Anti-inflammatory therapy for COVID-19 infection: the case for colchicine

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ABSTRACT
The search for effective COVID-19 management strategies continues to evolve. Current understanding of SARS-CoV-2 mechanisms suggests a central role for exaggerated activation of the innate immune system as an important contributor to COVID-19 adverse outcomes. The actions of colchicine, one of the oldest anti-inflammatory therapeutics, target multiple mechanisms associated with COVID-19 excessive inflammation.

While many COVID-19 trials have sought to manipulate SARS-CoV-2 or dampen the inflammatory response once patients are hospitalised, few examine therapeutics to prevent the need for hospitalisation. Colchicine is easily administered, generally well tolerated and inexpensive, and holds particular promise to reduce the risk of hospitalisation and mortality due to COVID-19 in the outpatient setting. Successful outpatient treatment of COVID-19 could greatly reduce morbidity, mortality and the demand for rare or expensive care resources (front-line healthcare workers, hospital beds, ventilators, biological therapies), to the benefit of both resource-replete and resource-poor regions.

INTRODUCTION
As of 27 October 2020, almost 1 year after the first reported cases, the SARS-CoV-2 had resulted in over 43 million people infected and over 1 million deaths from COVID-19 worldwide.1 Clinical experience and data underline the role of excessive inflammation in the pathophysiology of the disease and suggest a potential role for colchicine, a drug with pleiotropic effects.

BIOLOGY OF COVID-19: THE ROLE OF INFLAMMATION
COVID-19 progression can be divided into three distinct phases (figure 1) including: (1) early infection phase, wherein the virus infiltrates host cells in the lung parenchyma; (2) pulmonary phase, in which viral propagation causes lung tissue injury as the host immune response is activated and (3) the inflammatory cascade, which is triggered by pathogen-associated molecular patterns (ie, viral RNA) and damage-associated molecular patterns (DAMPs, ie, cellular debris released during pyroptosis) exposed during active viral replication and release. This third phase of the inflammatory cascade may occur even as viral titers are falling and is comprised of components targeted by colchicine (activation of the inflammasome that drives the cytokine storm, activation of neutrophils and the neutrophil/thrombosis interface)2 (figure 2).

Activation of the inflammasome
Signals driven by SARS-CoV-2 act on macrophages and other myeloid cells to drive assembly of a proinflammatory protein complex, the nod-like receptor protein 3 (NLRP3) inflammasome,3 composed of NLRP3, apoptosis-associated speck-like protein adaptor and cysteine-dependent aspartate-directed protease-1 (caspase-1).4 Activated caspase-1 activity then converts the precursors pro-interleukin (IL)-1β and pro-IL-18 to their active forms. Additionally, caspase-1 activates Gasdermin-D, forming pores in the cell membrane permitting large-scale secretion of IL-1β that, among other actions, induces macrophages to release large quantities of additional pro-inflammatory cytokines.5 6 IL-1β, IL-6, tumour necrosis factor (TNF) and ligation of toll-like receptors activate NF-κB and further upregulate the inflammasome. IL-1β and other cytokines additionally recruit large numbers of leukocytes from the marrow, which in turn undergo activation and cytokine production in an accelerating spiral.

In the related SARS-CoV-1, a small envelope (E) protein augments this reaction by self-assembling into an ion channel within the host cell membrane, causing calcium dysregulation that promotes further assembly and activation of the NLRP3 inflammasome.7 More study is needed to determine if the E protein of SARS-CoV-2 has a similar effect on the inflammasome.

The production of IL-1β drives the synthesis of IL-6, a cytokine that induces C reactive protein (CRP) and has been especially implicated as a major proinflammatory agent in the COVID-19 cytokine storm.8-11

Activation of neutrophils
Cytokines including IL-1β and IL-6 prime neutrophils for activation by chemoattractants and upregulate intercellular adhesion molecules on endothelial cells. The resulting neutrophil adhesion to the vasculature promotes neutrophil diapedesis and infiltration into the affected tissues—in COVID-19 infection, initially into lung parenchyma, but later into other organs. Once neutrophils migrate to sites of inflamed tissue, they degranulate and release proinflammatory cytokines and chemokines, proteases, antiviral proteins and toxic oxygen radicals. In the myocardium, neutrophils—play a prominent role in the development of myocarditis and cardio-myogenic shock.12-14

Neutrophil/thrombosis interface
Neutrophils trigger a cascade of events in arteries that promote plaque destabilisation/rupture and...
thrombosis. Neutrophils release the serine protease neutrophil elastase, which inhibits tissue factor pathway inhibitor and leads to generation of thrombin, the most potent activator of platelets. Neutrophil extracellular traps provide a platform to activate coagulation via active neutrophil elastase adherent to extracellular neutrophil DNA. Activated neutrophils and other leukocytes also aggregate with platelets directly to further exacerbate inflammothrombosis. In the setting of extreme inflammatory states, activated neutrophils adhere directly to each other (leukoaggregation), producing effective but usually transient vascular occlusions. Finally, neutrophils contribute to thrombosis via cytokine-induced release of α-defensin from neutrophil granules. Murine studies suggest that α-defensin, at concentrations similar to those observed in inflammatory conditions, results in accelerated, larger and denser thrombus formation. Human data suggest that patients with COVID-19...
infection have elevated levels of serum α-defensin proportional to COVID-19 disease severity.30

Clinical implications
The connections between inflammation, thrombosis and poor COVID-19 outcomes are well established. On admission, patients from our own institution who were admitted to regular floors but subsequently transferred to the intensive care unit (ICU) had higher CRP concentrations (159±86 mg/L) than patients admitted to the regular floors overall (114±81 mg/L). On transfer to the ICU, CRP concentrations (184 mg/L±104) were higher still (unpublished, figure 3). Manifestations of profound inflammation in severe COVID-19 include acute respiratory distress syndrome and distributive shock.14 15 17 Myocardial injury due to acute coronary syndrome (type 1) and/or supply-demand mismatch in the setting of profound inflammatory response and haemodynamic changes (type 2) is also significantly greater in those with severe COVID-19.31 Vascular inflammation is associated with a large burden of both venous (deep venous thrombosis, pulmonary embolism) and arterial (myocardial infarction, stroke) thrombus.

Severe COVID-19 has also been characterised by extrapulmonary and extravascular manifestations. Acute kidney injury may be a result of direct inflammatory injury, given evidence of acute tubular necrosis with lymphocyte and macrophage infiltration of the tubulointerstitium on histopathology.32 The mechanism(s) of COVID-related hepatic injury remains unclear but preliminary studies suggest that the ACE2 receptor is preferentially expressed in cholangiocytes, suggesting that liver involvement may require direct SARS-CoV-2 infection and injury of cholangiocytes.33 34 Cytokine storm itself can drive multisystem organ injury overall. Together, these observations suggest that an anti-inflammatory agent with limited immunosuppressive potential could prove useful in preventing severe inflammatory injury and promoting improved patient outcomes.

COLCHICINE
Historical perspective
Although colchicine first received approval from the US Food and Drug Administration in 2009, its modern use dates back two centuries. Indeed, papyri dating from 1500 BC describe the use of colchicine’s source plant—Colchicum autumnale—for pain and inflammation, making colchicine one of the world’s oldest anti-inflammatory therapeutics.35 Currently, colchicine is approved for treating and preventing acute gout and familial Mediterranean fever, and is used off label in Behçet’s disease, pericarditis and other inflammatory conditions.36

Colchicine and microtubules: inhibition of neutrophil activity
Microtubules are dynamic proteins that form via polymerisation of α-/β-tubulin dimers. Colchicine irreversibly intercalates into free α/β dimers that incorporate into and block microtubule extension.37 During inflammation, microtubules facilitate the movement of adhesion molecules onto cell surfaces. Colchicine concentrations are much higher in neutrophils than other leukocytes due to diminished activity of the P-glycoprotein membrane efflux pump that serves as an energy-dependent colchicine efflux
transporter. Thus, neutrophils appear to be more sensitive than other cells to lower serum concentrations of colchicine. Cronstein et al demonstrated that colchicine causes a quantitative decrease in leukocyte (L)-selectin expression and diminishes qualitative expression of endothelial (E)-selectin, two proteins involved in rolling and adhesion of neutrophils on endothelium. Disruption of microtubules also inhibits neutrophil rheologic capacity, inhibiting their transmigration out of blood vessels.

Additional studies show that colchicine directly inhibits intracellular neutrophil signalling and lysosomal enzyme release during phagocytosis. Colchicine-mediated inhibition of chemotaxin release (eg, leukotriene B4) suppresses neutrophil adhesion to inflamed endothelium. Colchicine also inhibits calcium influx, which raises intracellular cyclic adenosine monophosphate (cAMP) levels and dampens neutrophil responses. In lipopolysaccharide-stimulated neutrophils, we observed that colchicine can dampen stimulated neutrophil metabolism as measured by extracellular acidification (unpublished, figure 4).

Colchicine and the inflammasome: inhibition of IL-1β and prevention of the cytokine storm

More recently, colchicine has been shown to decrease cytokine production by inhibiting activation of the NLRP3 inflammasome (figure 5). The mechanism(s) of colchicine’s action on the inflammasome remain an area of ongoing investigation. Colchicine’s interruption of inflammasome activation reduces IL-1β production, which in turn prevents the induction of IL-6 and TNF and the recruitment of additional neutrophils and macrophages. Whereas the effect of specific anti-IL-6 inhibition for COVID-19 treatment is somewhat controversial (online supplemental text 1), the ability of colchicine to affect multiple cytokines may offer unique advantages.

Colchicine and the inflammation/thrombosis interface

Murine models show that colchicine inhibits neutrophil release of α-defensin, thereby potentially preventing large thrombus burdens. At supratherapeutic concentrations, colchicine, through its microtubule effects, converts normal discoid platelets to rounded, irregular structures and inhibits platelet activation by decreasing calcium entry. These mechanisms diminish in vitro platelet-to-platelet aggregation. In contrast, we demonstrated that standard clinical doses of colchicine do not decrease platelet-to-platelet aggregation but do diminish neutrophil-to-platelet aggregation, suggesting that colchicine at physiological doses may provide an inhibitory role at the inflammation/thrombosis interface without compromising homeostatic platelet-to-platelet function. Indeed, in vivo colchicine has not been shown to inhibit non-inflammatory-related thrombosis.

Adverse effects of colchicine

Colchicine metabolism occurs primarily inside hepatocytes via the cytochrome P450 3A4 (CYP3A4). Medications that strongly

Figure 4  Neutrophil metabolism in the presence of colchicine. Neutrophils were purified from healthy volunteer whole blood using the MACSxpress whole blood neutrophil isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and separated into four aliquots. Neutrophils were coincubated with and without lipopolysaccharide (LPS) and with and without colchicine. In vitro quantification of neutrophil metabolism, measured as extracellular acidification rate (ECAR) (mpH/min), was evaluated using a glycolysis stress test using a Seahorse XFe24 analyzer (Agilent Technologies, Santa Clara, California, USA). Using a modified assay, cells were first incubated with activators (LPS 10 ng/mL with or without colchicine 15 nM) for 10 min.

Figure 5  Colchicine inhibits inflammasome action and reduces supernatant levels of IL-1β. THP1 cells (macrophage cell line) were stimulated with monosodium urate (MSU) or calcium pyrophosphate dihydrate (CPPD) crystals in the presence or absence of colchicine. Supernatants were analysed for IL-1β by Western blot. For the purposes of this figure, the original published blot was quantified using Image J. Adapted from Martinon et al.
inhibit CYP3A4 metabolism (eg, ritonavir, ketoconazole, clarithromycin, cyclosporine, diltiazem, verapamil) pose a risk of drug-drug interactions. A small number of publications report cases of death after coadministration of clarithromycin and colchicine in patients with severe chronic renal disease.\textsuperscript{50} \textsuperscript{51} Similar cases have been rarely reported in patients receiving atorvastatin, a statin that is also processed by CYP-3A4, but not with statins that are not metabolised through CYP3A4. In a recent placebo-controlled randomised trial of 4745 patient with a recent myocardial infarction, patients receiving daily colchicine experienced no adverse effects related to the coadministration of statins, including atorvastatin.\textsuperscript{52} In another recent placebo-controlled randomised trial of 5522 patients with stable coronary artery disease, daily colchicine resulted in numerically higher rates of myalgia (HR 1.15, 95% CI 1.01 to 1.31) and one case of rhabdomyolysis (the patient made a full recovery).\textsuperscript{53} However, a non-significant trend towards increased non-cardiovascular death was observed that requires further investigation. Overall, reports of severe colchicine toxicity tend to occur in the setting of errors in colchicine prescribing.

Approximately 10\%-20\% of colchicine is excreted renally.\textsuperscript{56} However, dose reductions may only be necessary in patients with severe renal impairment.\textsuperscript{54} As a lipophilic molecule, colchicine is usually protein-bound in plasma, with P-glycoprotein in the intestinal lining serving as the primary protein for gut excretion of colchicine. Cyclosporine and ranolazine compete for the ligand site on P-glycoprotein and can therefore lead to delayed elimination. At higher concentrations for longer durations, particularly in the setting of kidney disease, colchicine has been reported to occasionally induce a reversible neumyopathy. Acute overdose may cause multiorgan system failure and death. Furthermore, increased adverse events may be noted in the simultaneous presence of moderate renal insufficiency with use of multiple CYP3A4 inhibitors.

A meta-analysis of 35 randomised trials of colchicine versus placebo found that the most common and significant adverse effect was diarrhoea.\textsuperscript{55} \textsuperscript{56} The only other adverse effect that occurred at a greater frequency than placebo was a set of pooled gastrointestinal symptoms including nausea, vomiting, diarrhoea, abdominal pain, loss of appetite, and bloating. A striking finding in this meta-analysis was the absence of increased infection rates in the colchicine compared with the placebo arm. However, in contrast to most available data, one retrospective and one prospective study did report increased pneumonia risk with colchicine (online supplemental table 1).

**COLCHICINE AND COVID-19: THE CLINICAL CASE**

Several of the biological therapies that have been studied and/or used in the setting of severe SARS-CoV-2 infection target some of the same pathways as colchicine, including IL-1β (ie, anakinra) and IL-6 (ie, tocilizumab and sarilumab).\textsuperscript{57} Colchicine differs from these agents in having pleotropic mechanisms of action, being less potent on any single target, and being an oral agent. In contrast to the biological agents used in the midst of cytokine storm, colchicine is not immunosuppressive, is not known to increase risk of infection, and is inexpensive. A review of the mechanisms of SARS-CoV-2 and colchicine in parallel reveals a potential intervention point that may prevent the progression from inflammatory activation (phase 2) to a hyperinflammatory state (phase 3). Taken together with the clinical data described herein, the potential benefits of colchicine are suggested to be maximised when used early in the disease process (ideally prior to phase 2, but certainly prior to phase 3), such as in non-hospitalised patients within a few days of diagnosis regardless of symptoms and/or within a few days of hospitalisation if not already critically ill. However, the optimal timing continues to require further investigation.

**Colchicine in non-rheumatological inflammatory conditions**

Multiple randomised studies have evaluated the use of colchicine in non-rheumatological inflammatory conditions. Two randomised trials in acute pericarditis demonstrated lower recurrence rate with colchicine versus conventional or placebo therapy.\textsuperscript{58} Colchicine reduced symptom persistence 72 hours after treatment initiation, and colchicine was beneficial even in the setting of recurrent pericarditis.\textsuperscript{59} Used after cardiac surgery, colchicine appears to prevent the inflammatory postpericardiotomy syndrome.\textsuperscript{60}

Colchicine may reduce risk of acute myocardial infarction (AMI). We demonstrated an association between daily colchicine use and decreased prevalence of AMI in patients with gout, a non-traditional cardiovascular risk factor.\textsuperscript{61} \textsuperscript{62} These findings were subsequently reproduced in an independent gout population.\textsuperscript{63} Two open-label prospective studies of daily colchicine use versus no colchicine use in patients with stable coronary artery disease already on aspirin and high-intensity statin therapy demonstrated a decrease in CRP levels with low-dose colchicine, and a significant reduction in cardiovascular events with daily colchicine vs no colchicine.\textsuperscript{64} \textsuperscript{65} The reduction in the primary clinical outcome was driven primarily by a reduction in AMI.\textsuperscript{65} The multicentre, double-blind COLChicine Cardiovascular Outcomes Trial (COLCOT) randomised 4745 patients within 30 days of AMI to colchicine or placebo and demonstrated a reduction in the primary composite endpoint of cardiovascular death, resuscitated cardiac arrest, AMI, stroke or urgent revascularisation with colchicine.\textsuperscript{52} The multicentre, double-blind Low Dose Colchicine 2 (LoDoCo 2) trial randomised 5522 patients with stable coronary artery disease and also demonstrated a reduction in the primary composite endpoint of cardiovascular death, AMI, stroke or urgent revascularisation.\textsuperscript{55} Finally, in cases where the thrombus burden remains refractory to standard antplatelet and anticoagulant therapies, colchicine has been shown to be associated with thrombus resolution.\textsuperscript{66}

Our 400-patient randomised Colchicine in Percutaneous Coronary Intervention (Colchicine-PCI) trial demonstrated that when given as a standard loading dose prior to tissue injury (coronary stent placement), colchicine significantly dampened the upregulation of IL-6 and CRP.\textsuperscript{55} These effects were observed 22-24 hours after the acute event, providing a rationale to administer colchicine earlier in the disease process to prevent clinical manifestations of cytokine-induced injury. Consistent with a possible preventive role, colchicine is effective to prevent cytokine-based disease flares in gout and familial Mediterranean fever.\textsuperscript{55} Finally, colchicine has also been shown to dampen the inflammatory response and reduce CRP levels among subjects with metabolic syndrome.\textsuperscript{68} These data support the general anti-inflammatory effect of colchicine, independent of a specific disease state.

**Colchicine trials in COVID-19**

The recent open-label, multicentre Randomised Evaluation of COVID-19 Therapy (RECOVERY) trial in the UK demonstrated a reduction in 28-day mortality with dexamethasone (n=2104) vs usual care (n=4321) in patients hospitalised with severe COVID-19.\textsuperscript{59} These data support the principle that an anti-inflammatory strategy in COVID-19 may be helpful. However,
glucocorticoids such as dexamethasone have intrinsic immunosuppressive drawbacks that colchicine does not share.

Several early studies have evaluated the benefit of colchicine in COVID-19 patients. A retrospective single-centre study of 87 ICU patients with COVID-19 demonstrated a lower risk of death in patients on colchicine (adjusted HR 0.41, 95% CI 0.17 to 0.98). The Greek Effects of Colchicine in COVID-19 (GRECO-19) trial was the first prospective open-label randomised trial evaluating colchicine versus usual care in early hospitalised patients. This study of 105 patients found a significant reduction in the primary clinical outcome of a two-point deterioration on WHO disease severity scale. The authors additionally noted suppression of D-dimer levels in the colchicine vs control group. An Italian study compared 122 hospitalised patients who received colchicine plus standard-of-care (lopinavir/ritonavir, dexamethasone or hydroxychloroquine) with 140 hospitalised patients receiving standard-of-care alone. Colchicine had a significant mortality benefit (84% vs 64% survival) vs controls.

A third prospective study randomised 38 hospitalised COVID-19 patients to colchicine or placebo in a double-blinded manner. Patients receiving colchicine had less need for supplemental oxygen at day 7 (6% vs 39%) and were more likely to be discharged at day 10 (94% vs 83%). Colchicine subjects also had greater reduction of CRP, and no increase in serious adverse events. Additional inpatient studies are ongoing (online supplemental table 2). Although the permitted use of other treatments could have biased the impact of colchicine in these studies, in the GRECO-19 trial no glucocorticoids were administered and other medications did not differ between the two groups; in the Italian study, there was no difference in outcomes among patients given colchicine who did or did not also receive dexamethasone.

Given its ease of use, tolerability and low cost, an argument for studying colchicine in the outpatient setting, to reduce hospitalisation and adverse outcomes, may be even more compelling. Unfortunately, data on the use of colchicine in the setting of outpatient COVID-19 cases are sparse. In a very small case series from Italy, nine outpatients with COVID-19 were administered colchicine, of whom only one subject was ultimately hospitalised. The hospitalised patient received 4 days of oxygen therapy and was discharged. Moreover, all patients experienced deference within 72 hours of colchicine initiation, suggesting an antipruritic effect. While these reports are insufficient to recommend colchicine for COVID-19 in clinical practice, they provide support for further study of colchicine in COVID-19, including in the outpatient setting. The ongoing ColCorona Trial (www.colcorona.net) is a large placebo-controlled trial of colchicine use within 2 days of COVID-19 diagnosis, regardless of symptoms, in patients with comorbidities that place patients at a higher risk of developing complications related to COVID-19 that may provide additional information.

**CONCLUSIONS**

Given the large body of data demonstrating colchicine’s inhibitory effects on neutrophil activity, cytokine generation and the inflammation/thrombosis interface, together with an overall lack of evidence for systemic immunosuppression, there is a rationale to study colchicine as a potential treatment for COVID-19. Given that colchicine is generally well tolerated, simple to take and inexpensive, demonstration of colchicine as a useful agent in COVID-19 would potentially spare patients morbidity and mortality, help to conserve valuable clinical resources (hospital floor and ICU beds, ventilators, etc), and dramatically reduce the cost of COVID-19 care. Colchicine might be of particular use in resource-poor rural and developing world settings, both of which have been increasingly affected by COVID-19. However, unless and until evidence is obtained from adequately designed and randomised placebo-controlled trials, this hypothesis must remain speculative.

The optimal dose of colchicine for daily use, even in well-established conditions such as gout, is unknown. Many but not all patients tolerate up to 1.2 mg daily in divided doses; whether lower doses such as 0.5 mg or less daily can be equally effective is unknown. The largest colchicine study for COVID-19 (ColCorona) is testing a dose of 0.5 mg daily based on prior cardiology trials. The duration of colchicine therapy for SARS-COV2 infection would also need to be determined. Most studies to date test a treatment duration of 2–4 weeks, concordant with the acute course of the infection; whether a shorter or longer treatment would be optimal is unknown. Finally, the timing of colchicine initiation is uncertain, with some studies beginning treatment in the outpatient setting, and others in the early inpatient setting. Given the recent track record of failure of treatment of severe COVID-19 treatment with anti-IL-6 biologics such as tocilizumab (a much more potent but also more specific immunosuppressive agent), it is likely that the severe inpatient setting is not the optimal condition under which to assess colchicine efficacy.
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