

Comparison of treatment of COVID-19 with inhaled bromhexine, higher doses of colchicine and hymecromone with WHO-recommended paxlovid, molnupiravir, remdesivir, anti-IL-6 receptor antibodies and baricitinib

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Abstract

Millions of publications and thousands of clinical trials have not led to the discovery of an effective treatment for COVID-19. We believe that the reason for this is the inaccurate strategy of inhibiting target molecules involved in the pathogenesis of the disease.

The leading cause of death in COVID-19 is the cytokine storm, which is caused by an NLRP3 inflammasome hyperreaction. WHO recommends for the outpatients treatment drugs blocking the replication of SARS-CoV-2. However, viral load and replication are not directly related to NLRP3 inflammasome hyperreactivity. This also explains the partial success of the WHO favorite paxlovid to reduce hospitalizations (51%). For hospital treatment, WHO suggests antibodies against the interleukin-6 receptor and Janus kinase (JAK) inhibition. Although important, IL-6 is one of dozens of cytokines elevated as a consequence of cytokine storm. The JAK inhibitor baricitinib inhibited the effect of not only IL-6 but also other elevated cytokines. But if the NLRP3 inflammasome is inhibited, the cytokines will not be elevated, and there will be no need for baricitinib. All medicines recommended by the WHO are distinguished by their very high prices.

Our therapeutic strategy is based on inhibition of SARS-CoV-2 entry into the cell and inhibition of the NLRP3 inflammasome. We offer two readily available, cheap and well-known medications - bromhexine hydrochloride and colchicine. The many studies on the treatment of COVID-19 so far have not produced the expected result. The devil is buried in the details.

For bromhexine, the reason is the way and its late application. Bromhexine is most effective when given prophylactically or started by inhalation after contact with a person with COVID-19. Its earliest possible application is crucial for its effect.

Increased doses of colchicine are necessary for COVID-19 treatment due to the fact that it accumulates in leukocytes, and this leads to inhibition of NLRP3. The high doses we administer have been given widely in the past and are completely safe. Our highest dose is about 5 times lower per kg of weight than the lowest severe toxic dose of colchicine described. Our results show about a 5-fold decrease in hospital mortality and almost complete prevention of hospitalizations if outpatients are treated with inhaled bromhexine and colchicine.

Keywords

COVID-19, cytokine storm, colchicine, bromhexine, hymecromone, paxlovid, molnupiravir, remdesivir, baricitinib, tocilizumab

Disease – COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes disease, COVID-19, which presents an array of clinical severity from asymptomatic through severe disease progressing to acute respiratory distress syndrome (ARDS) with systemic inflammatory response syndrome (SIRS), shock and multiorgan dysfunction, coagulopathy, and death (Zhou et al. 2020a).

Flow

Around 80% of patients have minor and nonspecific symptoms and recover without specific treatment (benign infection/“mild” COVID-19). Around 7–10 days from the onset of the disease about 20% of patients have sudden and rapidly progressing clinical deterioration, requiring hospitalization. Of these, 15% develop “severe” COVID-19 (systemic hyperinflammation and ARDS), and 5% “critical” COVID-19 requiring special assistance critical care management (Fu et al. 2020a; Siddiqi and Mehra 2020; Wu and McGoogan 2020; Zhou et al. 2020b).

Laboratory characteristics of COVID-19 complications

The unexpected aggravation of symptoms (fever, dyspnea) correlates with increased levels of acute phase reactants (ESR, CRP, ferritin), coagulopathy (elevated titers of D-dimers, disseminated intravascular coagulation), and cell lysis (CK, LDH) (Grasselli et al. 2020; Huang et al. 2020; Wang et al. 2020; Zhang et al. 2020b; Zhou et al. 2020a). In the most severe patients, clinical and laboratory parameters correlate with increased levels of proinflammatory cytokines (IL-1 β , IL-1Ra, IL-6, TNF- α , sIL2-R α etc.), evocative of a cytokine storm (Chen et al. 2020; McGonagle et al. 2020; Mehta et al. 2020; Zhou et al. 2020a).

Etiological agent – SARS-CoV-2

The viruses enter cells by binding to their cognate cell surface receptors. The expression and distribution of these receptors therefore regulates their tropism, determining the tissues that are infected and thus disease pathogenesis (Matheson and Lehner 2020). SARS-CoV-2 is the third human coronavirus who uses the peptidase angiotensin-converting enzyme 2 (ACE2) for cell entry (Andersen et al. 2020).

Like other coronaviruses, SARS-CoV-2 cell entry is carried out by the 180-kDa spike (S) protein, which mediates the binding to ACE2 by the amino-terminal region, and the fusion of viral and cellular membranes through the carboxyl-terminal region (Li 2015). Infection of lung cells requires host proteolytic activation of spike protein at a polybasic furin cleavage site which is absent from SARS-CoV (Hoffmann et al. 2020a).

Membrane fusion also requires cleavage by the transmembrane protease serine subtype 2 (TMPRSS2), a host

cell surface protease that cleaves spike protein shortly after binding ACE2 (Hoffmann et al. 2020a). SARS-CoV-2 tropism is therefore dependent on expression of cellular proteases, as well as ACE2.

Cause of mortality

The main cause of mortality is the cytokine storm (CS) leading to the severe symptoms of ARDS, which has been shown to be the cause of mortality in 70% of COVID-19 deaths (Hojo et al. 2020; Mehta et al. 2020; Ruan et al. 2020).

Following the binding of SARS-CoV-2 to ACE2, the levels of bradykinin (BR) and des-Arg (9)-bradykinin (DABK) increase (bradykinin storm/BS), resulting in vasodilation and leakage of hyaluronic acid (HA) into the alveoli (HA storm). The BR storm is also associated with the upregulation of proinflammatory cytokines, strengthening the CS. The high cytokine levels are strong inducers of HAS2 (HA synthase 2) leading to a dramatic increase in HA. These feedback loops are a typical vicious circle (Mondeshki et al. 2022).

COVID-19 mortality is approximately 3.7%, compared with a mortality rate of less than 1% from influenza (Schulert and Grom 2015; Mehta et al. 2020).

Cytokine storm

While the hypercytokinemia should be regarded as a general marker of SARS-CoV-2, the CS is a critical life-threatening condition with overwhelming systemic inflammation leading to ARDS, hemodynamic instability, disseminated intravascular coagulation or multiple organ failure having a quite high mortality. The trigger for CS is an uncontrolled immune response resulting in continuous activation and expansion of immune cells, lymphocytes, and macrophages, which produce immense amounts of cytokines, including the proinflammatory cytokines IL-1, IL-6, IL-18, IFN- γ , and TNF- α (Shimizu 2019). Thus, the CS is an abnormal inflammatory response causing organ injury (Vitiello and Ferrara 2021).

Cytokines are very powerful mediators which level is increased in COVID-19. Compared to healthy subjects, 38 out of 48 cytokines were remarkably elevated in patients with COVID-19 including IL-6, IL-2, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), IFN- γ , inducible protein (IP)-10, TNF- α , MCP-1, macrophage inflammatory protein (MIP)-1 α , which play a crucial role in the pathogenesis of COVID-19 (Hirano and Murakami 2020). In addition, there was a strong linear association between severe lung injury and the level of 15 cytokines including, IFN- γ , IFN- α 2, IL-1ra, IL-2, 4, 7, 10, 12, and 17, as well as chemokines such as IP-10, macrophage colony-stimulating factor (M-CSF) and G-CSF. The levels of Th1, Th2, and Th17 cells were increased, too (Liu et al. 2020).

The circulating levels of interleukins (such as IL-1 β , IL-2R, IL-4, IL-6, IL-7, IL-8 (CXCL8), IL-10, IL-12, IL-17, and IL-18), interferon- γ (IFN- γ), tumor necrosis factor- α

(TNF- α), TGF- β and NF- κ B play major roles in the body's inflammatory response to SARS-CoV-2 infection and are potential risk factors for severity and high mortality in COVID-19. (Hasanvand 2022; Hu et al. 2022).

Elevated circulating levels of IL-9, IL-22, IL-33, granulocyte-colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), chemokine (C-X-C motif) ligand (CXCL)10, monocyte chemoattractant protein 1 (CCL2 or MCP-1), macrophage inflammatory protein 1 (MIP-1)A (CCL3), CX3CL1 and MIP-1B (CCL4) are also reported (Hsu et al. 2020).

However, it seems that the main contributors to the interplay of the cytokine storm are IL-6 and TNF- α , which also partake in the molecular pathogenesis of fibrosis (Kovalchuk et al. 2021).

The aberrant activated NLRP3 inflammasome cause the cytokine storm

A large amount of data demonstrates the central role of NLRP3 Nod-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome for induction of cytokine storm in the pathogenesis of severe COVID-19. Generally, NLRP3 inflammasome activation intensely induces cytokine production as an inflammatory response to viral infection (Zhao et al. 2021). The magnitude of NLRP3 inflammasome activation directly correlates with COVID-19 outcomes (Courjon et al. 2021; Ferreira et al. 2021; Rodrigues et al. 2021; Gholaminejhad et al. 2022; Junqueira et al. 2022; Maes et al. 2022; Sefik et al. 2022; Silva et al. 2022; Sun et al. 2022). There are numerous studies that implicate the NLRP3 inflammasome and IL-1 β in mediating inflammation during lung injury and ARDS (Patton et al. 1995; Ganter et al. 2008; Kolb et al. 2021). The aberrant activated NLRP3 inflammasome plays a key role for the massive production of cytokines and the generation of CS (Vitiello and Ferrara 2021).

The NLRP3 inflammasome is a critical component of the innate immune system and its aberrant activation has been linked with several inflammatory disorders (Merad and Martin 2020) induced by COVID-19, causing lung injury, cardiac injury, renal injury (Kelley et al. 2019).

Can SARS-CoV-2 activate NLRP3 inflammasome?

SARS-CoV and SARS-CoV-2 can activate NLRP3 inflammasome directly or indirectly (via diverse cellular signaling mechanisms). SARS-CoV viroporins E, ORF3a and ORF8a have ion channel activity. In general, K⁺ efflux is a crucial step for NLRP3 inflammasome activation. SARS-CoV-2 ORF3a protein induces K⁺ efflux and ROS production, thus hyperactivating NLRP3 inflammasome (Xu et al. 2022), while Ca²⁺ transport through the SARS-CoV E protein channel does the same. In macrophages, SARS-CoV

ORF8b protein induces NLRP3 inflammasome activation directly by binding to the NLRP3 LRR domain (Zhao et al. 2021). SARS-CoV-2 ORF8b and N proteins activate NLRP3 inflammasome and subsequent hyperinflammation through direct interactions (Kaivola et al. 2021; Pan et al. 2021). Ang II accumulation and the complement cascade induced by SARS-CoV-2 infection activates the NLRP3 inflammasome. Notably, NLRP3 inflammasome activation has been considered a vital function of endothelial cells in COVID-19 inflammation (Zhao et al. 2021). In addition, NSP6 (non-structural protein 6) of SARS-CoV-2 activates the NLRP3 inflammasome by targeting a vacuolar ATPase proton pump component (Sun et al. 2022).

Which cells are responsible for the development of the cytokine storm?

Myeloid cells (granulocytes, monocytes, macrophages, and dendritic cells) are a major source of dysregulated inflammation in COVID-19. In COVID-19 lungs, myeloid cells were more prevalent there than in control lungs (Melms et al. 2021). Granulocytes (neutrophils, eosinophils, and basophils) are the most abundant type of myeloid cells in the blood stream as neutrophils are the most frequent granulocytes (De Kleer et al. 2021). SARS-CoV-2 efficiently infects human monocytes and macrophages (Knoll et al. 2021). Macrophages are possibly the most effective cell types that trigger inflammasome activation (Broz and Dixit 2016; Merad and Martin 2020). Thus, in COVID-19 patients there is high level of viral RNA in lung monocytes and macrophages (Delorey et al. 2021; Pontelli et al. 2022), as well as pronounced NLRP3 inflammasome activation in macrophages (Rodrigues et al. 2021; Junqueira et al. 2022; Sefik et al. 2022). The NLRP3 inflammasome activation in macrophages is closely associated with the coagulopathy that is observed in severe COVID-19 (Keyla et al. 2022).

Dysregulated activation of the mononuclear phagocyte (MNP) compartment contributes to COVID-19-associated hyperinflammation (Schulert and Grom 2015; Mehta et al. 2020).

It is recognized that neutrophils play a major role in lung tissue injury by their rapid recruitment and their release of proteases (myeloperoxidase (MPO), elastase, matrix metalloproteases (MMP), free oxygen radicals and neutrophils extracellular traps (NETs) (Prame et al. 2018). While neutrophils play a critical role in acute lung injury (ALI), macrophages and monocytes orchestrate resolution of inflammation and tissue repair (Herold et al. 2011).

Why does SARS-CoV-2 spread so easily?

SARS-CoV-2 must initially enter cells lining the respiratory tract where ACE2 and TMPRSS2 expression is highest

in ciliated nasal epithelial cells, with lesser amounts in ciliated bronchial epithelial cells and type II alveolar epithelial cells (Hou et al. 2020). This translates to greater permissivity of upper versus lower respiratory tract epithelial cells for SARS-CoV-2 infection in vitro and fits disease pathology: Upper respiratory tract symptoms are common early in disease (Wölfel et al. 2020).

In contrast to SARS-CoV, infectivity of patients with SARS-CoV-2 peaks before symptom onset. Thus, about 44% of secondary cases were infected during the index cases' presymptomatic stage. Because at first the infected person is clinically healthy and infects others, and when the first symptoms appear, the viral load is already at its maximum. Indeed, presymptomatic transmission makes SARS-CoV-2 impossible to contain through case isolation alone and is a key driver of the pandemic (He et al. 2020). This alteration in the pattern of disease may relate to the acquisition of the furin cleavage site in spike protein or increased binding affinity for ACE2 in SARS-CoV-2, compared with SARS-CoV (Wrapp et al. 2020).

Is there a connection between viral load, replication and the hyperactivation of NLRP3 inflammasome?

There is no direct relationship between viral load/titers and the hyperactivation of the NLRP3 inflammasome, leading to cytokine storm and tissue injury. It is not viral replication that causes cytokine storm and multiorgan failure; rather, it is the result of the abnormal response of the NLRP3 inflammasome (Freeman and Swartz 2020; Fu et al. 2020b; Fung et al. 2020; Mehta et al. 2020; Zhang et al. 2020a; Zhao 2020; Zhao et al. 2021). The viral load in the lungs of COVID-19 patients is inversely proportional to the duration of the disease, but the NLRP3 inflammasome activation occurs not as a result of the viral infection itself and the inflammation can persist, whereas the viral load decline (Keyla et al. 2022).

Interestingly, ARDS occurs in SARS-CoV patients despite a diminishing viral load, suggesting that exuberant host immune response may be responsible for this outcome rather than viral virulence (Jamilloux et al. 2020).

In consideration of direct acting anti-viral agents, viral load appears non- or minimally consequential in determining SARS-CoV and SARS-CoV-2 disease outcomes. Thus, it is not viral replication or infection that causes tissue injury; rather, it is the result of dysregulated hyperinflammation in response to viral infection. Hyperinflammation is due to hyperactivation of the NLRP3 inflammasome mostly in white blood cells (Freeman and Swartz 2020; Fu et al. 2020b; Fung et al. 2020; Mehta et al. 2020; Zhang et al. 2020a). In conclusion, NLRP3 hyperactivation may occur at a lower viral load and conversely, at a higher viral load there may be no hyperactivation of NLRP3 inflammasome.

You are patient with confirmed Covid-19 - what doing?

100 years ago (1918–1920) the world was ravaged by a pandemic caused by the H1N1 virus ("spanish flu"). The death rate is many times higher than during the first world war. Figures range from 20 to 150 million, with the most reliable being 50 million deaths, for an earth population of 1.8 billion (Barry 2004). Now, with tremendously higher medical development, the official SARS-CoV2 victims so far are over 6,955,497 million (from 769,806,130 confirmed cases of COVID-19) for an earth population of 8.1075 billion (<https://covid19.who.int>). But according to the WHO, the deaths from COVID-19 in the world are three times more or 14.9 million for the period 2020–2021 (<https://www.whitehouse.gov/cea/written-materials/2022/07/12/excess-mortality-during-the-pandemic-the-role-of-health-insurance/>). What both H1N1 and SARS-CoV-2 viruses have in common is their ability to hyperactivate the NLRP3 inflammasome, leading to a CS, pneumonia, ARDS, multi-organ damage and death. The H1N1 virus was particularly deadly because it triggered a CS, ravaging the stronger immune system of young adults (Barry 2004). It is extremely interesting that during the Spanish flu, mostly young individuals died (Gagnon et al. 2013), while now mostly adults are affected. An enormous intellectual and financial resource was thrown into the fight against Covid-19 and still there is no satisfactory answer to this key age distribution question. Perhaps there is some "age factor" that was protective for adults during the Spanish flu and lethal for them during the COVID-19 pandemic. Only the viruses are different, the lethal pathway is the same – hyperactivated NLRP3 inflammasome – cytokine storm – death.

If a reference is made in Google Scholar with the keyword "COVID -19" they come up above 4 620 000 results. Thousands of clinical trials (including Drug Studies) of potential COVID-19 treatments and interventions have been launched worldwide (<https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-therapeutics-2022.4/>; <https://ecrin.org/covid-19-trials-registries>; <https://www.who.int/clinical-trials-registry-platform>). The practical result of the vast number of Covid-19 studies is extremely disappointing.

World Health Organization (WHO) recommends drugs that inhibit SARS-CoV-2 replication, the IL-6 receptor, and the tyrosine kinase JAK. Our treatment strategy targets the inhibition of SARS-CoV-2 entry into the cell and the NLRP3 inflammasome as the source of the cytokine storm (Fig. 1).

World Health Organization recommendations for Covid-19 treatment

WHO recommends for outpatient treatment paxlovid, molnupinavir and remdesivir and for inpatient treatment corticosteroids, IL-6 receptor blockers (tocilizumab and sarilumab), the immunomodulator baricitinib (olumiant)

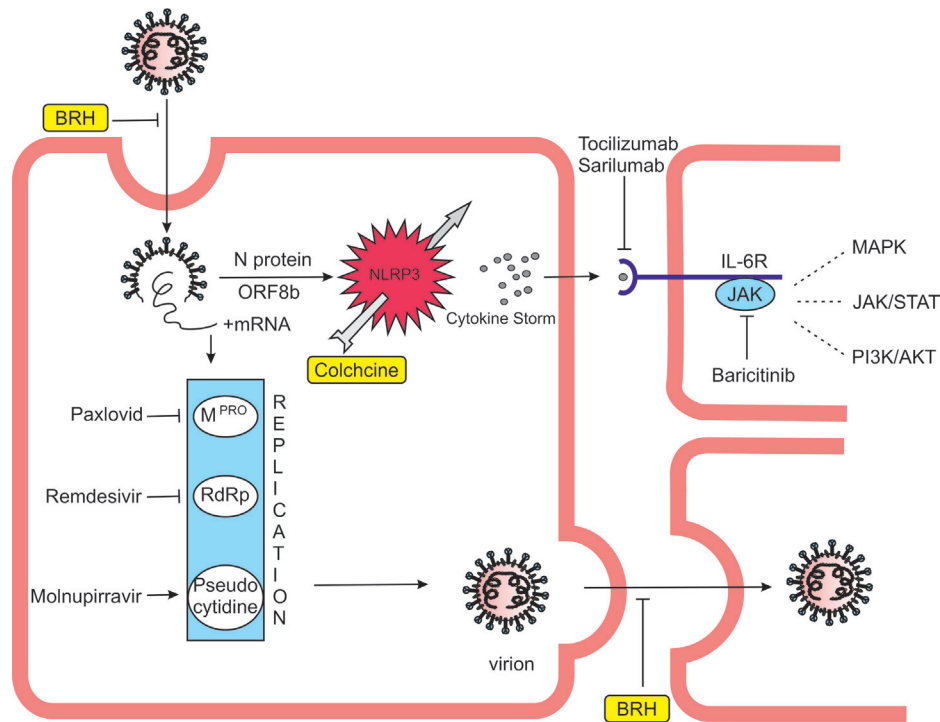


Figure 1. Schematic representation of two different strategies for treating COVID-19. WHO recommends drugs that inhibit SARS-CoV-2 replication, the IL-6 receptor, and the tyrosine kinase JAK. Our treatment strategy targets the inhibition of SARS-CoV-2 entry into the cell and the infection of other cells by the virion, and as well as inhibition of the NLRP3 inflammasome, as the source of the cytokine storm.

and remdesivir. That's all (<https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-therapeutics-2022.4/>).

Outpatients treatment

Paxlovid – the WHO favorite

According to the recommendations of World Health Organization (WHO), if we have non-severe Covid-19 and if we are with high risk of hospital admission, ritonavir-boosted nirmatrelvir (paxlovid) is “strongly recommended in favor”. According to the manufacturer Pfizer Inc. (New York, NY, USA), paxlovid had an 89% reduction in the risk of hospitalization and death in unvaccinated people (Mahase 2021). However, “Centers for Disease Control & Prevention (CDC)” is more pessimistic, reporting on a real-world study that showed adults (vaccinated or with a previous infection), who took paxlovid had a 51% lower hospitalization rate within the next 30 days than those who were not given the drug. (Shah et al. 2022). There have been reports of a “rebound” of COVID-19 symptoms in some people within 2 to 8 days after completing the five-day course of paxlovid. It should be also noted that there is a long list of medications Paxlovid may interact with, and in some cases, doctors may not prescribe paxlovid because these interactions may cause serious complications (<https://www.yalemedicine.org>)

Molnupiravir (Lagevrio)

In the column “weak or conditional recommendation in favour” is the low-potency (30% reduction in the risk for

hospitalization and death) and mutagenically questionable but expensive molnupiravir (lagevrio) from Merck&Co Inc. (Rahway, NJ, USA) (<https://www.merck.com/news/merck-and-ridgeback-biotherapeutics-provide-update-on-results-from-move-out-study-of-molnupiravir-an-investigational-oral-antiviral-medicine-in-at-risk-adults-with-mild-to-moderate-covid-19/>). That's why the WHO is insured with: “Mitigation strategies to reduce potential harms should be implemented”. However, molnupiravir (pseudo cytidine) has already been fully compromised and rejected by the European Medicines Agency (EMA). Logically, On 21 June 2023, Merck Sharp & Dohme B.V. withdrew its application for a marketing authorisation of Lagevrio for the treatment of COVID-19 in adults (<https://www.ema.europa.eu/en/medicines/human/withdrawn-applications/lagevrio>).

Remdesivir

In the same column is the resurrected RdRp inhibitor remdesivir (an agent administered intravenously), rejected earlier by the WHO (WHO Solidarity Trial Consortium 2021), whose course of treatment is more expensive than that of molnupiravir. Remdesivir, approved for use in patients hospitalized for Covid-19, has shown an 87% reduction in the risk of progression to severe disease in the outpatient setting (Gottlieb et al. 2022).

Remdesivir may cause serious allergic reactions, including infusion-related reactions and anaphylaxis, drug-induced liver injury (DILI) leading to acute liver failure (ALF) and death (Ahmed-Khan et al. 2023).

According to the instructions of Mayo Clinic (Rochester, MN, USA), “this medicine is to be given only by or under the immediate supervision of your doctor” (<https://www.mayoclinic.org/drugs-supplements/remdesivir-intravenous-route/side-effects/drg-20503608>). It is difficult to imagine how the treatment of outpatients with the very expensive remdesivir will be organized.

Inpatient treatment

For severe and critical Covid-19 WHO *strongly recommend in favor* corticosteroids, IL-6 receptor blockers (tocilizumab and sarilumab) and the immunomodulator baricitinib (olumiant), which is a JAK1/2 inhibitor. Corticosteroids are the only generally accepted low-cost medications for the treatment of hospitalized patients with Covid-19.

Tocilizumab and sarilumab

Interleukin (IL)-6 is a pleiotropic, proinflammatory cytokine produced by a variety of cell types, including lymphocytes, monocytes, and fibroblasts in large amounts, due to the hyperactivated NLRP3 inflammasome in severe Covid-19 (Kovalchuk et al. 2021). IL-6, as several other cytokines increased during the cytokine storm activate JAK/STAT signal transduction pathway, contributes to the exacerbation of the cytokine storm. If the NLRP3 inflammasome is inhibited, the cytokine level normalizes and there is nothing to hyperactivate the JAK. And what would happen after stopping administration of anti-IL-6R antibodies, given that the NLRP3 inflammasome remains hyperactive, as well as the high levels of IL-6?

In other words, inhibiting the NLRP3 inflammasome renders the use of IL-6 receptor blockers and baricitinib pointless.

Baricitinib

Baricitinib is an ATP competitive kinase inhibitor that inhibits selectively, effectively, and reversibly JAK1/JAK2. The ACE2 receptor has several regulators among which AP2-associated protein kinase-1 (AAK1) and cyclin G-associated kinase (GAK), two pivotal regulators, mediate clathrin-dependent endocytosis. Baricitinib was expected to have a high binding affinity to AAK1 and GAK and interrupt the passage and intracellular assembly of SARS-CoV-2 into the target cells (Lu et al. 2020).

Remdesivir is with weak or conditional recommendation in favour

The RNA-dependent RNA polymerase (RdRp) intravenous inhibitor remdesivir may cause serious allergic reactions, gastrointestinal symptoms (e.g., nausea), an increase in prothrombin time, elevated transaminase levels, DILI leading ALF and death. Before starting patients on remdesivir, the FDA recommends performing liver function and prothrombin time tests as clinically appropriate and repeating these tests during treatment as clinically indicated (Ahmed-Khan et al. 2023; <https://www.covid19treatmentguidelines.nih.gov/therapies/antivirals-including-antibody-products/remdesivir/>).

The Solidarity trial among COVID-19 inpatients reports that remdesivir has no significant effect on patients with COVID-19 who are already being ventilated. Among other hospitalised patients, it has a small effect against death or progression to ventilation (or both) (WHO Solidarity Trial Consortium 2022).

In conclusion, none of these WHO-recommended medications have been proven to be a definitive cure.

Price list of WHO-recommended Medicines

The cheap colchicine belongs to the WHO group “*strong recommendation against*” (<https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-therapeutics-2022.4/>).

The cost for colchicine oral tablet 0.6 mg is around \$24 for a supply of 30 tablets for cash-paying customers [<https://www.drugs.com/price-guide/colchicine/>].

Table 1. Prices of WHO-recommended anti-COVID-19 drugs.

Medicine	Price
Paxlovid	\$530
Molnupiravir	\$700
Remdesivir	\$2340
Tocilizumab	\$410–3635
Baricitinib	\$1307

Table data sources: [Tocilizumab cost effective in reducing COVID-19-related deaths. Pharmacoecon Outcomes News. 2021; 879(1): 28. <https://doi.org/10.1007/s40274-021-7751-7>; <https://reliefweb.int/report/world/tocilizumab-second-drug-ever-recommended-who-covid-19-will-remain-unaffordable-and>; <https://www.fiercepharma.com/marketing/gilead-prices-covid-19-drug-remdesivir-line-cost-analysis>; <https://www.reuters.com/business/healthcare-pharmaceuticals/price-covid-treatments-pfizer-merck-gsk-align-with-patient-benefits-report-2022-02-03/>; <https://pubmed.ncbi.nlm.nih.gov/34807369/>].

Treatment of Covid-19 with inhaled bromhexine, higher doses of colchicine and hycromone

Why Bromhexine hydrochloride?

The mucolytic cough suppressant bromhexine hydrochloride (BHH) is an over-the-counter, non-invasive, effective, well-tolerated, safe, affordable and low cost drug introduced in 1963. It has been used for treatment of ARDS, bronchitis, asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and children with respiratory disorders. (Al-Kuraishy et al. 2021; Barzegar et al. 2021).

BHH has a selective inhibitory effect on TMPRSS2 due to its bromide derivative, thus inhibiting the SARS-CoV-2 entry into the cell (Lucas et al. 2014; Hoffmann et al. 2020b). In addition, BHH demonstrates an antiviral effect because it has a high binding affinity to the cysteine protease 3Clpro, inhibiting its replication and disrupting the viral replication (Bahadoram et al. 2022).

Thus, is possible that BHH, by inhibiting TMPRSS2, can prevent the SARS-CoV-2 cell entry and disrupt the viral replication by blocking 3Clpro.

What are the results so far for treating COVID-19 with BHH?

There is conflicting data about the role of BHH in the prevention and treatment of COVID-19 (Ansarin et al. 2020; Depfenhart et al. 2020; Fu et al. 2021; Tolouian and Mulla 2021; Tolouian et al. 2021, 2022; Vila Méndez et al. 2022).

In sum, there is no convincing evidence for the effect of BHH in the treatment of COVID-19.

What accounts for the discrepancy between expected and actual results in the use of BHH for the treatment of COVID-19?

The main reasons are not the doses, but the delayed reception and the mode of BHH administration (Mitev 2022; Mitev and Mondeshki 2022, Mitev et al. 2022, 2023; Mondeshki et al. 2022). As noted above, when the first symptoms appear, the viral load is already at its maximum (He et al. 2020). Therefore, even if the treatment with BHH starts from the first day of symptom onset, the virus has already penetrated the cells! This significantly reduces the efficacy of BHH, especially if not inhaled (Mitev and Mondeshki 2022).

When and how to apply BHH to achieve maximum effect in the treatment of COVID-19

A) It is most effective when taken prophylactically during another COVID-19 wave. For example, 3×1 pill daily for 1 month.

We have reported a number of cases of taking BHH prophylactically in the dose and duration mentioned. Upon contact with infected people, they either do not get sick or pass the disease mildly. It is remarkable that in a family of five, all taking prophylactic BHH, only the husband (67 years old) fell slightly ill, while the wife and children did not fall ill [Authors personal communications: Mitev V. Prevention and outpatient treatment of COVID-19. “23rd Medicine and Football International Scientific Conference”, 15 November 2021; oral presentation; Mitev V. COVID-19. “26th Medicine and Football International Scientific Conference”, 7 April 2023; oral presentation].

Other authors also report the benefit of prophylaxis with BHH (Mikhaylov et al. 2022). The prophylactic effect of BHH, which is achieved by its ability to prevent viral penetration into host cells can distinguish its effective mechanism against SARS-CoV-2 infection from other standard treatments (Tolouian et al. 2021).

B). When someone has been in contact with an infected or sick person, bromhexine inhalations should be started immediately. The effect is very good.

In this case, the response time and the mode of application is of leading importance. If the BHH inhalations start immediately, the probability that the contact person will not get sick is high [Authors’ personal communication: Mitev V. COVID-19. “26th Medicine and Football

International Scientific Conference”, 7 April 2023; oral presentation]. The immediate initiation of BRH nebulizers is of paramount importance for successful prevention and treatment of COVID-19 outpatients. The inhalatory form of BRH applies as 4×1 or 3×1 ampoules (one ampoule of 2 ml contains 4 mg BRH), diluted 1:1 (up to 1:3) with saline (Mitev et al. 2022, 2023; Mondeshki et al. 2022). As noted by others, the therapeutic window to prevent replication and spread of SARS-CoV-2 is relatively short and direct acting antivirals, such as TMPRSS2 inhibitors, should be administered as early as possible. (Jamilloux et al. 2020; To et al. 2020). In support of our BRH treatment strategy, Wettstein L, et al. suggest that targeted administration of TMPRSS2 inhibitors via inhalation might suppress SARS-CoV-2 more efficiently than systemic administration (Wettstein et al. 2022).

C). The COVID-19 patient should immediately start bromhexine inhalations, as we have described. The effect is good because the spread of the virus from cell to cell will be limited. The effect of BHH on 3CLpro and SARS-CoV-2 replication should also be considered. (Bahadoram et al. 2022; Mitev 2022; Mitev and Mondeshki 2022).

D). In hospital conditions, inhalations with BHH will be useful, although there, in the first place, the question of inhibition of NLRP3 inflammasome is leading (Mondeshki et al. 2022; Mitev et al. 2023b). As noted there, “In clinical practice, the benefits of using bromhexine in COVID-19 far outweigh the risk for now” (Tolouian and Mulla 2021).

Colchicine

In the Ebers papyrus of ancient Egypt in 1550 BC colchicine first has been described as a treatment for pain and swelling (Nerlekar et al. 2014).

Colchicine properties and mechanisms of action

Colchicine is an affordable low-cost drug, proven effective in the treatment and prevention of inflammatory disorders characterized by dysregulated inflammation including gout, familial Mediterranean fever, Behçet’s disease and pericarditis. Colchicine prevents recurrent events after acute coronary syndrome, characterized by inflammation with neutrophils and macrophages rich plaques (Tardif et al. 2019), abrogated NETs formation in acute coronary syndrome patients after percutaneous coronary intervention (Vaidya et al. 2021) and in patients with active Behçet’s disease (Safi et al. 2018).

In vitro, at micromolar concentrations, colchicine inhibits NLRP3 inflammasome, blocks the release of IL-1 β , and suppresses the expression of genes involved in cell regulation (Ding et al. 1990; Martinon et al. 2006; Jackman et al. 2009; Misawa et al. 2013).

At higher concentrations colchicine (IC₅₀ = 300 nM) diminishes the quantitative expression of E-selectin on neutrophils (Cronstein et al. 1995).

At nanomolar concentrations (IC₅₀ = 3 nM), colchicine can decrease IL-1–induced L-selectin expression on neutrophils (Cronstein et al. 1995) and increase the expression of E-selectin at the cell surface on endothelial cells

by inhibition of the internalization process (Kuijpers et al. 1994), can modulate cytokine maturation and release, and diminishes neutrophil chemotaxis to cytokines (Paschke et al. 2013).

Colchicine can also suppress the expression of NF- κ B (Jackman et al. 2009), decrease the number of TNF- α receptors on the surface of macrophages and endothelial cells (Ding et al. 1990), inhibit superoxide anion production (Roberge et al. 1996; Chia et al. 2008), interrupt mast cell degranulation process (Oka et al. 2005) increase the level of TGF- β 1 (Yagnik et al. 2004; Sayarlioglu et al. 2006).

In circulating monocytes 1 mg colchicine followed by 0.5 mg 1 h later markedly suppresses caspase-1 activity and monocyte secretion of IL-1 β is reduced (Robertson et al. 2016).

It also reduces leukocytes inflammation by inhibition of NLRP3 inflammasome and caspase-1, and therefore leading to secondary reductions in cytokines such as IL-1 β , TNF- α and IL-6 (Martinon et al. 2006; Martínez et al. 2015; Otani et al. 2016). Colchicine's effect on NLRP3 inflammasome inhibition may be upstream of, rather than directly on this complex (Martinon et al. 2006).

In leukocytes, colchicine binds to free tubulin dimers, thus inhibiting microtubule polymerization reducing their adhesion, recruitment and activation. This leads to inhibition of vesicle transport, cytokine secretion, phagocytosis, migration and division. At higher concentrations colchicine may also induce some microtubule dissociation (Taylor 1965).

In neutrophils, colchicine inhibits intracellular signaling molecules, inhibits chemotaxis and lysosomal enzyme release during phagocytosis. (Terkeltaub 2009), modulates

their deformability to suppress neutrophil extravasation (Paschke et al. 2013), inhibits superoxide anion production and increases leukocyte cAMP, which suppress neutrophil function (Rudolph et al. 1997).

In macrophages colchicine inhibits activation of P2X2 and P2X7 receptors, NLRP3 inflammasome, the RhoA/Rho effector kinase (ROCK) pathway, the activation of caspase-1, the release of reactive oxygen (ROS), nitrite oxide (NO) and tumor necrosis factor (TNF) α (Leung et al. 2015). (Fig. 2)

Colchicine accumulates in leukocytes!

While it is recognized that polymorphonuclear neutrophils play a critical role in acute lung injury (ALI), macrophages and monocytes orchestrate resolution of inflammation and tissue repair, having a key role in the pathology of COVID-19 (Herold et al. 2011; Wong et al. 2019; Dupuis et al. 2020).

NLRP3 inflammasome inhibition has been assessed at colchicine concentrations 10- to 100-fold higher than those achieved in serum (Cronstein and Sunkureddi 2013). However, the nonspecific inhibitor of the NLRP3 inflammasome, colchicine, is unique in that it can accumulate in white blood cells. Peak plasma concentrations occur 1h after administration and maximal anti-inflammatory effects develop over 24–48h based on intra-leukocyte accumulation. Colchicine reaches much higher concentrations within leukocytes than in plasma and persists there for several days after ingestion (Dupuis et al. 2020). Whereas plasma concentration after single dosing of 0.6-mg colchicine is approximately 3 nmol/L, it has been shown to accumulate in a saturable manner in neutrophils

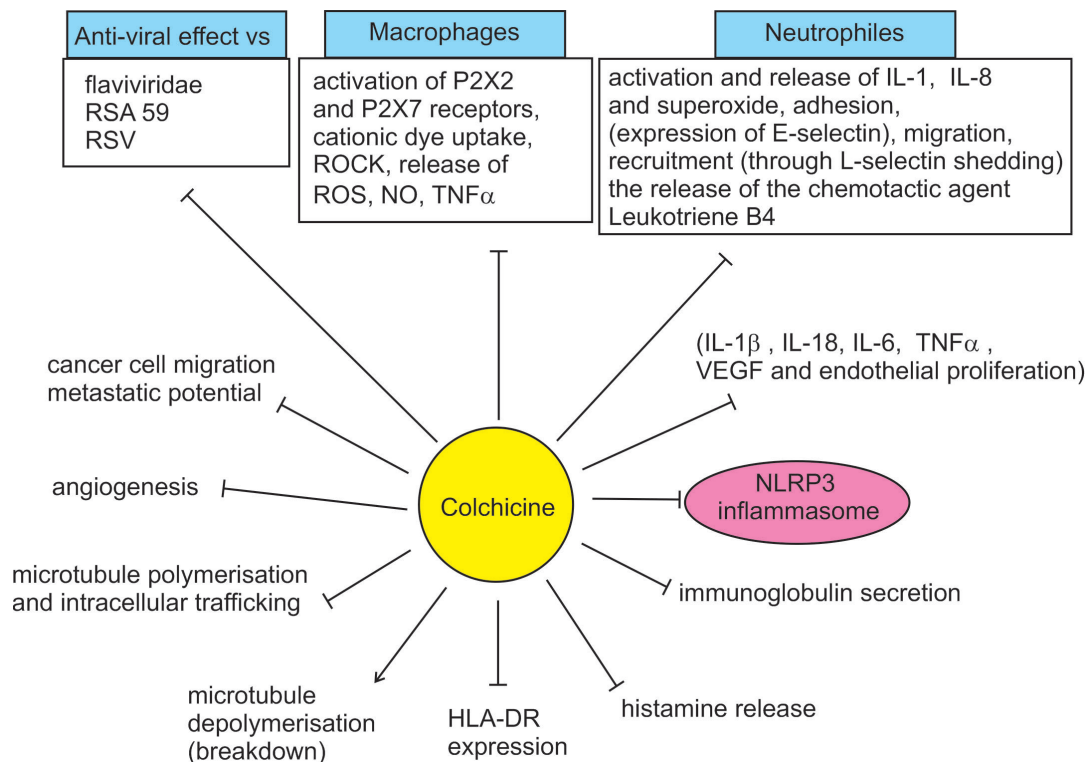


Figure 2. Effects of colchicine.

to 40 to 200 nmol/L, well above its K_i of 24 nmol/L for microtubule polymerization (Sherline et al. 1975; Chappey et al. 1994).

Why attempts to prove a curative effect of colchicine against COVID-19 have failed?

Given the multiple anti-inflammatory and anti-Covid-19 effects of colchicine *in vitro*, several case reports, over 50 observational studies and randomized clinical trials, small randomized non-controlled trials and retrospective cohort studies were initiated to test its healing effect *in vivo*, leading to conflicting, rather negative results (Mikolajewska et al. 2021).

The early advocates of the positive effect of colchicine (Scarsi et al. 2020) were later unable to confirm the curative effect of colchicine at standard colchicine doses (Perricone et al. 2023).

Common to all available publications studying colchicine as part of COVID-19 treatment regimens is that they are based on standard colchicine doses. The current maximum loading dose is 1.5 mg followed by 0.5 mg in 60 minutes. The maintenance daily dose does not exceed 1 mg (Mikolajewska et al. 2021).

As we noted before, the use of only low doses of colchicine once again confirms what Einstein said that “Insanity is doing the same thing over and over and expecting different results” (Mitev et al. 2023b).

Of all clinical trials with colchicine, only one gave two different doses – low (1.6 mg) and high (4.8 for 6 hours), but this was for the treatment of a gout attack (Terkeltaub et al. 2010). Since it is concluded that there is no difference in the effects between low and high dose colchicine, it is appropriate to use the low dose. Thus the incidence of diarrhea will drop from 76.9% to 23 %. But COVID-19 is not gout!

Given the well-known fact that colchicine accumulates in leukocytes (Sherline et al. 1975; Chappey et al. 1994; Dupuis et al. 2020), it is inexplicable and puzzling to us why in large and noisily advertised clinical studies (Recovery Collaborative Group 2021; Tardif et al. 2021) they have not tested the effect of high doses of colchicine as it was done by Terkeltaub RA et al. (Terkeltaub et al. 2010). Moreover, the dose of 4.8mg of colchicine within 6 hours has no serious side effects, but an increase in diarrhea (Terkeltaub et al. 2010), which is not a high price to pay to save a human life (Mondeshki et al. 2023).

It should be noted in particular that in none of the studies was the weight of the patients taken into account. It is not the same to give a loading dose of 2 mg to a 50 kg patient and a hundred kg patient. The fact that obese patients have higher mortality in COVID-19 infection than non-obese patients (Tartof et al. 2020), clearly states that the result of treatment with colchicine is closely related to the dose per kg of weight. Five mg of colchicine had a dramatic effect on severely obese individuals worsening by day 7 of the onset of COVID-19 [Mitev V. COVID-19. “26th Medicine and Football International Scientific Conference”, 7 April, 2023; oral presentation].

This is why most of these studies report no or minor colchicine effect.

Our therapeutic regimen

Our therapeutic regimen is based on the assumption that safe increase in colchicine doses will result in colchicine accumulation in leukocytes sufficient to block the NLRP3 inflammasome (Mitev et al. 2022, 2023; Mondeshki et al. 2022). As the NLRP3 inflammasome is expressed largely in cells of the myeloid lineage (Centola et al. 2000), colchicine is useful mainly in treating diseases associated with neutrophils and monocytes/macrophages (i.e. innate immune system) rather than those of the adaptive immune system. However, as leukocytes accumulate colchicine, and intracellular neutrophil colchicine concentrations have been demonstrated to be much higher than plasma concentrations, clinically used colchicine doses may still be sufficient for inflammasome suppression (Wallace and Ertel 1970; Chappey et al. 1994; Leung et al. 2015).

Mortality in 452 inpatients treated with higher colchicine doses was about 5 times lower than the control group

Mortality in 452 patients from two hospital centres treated with higher colchicine doses was 4.06% compared with the control group of 213 patients without colchicine where the mortality rate was 18.3% (Combined Odds and Risks from both centres RR (0.23, 95%CI 0.136–0.387); OR (0.23, 95%CI = 0.1296–0.4068) $p < 0.0001$). Both OR (Odds ratio) and RR (Relative Risk) show a 77% reduction in the risk and odds of death in the colchicine group, which was statistically significant. The robustness of the results is confirmed by the confidence intervals (Mitev et al. 2023b).

The inhibitory effect of colchicine on cytokine storm occurs at higher doses, such as [0.5mg per 10kg body weight]-0.5mg, but not more than 5mg loading dose. This makes 0.04–0.045 mg/colchicine/kg (Mitev and Mondeshki 2022; Mitev et al. 2022, 2023; Mondeshki et al. 2022).

Another 600 inpatients were treated with similar doses of colchicine with similar results. Our data, including also more than 1000 COVID-19 outpatients, showed that nearly 100% of patients treated with this therapeutic regimen escaped hospitalization. These results have been announced in several national and international conferences [Mitev V. Prevention and outpatient treatment of COVID-19. “23th Medicine and Football International Scientific Conference”, 15 November 2021; oral presentation; Mitev V and Mondeshki T. Treatment of COVID-19 with colchicine and inhaled bromhexine. “24th Medicine and Football International Scientific Conference”, 11 April 2022; oral presentation; Mondeshki T. Therapeutic combination of inhalations with bromhexine, higher doses of colchicine, and hymecromone for COVID-19 treatment (case report). “National Cardiology Conference on Covid-19” 19–20 November, 2021, Sofia, Bulgaria) oral presentation; Marinov K. Results of treatment of 322 inpatients with colchicine. “National Cardiology Conference on Covid-19” 19–20 November, 2021, Sofia, Bulgaria) oral presentation. Mitev V. Effective hospital treatment of COVID-19. “25th Medicine and Football International Scientific Conference”,

7 November 2022; oral presentation; Mitev V. COVID-19. “26th Medicine and Football International Scientific Conference”, 7 April, 2023; oral presentation].

We described a unique effect of a wrongly taken overdose of colchicine (15 mg over 10 hours) that was sufficient to the very quick resolution of bilateral pneumonia and pericardial effusion. Two similar cases with 12 mg of colchicine were also presented and this overdose of colchicine was sufficient for the complete recovery of the two outpatients. These cases demonstrate the possibility that high colchicine doses may have a major role and a dramatic effect in the treatment of COVID-19 patients (Mondeshki et al. 2023).

Are the higher doses of colchicine dangerous?

The doses we used expectedly increased the incidence of diarrhea, but this is a small price to pay for saving human life (Mitev et al. 2022, 2023). Such doses have been used extensively in the past without life-threatening side effects for treatment of gout and familial Mediterranean fever (Pascart et al. 2016). For example, in FMF colchicine can be used up to a daily dose of 2 mg in children and 3 mg in adults, or “**the maximum tolerated dose if this cannot be appropriate**” (Ozen et al. 2016). In a number of cases, high doses of colchicine have been used or recommended (Terkeltaub et al. 2010; Ahern et al. 1987). Pediatric daily doses that range from 0.03 to 0.07 mg/kg (Pérez et al. 2021) are 2 times higher than the so-called “standard” doses between 0.015 and 0.03 mg/kg (Stack et al. 2015).

Of the thousands of outpatients and inpatients treated with our regimen, we have not had a single case of colchicine intoxication (Mitev et al. 2022, 2023). Deaths described in the distant past with colchicine doses of 7–7.5 mg were due to drug interactions (Macleod and Phillips 1947; Jarvie et al. 1979; Mitev et al. 2023a). We suggest that the sentence “The lowest reported lethal doses of oral colchicine are 7–25/26 mg” (Baud et al. 1995; Finkelstein et al. 2010; Aghabiklooei et al. 2013) needs to be redone as follows: “The lowest reported lethal doses of oral colchicine are 15–25/26 mg” (Mitev et al. 2023a). This means that the described minimum severe toxic dose of 15 mg (0.2mg/kg) (Hirayama et al. 2018) is about 5 times higher than what we used - our maximum loading dose is 5mg (0.045 mg/kg).

When administering colchicine, special attention should be paid to patients with liver and kidney damage and incompatible drug interactions.

Colchicine toxicity in drug-drug interactions

Certain drugs increase the potential for colchicine toxicity via modulation of P-glycoprotein 1 (P-gp1) and cytochrome P450 3A4 (CYP3A4) activity, because colchicine is their substrate. A typical case of toxicity at drug–drug interaction is at colchicine and clarithromycin (Yahia et al. 2018). For full list see New Zealand Formulary (NZF) [NZF v109. 2021. Available from: www.nzf.org.nz (Accessed Jul, 2021) www.nzformulary.org]. Adverse colchicine drug interactions can almost always be prevent-

ed through proper management and patient education (Hansten et al. 2023).

Detailed descriptions of dangerous life-threatening drug-drug interactions, such as pancytopenia, multiorgan failure, and cardiac arrhythmias are given in Hansten PD et al. (Hansten et al. 2023). The risk of colchicine toxicity is detailed in Colchicine – NEW ZEALAND DATA SHEET (20.11.2022) <https://www.medsafe.govt.nz>.

In conclusion, the doses we used are safe and already administered without severe side effects or life-threatening conditions. The attending physician must be familiar with the side effects of colchicine and unwanted interactions with other drugs. As colchicine is primarily eliminated by hepatobiliary and renal (10–20%) excretion, the patient should be questioned about diseases of the liver and kidneys. Other exclusion criteria should also be considered (Perricone et al. 2023). The patient should be well informed about possible risk conditions during treatment with colchicine. All this will bring the risk of colchicine intoxication to practically zero.

As the colchicine doses increase, the incidence of diarrhea also increases (Ahern et al. 1987; Terkeltaub 2009). However, the health benefits of higher doses of colchicine for the treatment of COVID-19 are many times greater than the non-life-threatening side effects.

Sometimes in severe COVID-19 patients the choice is: high doses of colchicine and diarrhea, but alive or death without diarrhea (Mondeshki et al. 2023; Mitev et al. 2023a).

The secret of colchicine’s success lies in the fact that it accumulates in leukocytes in high concentrations (Sherline et al. 1975; Chappey et al. 1994) sufficient to inhibit the NLRP3 inflammasome (Cronstein and Sunkureddi 2013) and to prevent the CS (Mitev and Mondeshki 2022; Mitev et al. 2022, 2023; Mondeshki et al. 2022, 2023). At the same time, its plasma concentrations are nanomolar, something that is unattainable for ivermectin (Momekov and Momekova 2020).

Hymecromone inhibits the hyaluronan synthesis and storm

In our therapeutic scheme for inpatients we also include the hyaluronan/hyaluronic acid (HA) synthesis inhibitor hymecromone (4 × 800 mg/d) (Mondeshki et al. 2022; Mitev et al. 2023b).

HA is an essential component of the extracellular matrix and is one of the leading factors associated with the lung edema, result in ARDS. Its overproduction and accumulation leads to absorption of high amount of water molecules due to their hygroscopic properties. Clear jelly liquid was found in lung autopsies of COVID-19 patients.

The high levels of cytokines during CS are strong inducers of *HAS2* (*HA synthase 2*) in endothelium, lung alveolar epithelial cells, and fibroblasts. This fits well with the notion that the state of “hyperinflammation” induces the production and accumulation of HA (clear jelly liquid) within the alveolar spaces of patients with severe Covid-19 (Xu et al. 2020; Yang et al. 2022).

The most susceptible population of SARS-CoV-2 have a high level of plasma HA (Ontong and Prachayasittikul 2021).

In conclusion, the treatment of COVID-19 with inhaled bromhexine, colchicine and hymecromone is much more effective, safer and cheaper than the drugs proposed by the WHO.

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