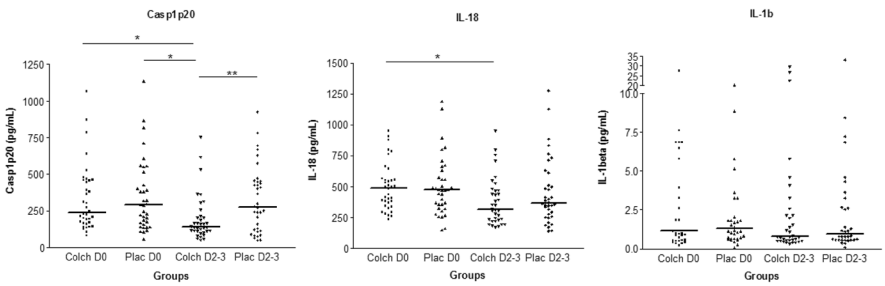
Discussion

The first studies evaluating the effect of colchicine on COVID-19 showed its utility in controlling systemic inflammation caused by SARS-CoV-2, resulting in shorter hospital stays and lower mortality [16]. Initial observations were followed by a greater number of studies, observational or controlled, in which this effect of colchicine was not confirmed by the majority [9–11]. Moreover, the two most prominent studies on colchicine for COVID-19, one recruiting outpatients [17] and the other recruiting inpatients [18], failed to demonstrate the efficacy of colchicine for their primary endpoints. Based on all studies available, the drug is not recommended as a treatment for COVID-19, regardless of its severity [12–14].

We focused on understanding whether the effect of colchicine on the NLRP3 inflammasome could explain the better outcomes we found in our RCT. It is worth mentioning that we studied only one of the multiple potential mechanisms of action of colchicine. We cannot exclude, for example, its negative effects on immune cell migration, neutrophil degranulation, platelet activation, and NETosis. By measuring the serum concentration of Casp1p20 and IL-18, we found that colchicine inhibited the activation of the NLRP3 inflammasome. To the best of our knowledge, this is the first demonstration in an RCT that NLRP3 inflammasome products were systemically reduced under colchicine treatment. We found no difference in serum IL-1β levels. There are potential explanations for this finding: IL-1β can be released in the absence of Casp1p20, especially by neutrophils, cleaved by their cytosolic enzymes [19]; there was a difference in serum IL-1β, but it was not detected [20]; and IL-1β has a very short serum half-life, which may limit its detection [21, 22].

Colchicine reduced systemic inflammation, as evidenced by the reduced serum CRP on Day 2. Colchicine was shown to reduce, among other effects, inflammasome activation in patients with chronic coronary disease [23, 24], acute and recurrent pericarditis [25], and low-grade inflammation related to obesity [26]. There was a concomitant reduction in circulating IL-6 and CRP in all these scenarios.

Previously, our group showed that along with serum Casp1p20, serum IL-18 was correlated with severity and poor clinical outcome in hospitalized COVID-19 patients [3]. Other groups also found the elevation of serum IL-18 as a marker of increased severity and mortality in COVID-19, as reviewed by Qin et al. [27]. In the present study, the reduction in serum IL-18 at Day 2 or 3 occurred in both groups of patients, but with a significant difference only for patients receiving SOC plus colchicine, which provides



	Colchicine – D0	Placebo - D0	Colchicine - D2-3	Placebo - D2-3	
Casp1p20 [pcg/mL; median (IQR)]	238.1 (168.1-459.4)	289.2 (162.8-471.1)	139.8 (111.2-207.4)	275.3 (118.3-451.0)	<0.05#
IL-18 [pcg/mL; median (IQR)]	1.1 (0.5-6.8)	1.2 (0.7-3.2)	0.8 (0.5-2.7)	1.0 (0.6-3.3)	>0.05#
IL-18 [pcg/mL; median (IQR)]	487.1 (353.0-558.8)	471.4 (337.9-601.6)	312.9 (230.1-465.0)	363.5 (265.1-560.0)	<0.05#

#Kruskal-Wallis test ; Dunn´s post test - *p<0.01; **p<0.05

Fig. 1 Measurements of serum Casp1p 20 and cytokines at day zero and day 2 or 3 for Colchincine and control groups

indirect evidence that the clinical benefit of colchicine is related to NLRP3 inhibition.

Our study has some limitations. Being conducted in one center resulted in a reduced number of individuals. We did not evaluate the function of NLRP3 inflammasome ex vivo nor whether colchicine would reduce the activation of NLRP3 inflammasome in vitro. The effect of colchicine in both situations [28, 29] has already been confirmed in other clinical and laboratory situations, but has not yet been tested in COVID-19. We did not enroll patients with mild symptoms to compare whether the magnitude of systemic inflammation correlates in a broader range with the levels of Casp1p20 and cytokines.

In conclusion, the activation of the NLRP3 inflammasome, as reflected by serum levels of activated Casp1 and IL-18, appeared to be reduced by colchicine in patients with moderate-to-severe COVID-19.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00011-023-01718-y.

Acknowledgements We are grateful to Muriel C. R. O. Berti, Basílica Botelho Muniz and Livia Maria C. S. Ambrósio for technical assistance.

Author contributions All authors contributed to the study design. RDRO contributed to randomization and administrative support. FCV, RCS, BAAS, MIFL, LPB, MCG, NBA, MNB, LLEF, SCLA, RLA, MAM, MCB, BAL, and CHMJ contributed to patients' selection and follow-up. UEC analyzed all 12 derivations ECG. MIFL, LPB, MCG, NBA, MNB, FPV, AS, and TSR contributed to blood collection and processing. All authors contributed to clinical data collection and interpretation of data. RDRO, PLJ, and CMDB contributed to statistical analysis. RDRO wrote the manuscript draft. All authors revised the manuscript. RDRO, FQC, JCAF, TMC, LDC, DSZ, and PLJ obtained funding. All authors approved the manuscript.

Funding This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (2013/08216–2, 2020/05601–6, 2020/04964-8, 2020/05288-6 and 2020/04826-4) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (425075/2016-8) grants. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil—dFinance Code 001.

Data availability Data are available on reasonable request. Data types: deidentified participant data. How to access data: renedroliveira@gmail.com. When available: with publication. Who can access the data: researchers whose proposed use of the data has been approved. Mechanisms of data availability: with investigator support, after approval of a proposal with a signed data access agreement

Declarations

Conflict of interest The authors declare they have no relevant conflicts of interest.

Ethical approval Protocols and Informed Consent Forms of both studies were approved by Ethics Board of Hospital das Clínicas de Ribeirão Preto (numbers—CAAE: 30248420.9.0000.5440 and CAAE: 38302520.7.0000.5440). All patients signed the Consent Form at enrollment.

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