

Cytokine storm and colchicine potential role fighting SARS-CoV-2 pneumonia

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Abstract

For some patients with SARS-CoV-2, the worst clinical damage is not caused by the virus itself, but by an overactive inflammatory state. In fact, in some people the immune system goes into overdrive and launches a large-scale assault on the tissue known as cytokine storm. This excessive inflammatory/immune reaction can damage tissue and eventually kill people. Evidence shows that blocking such cytokine storms can be effective and trials are underway to test drugs that act by reducing cytokine response, such as tocilizumab and sarilumab which bind interleukin 6 (IL-6), or anakinra which is the interleukin 1 receptor antagonist (IL-1). However, other drugs that block the cytokine cascade can also be considered. In this article we describe the scientific and molecular motivation for the use of drugs that act by modulating the hyperactive inflammatory system in severe patients suffering from SARS-CoV-2, considering in particular an old drug that has been in use for many years for other therapeutic indications such as colchicine, and that could be favourable to its use, with low cost and good tolerability.

Introduction

In December 2019, a group of pneumonia cases occurred in Wuhan, China, caused by a newly identified coronavirus (SARS-CoV-2). This coronavirus rapidly spread to China and other countries causing a global pandemic. SARS-CoV-2 is a β -coronavirus, virus that can lead to serious and potentially fatal respiratory tract infections. It was found that the genomic sequence of SARS-CoV-2 shares 79.5% of the identity with the SARS-CoV responsible for the epidemic in 2003. It is now known that SARS-CoV-2 could use the angiotensin 2 conversion enzyme (ACE2), the same receptor as SARS-CoV, to infect humans and penetrate cells.

Clinical characteristics of patients infected with SARS-CoV-2

Based on ongoing epidemiological investigations, the incubation period of the virus is 1-14 days, mostly 3-7 days. SARS-CoV-2 is contagious during the latency period. Based on the knowledge acquired during these months the infection has been divided into three phases, the first asymptomatic or with mild symptoms, the second and third characterized by hyperactive inflammatory state responsible for

lung lesions and that in some patients can rapidly develop acute respiratory distress syndrome, respiratory failure, multiple organ failure, and even death.

The most common clinical manifestations recorded in the three stages of infection are fever, cough, fatigue, dyspnea, sore throat. In addition, some patients experience gastrointestinal symptoms such as diarrhea and vomiting.

Elderly people or those with underlying diseases (e.g. hypertension, chronic obstructive pulmonary disease, diabetes, cardiovascular disease) are at greater risk of rapidly developing acute respiratory distress syndrome, septic shock, metabolic acidosis and clotting dysfunction, which quickly lead to death. Laboratory tests vary depending on the stages of infection, in the most severe stages neutrophil count, D-dimer, blood urea and creatinine are significantly higher and the lymphocyte count is down. In addition, inflammatory factors IL-6, IL-10, IL-1 increase, indicating the altered inflammatory and immune status of the patients. The data also showed that patients have higher plasma levels of IL-2, IL-7, the granulocyte colony stimulation factor (GCSF).

The disease tends to progress more rapidly in older people, with a shorter median number of days among people aged 65 years or older, from the onset of the first symptoms until death. Infants and the elderly need more attention and care because of their immature or weak immune system. (1-6)

Inflammatory cytokine storm in patients with severe SARS-CoV-2

Phase two and three of the infection is characterized by a hyperactive inflammatory state that can result in a cytokine storm (CS). CS refers to the excessive and uncontrolled release of pro-inflammatory cytokines. The syndrome can be caused by a variety of diseases, including infectious diseases, rheumatic diseases and cancer immunotherapy such as CAR-T. Clinically, it presents as systemic inflammation, multiple organ failure and elevated inflammatory parameters. In infectious diseases, CS usually originates from the infected focal zone, spreading throughout the body through the circulation. The accumulation of evidence has revealed that a percentage of patients with severe SARS-CoV-2 have a high cytokine profile similar to CS. Studies have reported the level of inflammatory factors in patients with SARS-CoV-2 during CS, cytokine levels in 41 hospitalized patients (including 13 in intensive care and 28 non-in intensive care patients), IL-1, IL-7, IL-8, IL-9, IL-10, fibroblast growth factor (FGF),

granulocyte and macrophage colony stimulating factor (GM-CSF), IFN γ , tumor necrosis factor (TNF α), vascular endothelial growth factor (VEGF) were significantly increased, including IL-2, IL-7, IL-10, G-CSF, TNF α were higher in severe patients. Several other studies confirmed increased levels of IL-6 in severely SARS-CoV-2 patients. One study analyzed lymphocyte subsets and cytokines in 123 patients, all patients had lymphocytopenia, the percentage of reduction in CD8 + T lymphocytes was 28.43% and 61.9% in mild and severe groups respectively, and the reduction in NK cells was 34.31% and 47.62% in mild and severe groups respectively.

In addition, serum levels of IL-6 in the severe group were significantly higher than in the mild group. It is possible that CS aggravates lung damage and leads to other fatal complications. At this stage, the markers of systemic inflammation therefore appear to be extremely high. Therefore, blocking CS and knowing when to start anti-inflammatory therapy is essential to reduce the mortality rate of SARS-CoV-2. Probably in phase one of the infection the inflammatory/immune response is important to fight the virus, in phase two and three a multi-organ systemic inflammation is probably responsible for the worsening of the health condition, in these phases therefore it could be useful to act with CS blocking agents. (6-20)

Therapeutic approaches to reduce cytokine storm

Based on the above, new strategies to attenuate inflammatory responses are likely to improve clinical outcomes in SARS-CoV-2 patients in stages two and three of infection. Here we describe the agents that have the potential to reduce inflammation and CS virus-induced. Undoubtedly, antiviral and supportive treatments are very important. The CS is relatively common in severe cases and often leads to fatal lung lesions, anti-inflammatory therapy can help prevent these damages. As we know, there are a variety of anti-inflammatory and immunomodulatory drugs, including nonsteroidal anti-inflammatory drugs, glucocorticoids, chloroquine/hydroxychloroquine, immunosuppressants, cytokine inflammatory antagonists (such as IL-6 inhibitor monoclonal antibodies, TNF inhibitors, IL-1 inhibitors, janus kinase inhibitors). Evidence suggests that the use of immunomodulatory agents reduces systemic inflammation before causing multi-organ dysfunction. At this stage, the use of corticosteroids may also be justified in combination with the use of cytokine inhibitors such as tocilizumab (IL-6 inhibitor) or anakinra (IL-1

receptor antagonist). However, is there a dilemma of anti-inflammatory and immunomodulatory therapy, when and at what doses? All these questions are still the subject of intense debate and do not reach a consensus. The main concern is that anti-inflammatory/immunomodulatory drugs, such as corticosteroid, may delay the elimination of the virus and increase the risk of secondary infections, especially in those with compromised immune systems. Second, biological agents targeting pro-inflammatory cytokines can only inhibit a specific inflammatory factor, and therefore may not be very effective in stopping CS in SARS-CoV-2, where other cytokines may be of significant importance. Finally, the time window of anti-inflammatory treatment is very important. According to the evidence, severe patients usually suffered a sharp deterioration in 1-2 weeks after the onset of infection, early initiation of anti-inflammatory therapy in this extremely short time window is likely to achieve a favourable response to treatment and avoid a worsening of the condition. During the SARS epidemic in 2003, glucocorticoid was the main drug of immunomodulatory therapy. However, some studies have shown no beneficial effect with glucocorticoid in SARS-Cov-2 infection leading to deterioration of the disease. In addition, there is no evidence of randomized clinical trials supporting glucocorticoid treatment for SARS-CoV-2. However, some evidence indicates that the benefit of glucocorticoid use is likely to outweigh the adverse effect. Among the interleukine inhibitors that have shown some evidence of efficacy are IL-6 inhibitors. Tocilizumab is an antibody that binds specifically to IL-6 thus blocking its signaling and its inflammatory mediated response. Tocilizumab is widely used in rheumatic diseases such as rheumatoid arthritis and on August 30, 2017, Tocilizumab was approved in the United States for severely life-threatening cytokine release syndrome caused by chimeric immunotherapy of T cell antigen receptors (CAR-T). Recent trials demonstrate the efficacy of Tocilizumab in the treatment of phase two or three patients with SARS-CoV-2. Together with basic anti-virus treatment, Tocilizumab was applied to 20 patients 400 mg once intravenously. Within a few days, the fever returned to normal and the other symptoms improved significantly. The opacity of the lung lesion on Tocilizumab scans was absorbed in 90.5% of patients. In addition, the percentage of peripheral lymphocytes returned to normal in 52.6% of patients. Their data suggest that Tocilizumab could be an effective treatment in severe SARS-CoV-2. So far, several clinical studies have been recorded on the safety and efficacy of Tocilizumab in the treatment of severe SARS-CoV-2 pneumonia in adult hospitalized patients. To date,

several clinical trials are underway in Italy to test its efficacy and safety in SARS-Cov-2 patients. Chinese health authorities have approved the use of this interleukin 6 inhibitor drug in SARS-Cov-2 patients. Sarilumab is of the same class. It is a human monoclonal interleukin 6 receptor antibody for the treatment of rheumatoid arthritis, has the same pharmacodynamic profile as tocilizumab and also shares the same tolerability profile, common side effects that occur in 1-10% of treated patients are lung tract infections, which could be an unfortunate factor for treatment in SARS-Cov-2 patients. Emapalumab is an anti-interferon-gamma antibody (IFN γ) used for the treatment of hemophagocytic lymphohistiocytosis (HLH). HLH causes excessive secretion of IFN- γ which contributes to the pathogenesis of the disease. Emapalumab binds and neutralizes IFN- γ , preventing it from inducing pathological effects. Anakinra is a biopharmaceutical drug used for the treatment of rheumatoid arthritis. It is a recombinant and slightly modified version of the human interleukin 1 receptor antagonist protein. It also appears to be effective in the treatment of macrophage activation syndrome (MAS), a form of cytokine storm. A study is currently underway to study the efficacy and safety of Emapalumab and Anakinra in reducing hyperinflammation and respiratory distress in patients with SARS-CoV-2 infection. Chloroquine (CQ) and hydroxychloroquine (HCQ) are first-line drugs for the treatment and prophylaxis of malaria and are also used for the treatment of autoimmune diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Some studies have reported that CQ/HCQ have a broad spectrum of antiviral effects on a variety of different viruses, such as human immunodeficiency virus (HIV), Marburg virus, and SARS-CoV-1. CQ and HCQ may interfere with the binding of viral particles to the receptor of their cell surface or with the pH-dependent endosome-mediated viral input of enveloped viruses to inhibit the viral cycle. They may also interfere with post-translational modification of viral proteins or compromise proper viral protein maturation through pH modulation. In addition, CQ and HCQ can regulate the immune system by affecting cell signaling and the production of pro-inflammatory cytokines. Although CQ or HCQ are often used for the treatment of rheumatic diseases due to its immunomodulatory and anti-inflammatory effects, the benefit in the treatment of SARS-CoV-2 can be attributed primarily to its antiviral effects. Recently, CQ and HCQ have been demonstrated in several studies to reduce the viral load of SARS-CoV-2 and shorten the duration of viremia. Whether their immunomodulatory effect also plays a role in the treatment of SARS-

CoV-2 still requires further investigation. (21-44)

Colchicine

One drug that could perhaps add value to counteract SARS-Cov-2 CS is colchicine. Colchicine is used for the treatment of gout, Behçet's disease, prevention and treatment of pericarditis and family Mediterranean fever, Sweet syndrome, scleroderma, amyloidosis. Perhaps the most effective results of colchicine treatment have been obtained in family prophylaxis of Mediterranean fever. The scientific hypothesis of the use of colchicine in SARS-CoV-2 is based on the anti-inflammatory properties of the drug and on the already widely demonstrated efficacy of familial Mediterranean fever and in pericarditis, considering that these diseases also demonstrate a phase of hyperactivation of the inflammatory system. Recently published data on colchicine seem to suggest a potential synergism in the treatment at different trigger point levels of cytokine storm. In fact, colchicine acts by decreasing inflammation through multiple mechanisms. The main mechanism of action is to bind the tubulin molecule and thus inhibit its polymerization. In particular, its anti-inflammatory effect has been attributed to the decomposition of microtubules into neutrophils thus inhibiting their migration. Furthermore, colchicine can also alter the distribution of adhesion molecules on the surface of both neutrophils and endothelial cells, leading to a significant inhibition of the interaction between white blood cells and endothelial cells interfering with their transmigration. Therefore, there is growing evidence that the anti-inflammatory effect of colchicine is multifaceted. Probably the main mechanism of action for cytokine reduction in patients with SARS-CoV-2 is the inhibition of IL-1, IL-6 and IL-18 interfering with the inflammatory protein complex NLRP3, a factor increasingly recognized for its role especially in recurrent idiopathic pericarditis and Mediterranean fever. (Figure 1)

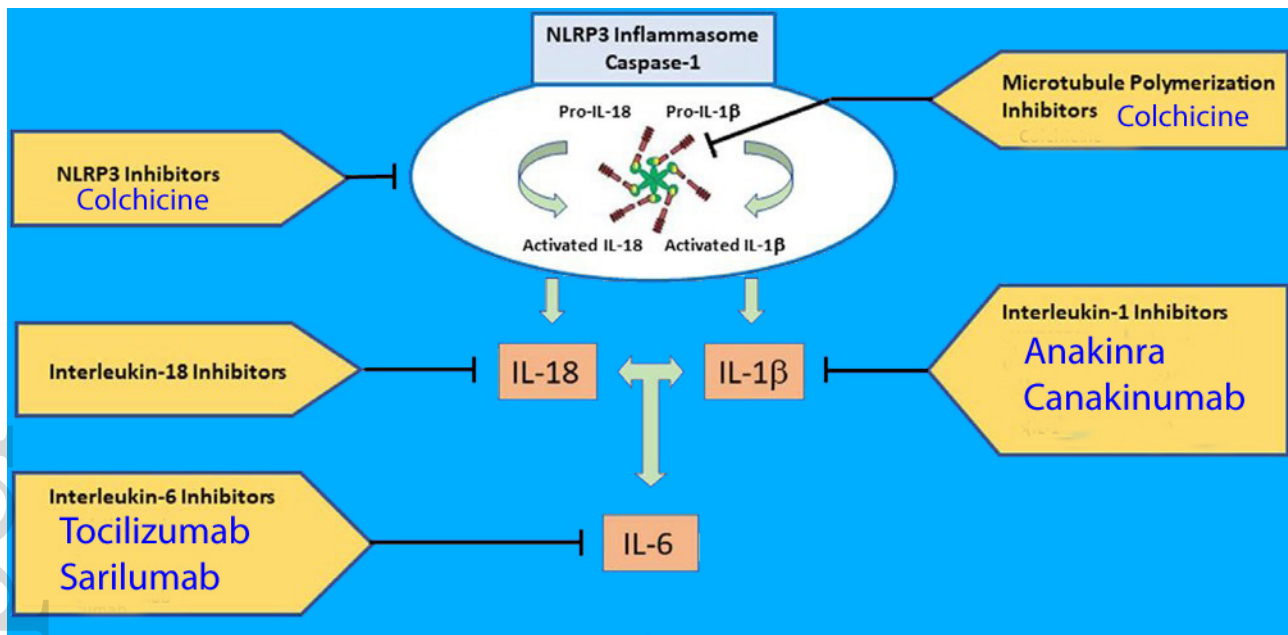


Figure 1. Potential therapeutic targets in the NLRP3 inflammasome and IL-1, IL-18, IL-6.

In addition, colchicine accumulates in white blood cells and affects them in various ways: by decreasing motility, loosening chemotaxis and adhesion, it inhibits the production of superoxide anions, interrupts the degranulation of mast cells. It is important to note that studies have shown that viroporine E, a component of the SARS-associated coronavirus (SARS-CoV), forms Ca^{2+} -permeable ion channels and activates NLRP3 inflammation. In addition, another viroporin 3a has been shown to induce activation of NLRP3 inflammation. The mechanisms are unclear. Colchicine counteracts the increased inflammation of NLRP3, thus reducing the release of IL-1 β and a number of other interleukins, including IL-6, the added value of this drug compared to IL-1 or IL-6 inhibitors is that it acts upstream of the cascade of cytokines and not only on one route, moreover at standard doses it shows a good tolerability profile. Several clinical trials are currently underway to study the efficacy of colchicine in patients with SARS-Cov-2, as shown in Table 1. In particular, two trials are underway in Italy to evaluate the efficacy and safety of colchicine in SARS-Cov-2 patients, the first "Colchicine Counteracting Inflammation in COVID-19 Pneumonia (ColCOVID-19)" and the second entitled "Treatment with colchicine of patients affected by Covid-19: a pilot Study". (44-68)

Table 1. Trials on going with colchicine in SARS-Cov-2 patients (Clinicaltrials.gov).

Row	Study Title	Conditions
1	Colchicine Coronavirus SARS-CoV2 Trial (COLCORONA)	Corona Virus Infection
2	The GReek Study in the Effects of Colchicine in Covid-19	Corona Virus Disease 19 (SARS-Cov 2)
3	Colchicine Efficacy in COVID-19 Pneumonia	Corona Virus Infections Pneumonia Viral
4	The ECLA PHRI COLCOVID TRIAL	SARS-Cov-2 infection
5	Colchicine Counteracting Inflammation in COVID-19 Pneumonia (ColCOVID-19)	SARS-Cov-2
6	Treatment with colchicine of patients affected by Covid-19:a pilot Study”	SARS-Cov-2

Conclusions

Inflammation is an indispensable part of an effective immune response, without which it is difficult to successfully eliminate an infectious agent. The inflammatory response begins with the initial recognition of a pathogen, which then mediates the recruitment of immune cells, eliminates the pathogens and ultimately leads to tissue repair and return to homeostasis. However, some viruses such as SARS-Cov-2 induce an excessive and prolonged cytokine response, known as "cytokine storms", which results in high morbidity and mortality due to immunopathology. Therefore, therapeutic interventions targeting these pro-inflammatory cytokines and chemokines could be useful to improve undesirable inflammatory responses. In addition, since high viral titers in the early and later stages of infection are strongly related to the severity of the disease in humans, strategies to control viral load and attenuate the inflammatory response may be useful. In conclusion, SARS-CoV-2 is a viral infectious disease that mainly manifests itself in fever and pneumonia, and antiviral therapies are certainly the mainstream, but we believe that treatments that reduce the cytokinic response may be effective especially for more severe cases. In this way, biological agents targeting pro-inflammatory cytokines can only inhibit a specific inflammatory factor, and therefore may not be very effective in stopping CS in SARS-CoV-2 where other cytokines may be of significant importance. The colchicine could result in a therapeutic treatment that acts upstream of the cytokine cascade in IL-6 and IL-1, bringing more

benefits, it is also a low cost and if used at the right doses with a good tolerability profile. In addition there is a fundamental aspect to add, biological drugs (such as tocilizumab, sarilumab etc..) can, with reference to the RCP and evidence of the drugs, can cause with a 'common' frequency secondary infections of the respiratory tract and therefore compromise paradoxically the clinical situation of patients infected SARS-CoV-2, therefore clinical evidence is needed to clarify their possible use and on which target of SARS-CoV-2 patients. For colchicine, however, with reference to the RCP, the clinical study and pharmacovigilance data, the risk of upper respiratory tract infections may not be an issue. However, data on the viral nature of SARS-CoV-2 and considering substantial damage to the host's immune system in severe cases, it is essential to balance the risk / benefit balance before starting anti-inflammatory therapy. Furthermore, the early anti-inflammatory treatment started at the right time is of fundamental importance and should be adapted to the individual patient to get the most out of it, however this would be an interesting area for future research, the data deriving from ongoing clinical studies will respond to our questions.

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