HOME PHARMACOLOGICAL THERAPY OF THE SYMPTOMATIC PATIENT

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Summary

The following paper will elaborate on the mechanisms of action and the main results of clinical trials, as of the date indicated at the foot of the page, of drugs and their combinations used in the treatment of COVID-19 in both home and hospital settings. For an update on treatment indications provided by institutions, please refer to the reference sites listed below.

Disclosure

The content of all the present writings is a contribution to the study and further study of the topics discussed and to scientific medical research. The proposed indications are not a substitute for medical advice, and therefore the diagnosis and prescription of remedies for therapeutic purposes should be made under the supervision of a qualified and licensed physician or practitioner. Therefore, no liability is accepted for improper use of these documents.

INTERNATIONAL GUIDELINES FOR THE PHARMACOLOGICAL TREATMENT OF COVID-19 DISEASE

Guidance from institutional bodies on drugs to be used for the treatment of COVID-19 can be found at the following links:

AIFA: *Drugs that can be used for the treatment of COVID-19 disease Clinical trials - COVID-19*
Compassionate Use Programs - COVID-19
Recommendations on the use of drugs in the population exposed to the virus

EMA: *COVID-19: latest updates*

FDA: *Coronavirus (COVID-19) | Drugs*

CDC: *Therapeutic Management of Patients with COVID-19*

WHO: *Country & Technical Guidance - Coronavirus disease (COVID-19)*

Early drug access and off-label use [1]

In some cases, free access to a drug therapy is allowed in Italy before AIFA authorizes its marketing or, for drugs already authorized, for indications other than those for which the drug has been authorized in Italy (off-label use).
The pathways for early access to a drug are:

Law 648/1996
Compassionate use
AIFA national fund (Law 326/2003 - "5% fund")
Non-repetitive use of advanced therapies

Law 648/1996 and AIFA Fund provide for drug reimbursement by the National Health Service and AIFA, respectively.
Compassionate use involves direct supply free of charge by the manufacturer of the medicine.
Non-repetitive use of advanced therapies involves the preparation of the drug directly from a *cell factory*, and the requesting clinical center bears the related costs.
The choice of the most appropriate pathway depends on the specific indication; the tools mentioned can also be applied in combination, for access to multi-drug treatment regimens.
Finally, it is possible to access treatment with a drug regularly on the market but for an indication other than the one for which it was authorized (**Law 94/98 art.3, paragraph 2 - former Di Bella Law**), even in the presence of regularly authorized therapeutic alternatives. In this case, however, the therapy is paid for by the patient or by the health care provider in the case of hospitalization. All these early access pathways take place under the responsibility of the prescribing physician.

Clarifications on definitions of compassionate use and related applications of Decree Law 18/2020 [2]

Following Decree Law No. 18 of March 17, 2020, specifically Article 17 (Urgent provisions on the testing of medicines and medical devices for the epidemiological emergency from COVID-19), it is deemed appropriate to recall the following definitions with reference to compassionate use:

- **Therapeutic use program**: predefined and identical clinical protocol for all patients, submitted by pharmaceutical companies, with application of unambiguous inclusion, exclusion and treatment scheme criteria for specific drugs administered to multiple patients (according to DM 7/9/2017).

[1] https://www.aifa.gov.it/accesso-precoce-uso-off-label
[2] https://www.aifa.gov.it/web/guest/-/covid-19-precisazioni-su-definizioni-di-uso-compassionevole-e-relative-applicazioni-del-decreto-legislativo-18-2020
https://www.lavoro.gov.it/documenti-e-norme/normative/Documents/2020/DECRETO-LEGGE-17-marzo-2020-n-18-Cura-Italia.pdf

- **Nominal therapeutic use**: all other uses of drugs within the scope of compassionate use on a nominal basis for individual patients within a single hospital facility, based on scientific evidence and not within a predefined clinical protocol of the drug-owning company.

The provision of Article 17 applies only to applications that fall under the first case or therapeutic use programs.

PRINCIPLES OF OUTPATIENT PHARMACOLOGICAL THERAPEUTIC APPROACH

COVID-19 presents a broad spectrum of disease progressing from asymptomatic infection to symptomatic infection to adult fulminant respiratory distress syndrome and multi-organ injury, and it is therefore necessary to tailor therapy based on what is learned about the pathophysiology of SARS-CoV-2 infection.

Peter A. McCullough et al in their study "*Pathophysiological Basis and Rationale for Early Outpatient Treatment of SARS-CoV-2 (COVID-19) Infection*"[3] propose a pharmacological therapeutic approach with appreciable and feasible clinical support for administration in the outpatient setting. SARS-CoV-2 infection as with many infections may be amenable to therapy in the early stages of its course, but probably does not respond to the same treatments in the advanced hospital and terminal stages of the disease.
For the outpatient with recognized early signs and symptoms of COVID-19, pending the outcome of laboratory diagnostics for SARS-Cov-2, the following principles could be applied in a stratified and escalating manner according to the clinical manifestations of suspected case of COVID-19 and confirmed infection:

1) Reduction of reinoculation,
2) Combination antiviral therapy,
3) immunomodulation,
4) antiplatelet / antithrombotic therapy
5) oxygen administration, monitoring and telemedicine Of

these points, the first four will be explored in depth.

Because the outcome of the tests may take several days, it is necessary to start treatment before the results are known.
For patients with features peculiar to the syndrome (i.e., fever, muscle pain, nasal congestion, loss of taste and smell, etc.) and suspected false-negative tests, treatment may be the same as for those with confirmed COVID-19.

1) REDUCTION OF REINOCULATION

The air exhaled by an infected person is considered to be "laden" with bioaerosol of particles and droplets containing the virus that can be re-inoculated with each exhalation and inhalation,[4] and this risk is increased if personal protective equipment such as a face mask is worn. [5]

[3] McCullough PA, Kelly RJ, Ruocco G, et al.
Pathophysiological Basis and Rationale for Early Outpatient Treatment of SARS-CoV-2 (COVID-19) Infection
[published online ahead of print, 2020 Aug 7]. Am J Med. 2020;S0002-9343(20)30673-2. doi:10.1016/j.amjmed.2020.07.003
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7410805/

[4] Chen LD.
Effects of ambient temperature and humidity on droplet lifetime - A perspective of exhalation sneeze droplets with COVID-19 virus transmission.
Int J Hyg Environ Health. 2020;229:113568. doi:10.1016/j.ijheh.2020.113568
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7274593/

[5] Chughtai AA, Stelzer-Braid S, Rawlinson W, et al.
Contamination by respiratory viruses on outer surface of medical masks used by hospital healthcare workers.
BMC Infect Dis. 2019;19(1):491. Published 2019 Jun 3. doi:10.1186/s12879-019-4109-x
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6547584/

https://aapsonline.org/covid-19-is-breathing-stale-air-the-standard-of-care/
https://aapsonline.org/mask-facts/

Therefore, to reduce re-inoculation, it is necessary to maintain good ventilation with open windows or the use of fans that exchange air with the outdoors, or to spend long periods of time outdoors away from others without covering one's face so as to disperse and not inhale the viral bioaerosol again.

For inpatients, reinoculation can be limited with isolation in rooms where air is maintained at negative pressure relative to surrounding areas and with a minimum of 6 air changes per hour. Air from these rooms should be exhausted directly to the outside or be filtered through a high-efficiency particulate air (HEPA) filter directly before recirculation. [6]

2) ANTIVIRAL THERAPY IN COMBINATION

Rapid and exponential viral replication is the hallmark of most acute viral infections.
The degree of direct viral injury to the respiratory epithelium, vascular system, and organs can be limited by reducing the rate, amount, or duration of viral replication. In addition, secondary processes that depend on the damage caused by the virus, including activation of inflammatory cells, cytokines, and coagulation can potentially be reduced if viral replication is attenuated.

Because no drug that has been made readily available is specifically designed to inhibit SARS-CoV-2 replication, the nonspecific agents listed below can be considered for "off-label" use.

The following will explore the mechanism of action of a drug combination [7] used by physicians around the world during the epidemic

Zinc sulfate 220 mg once daily for 5 days

Hydroxychloroquine 200 mg twice daily for 5 days

Azithromycin 500 mg once daily for 5 days

ZINC

Zinc is a known inhibitor of coronavirus replication. Clinical trials conducted with zinc for the treatment of the common cold have demonstrated a modest reduction in the duration and/or severity of symptoms. By extension, this readily available nontoxic therapy can be used at the first signs of COVID-19. [8]

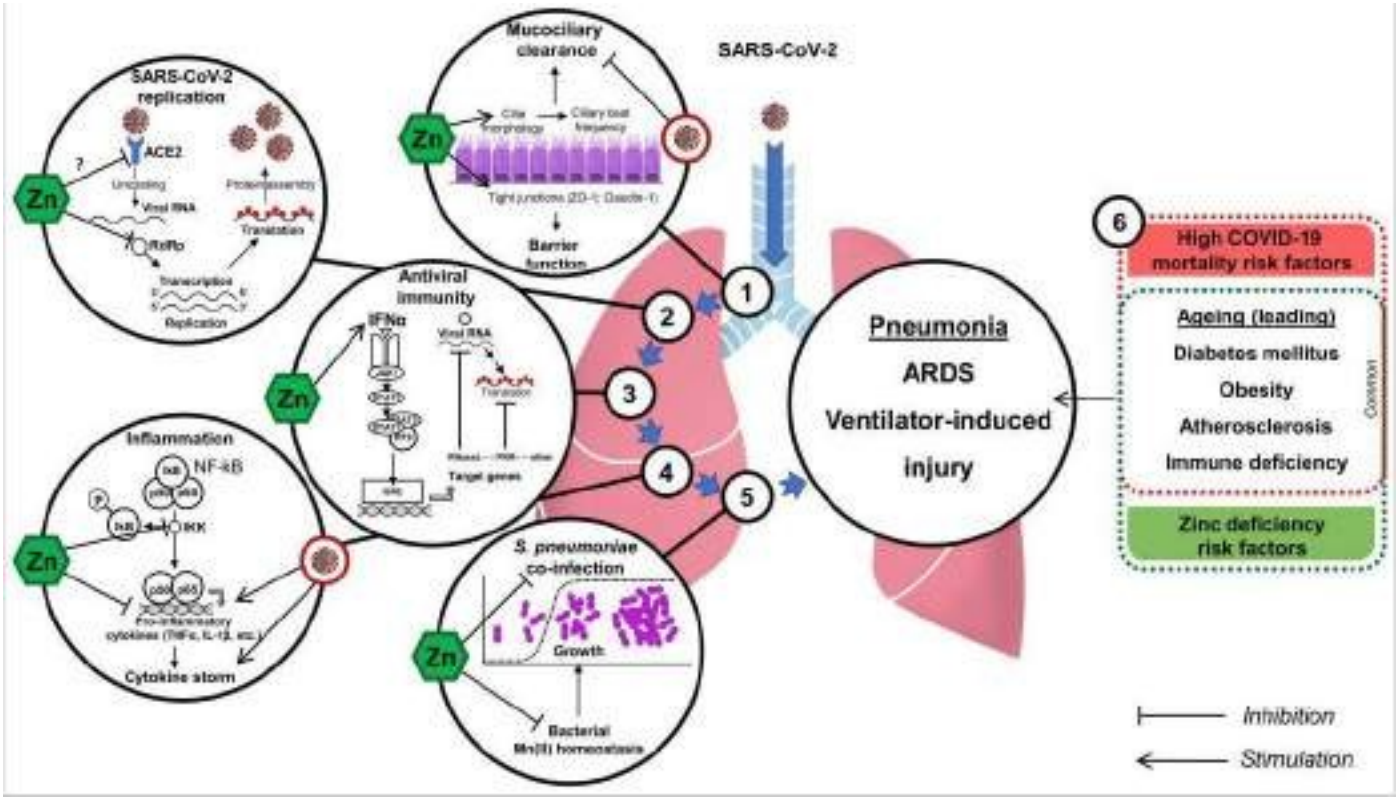
https://www.spandidos-publications.com/10.3892/ijmm.2020.4575
The proposed protective mechanisms of zinc in COVID-19. Zinc significantly enhances cilia morphology and increases ciliary beat frequency thereby improving mucociliary clearance and removal of bacteria and virus-containing particles **2.** By up-regulating the tight junction proteins ZO-1 and claudin-1 and increasing the antioxidant activity of respiratory epithelia, zinc also increases the barrier function of respiratory epithelia. In turn, coronavirus infection has been shown to impair mucociliary clearance by predisposing the lung to further viral and bacterial aggression. **2**. Zinc may also possess antiviral activity through inhibition of RdRp and blocking further viral RNA replication as demonstrated for SARS-CoV. Indirect evidence also indicates that Zn^{2+} may decrease the activity of ACE2, known to be the receptor for SARS-CoV-2. **3**. Modulation of antiviral immunity by zinc may also limit SARS-CoV-2 infection at least through up-regulation of IFNα production and by increasing its antiviral activity. The latter may be mediated through IFNα-induced JAK1/STAT1 signaling and up-regulation of antiviral proteins (RNaseL and PKR) known to degrade viral RNA and inhibit its translation. **4**. Excessive inflammatory response resulting in overproduction of pro-inflammatory cytokines and cytokine storm is known to play a significant role in the pathogenesis of COVID-19. In turn, zinc possesses anti-inflammatory activity through inhibition of IKK activity and subsequent NF-κB signaling resulting in down-regulation of proinflammatory cytokine production. Modulation of T-lymphocyte regulatory functions by zinc may also limit excessive inflammatory response as well as down-regulation of proinflammatory cytokine production. **5.** Given a high risk of bacterial co-infection in viral pneumonia, Zn-induced inhibition of S. pneumoniae growth through modulation of bacterial Mn(II) homeostasis may also be useful. **6**. Zn status is also associated with risk factors for high COVID-19 mortality. Specifically, aging, immune deficiency, and metabolic diseases such as obesity, diabetes, and atherosclerosis are both known to be risk factors for high disease mortality and zinc deficiency. In turn, Zn supplementation can have a beneficial effect on.

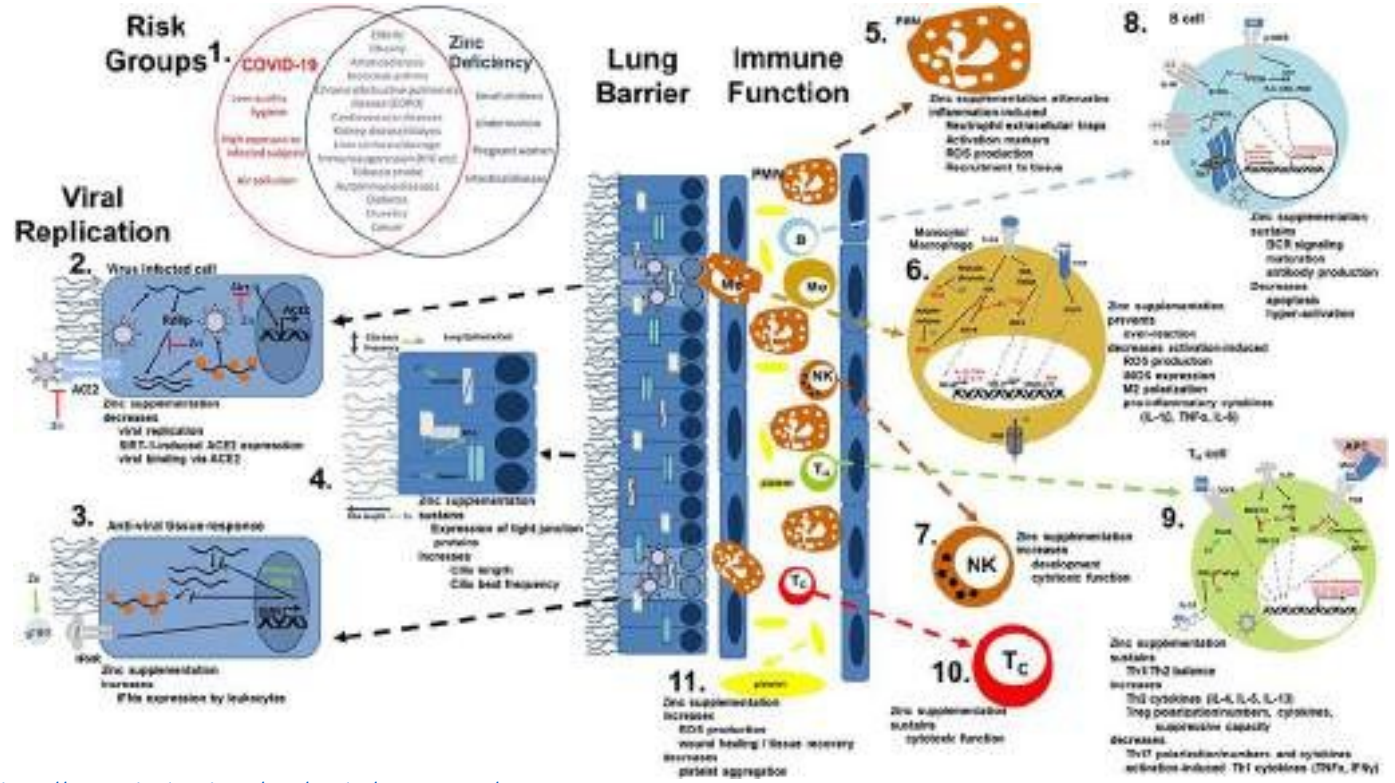
[6] https://www.health.state.mn.us/communities/ep/surge/infectious/airbornenegative.pdf

[7] Dr. Vladimir (Zev) Zelenko M.D.
https://docs.google.com/document/d/1pjgHlqI-ZuKOziN3txQsN5zz62v3K043pR3DdhEmcos/edit

[8] Rahman MT, Idid SZ.
Can Zn Be a Critical Element in COVID-19 Treatment?
[published online ahead of print, 2020 May 26]. Biol Trace Elem Res. 2020;1-9. doi:10.1007/s12011-020-02194-9
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7250542/

In modulating at least some of these risk factors. ACE2, angiotensin-converting enzyme 2; IFN, interferon; IKK, IκB kinase; NF-κB, factor nuclear-κB; ARDS, acute respiratory distress syndrome.





https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7365891/

viral mechanisms of COVID-19 and how they might be counteracted by zinc data. **(1)** There is a striking intersection of known risk factors for zinc deficiency (blue circle) and susceptibility to severe COVID-19 infection (red circle). **(2,3)** Zinc (Zn) supplementation could already p r e v e n t viral entry and also suppress its replication, while supporting the antiviral response of host cells. **(4)** Since zinc is known to increase ciliary length and movement and also supports tissue integrity, virus entry is prevented. **(5-10)** The importance of zinc on immune cell development and function is manifold. It should be emphasized that the effects of zinc should not generally be described as activating or inhibiting, since zinc in various cases normalizes immune overstimulation reactions and balances the ratios of various immune cell types. Zinc thus prevents, for example, high levels of mediators of inflammation, including reactive oxygen and nitrogen species, from destroying host tissue. **(11)** At first glance.

It seems contradictory that zinc increases the production of reactive oxygen species in platelets, because it is generally considered an antioxidant. However, in the case of platelets, up to a certain threshold, ROS production is critical, as it may prevent platelet aggregate formation. In summary, zinc might therefore be able to prevent the vascular complications observed in COVID-19 patients. Details for each point can be found in the text. ACE2, angiotensin-converting enzyme 2; AG, antigen; IFN, interferon; IFNR, interferon receptor; ISRE, interferon-sensitive response element; APC, antigen-presenting cell; IKK, IκB kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; IRF3, IFN regulatory factor 3; MHC, major histocompatibility complex; MEK1 / 2, mitogen-activated protein kinase kinase 1/2; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase; NFAT, nuclear factor of activated T lymphocytes; NF-κB, nuclear factor kappa B; PKR, protein kinase R; Akt, protein kinase B; PI3K, phosphatidylinositol-3 kinase; ROS, reactive oxygen species; RdRP, RNA-dependent RNA polymerase; RNase L, ribonuclease L; Sirt-, Sirtuin 1; STAT, signal transducer and activators of transcription; TCR, T cell receptor; Tc, cytotoxic T cell; TH, T helper cell; TGF, transforming growth factor; TRAM, adaptor molecule related to TRIF; TRIF, adaptor-inducing interferon β containing TIR domain; TLR, Toll-like receptor; TNF, tumor necrosis factor; Zip, Zrt- and Irt-like proteins; ZO-1, occludens zone.

Zinc tablets can be administered 5 times a day for up to 5 days and extended if necessary if symptoms persist. The amount of elemental zinc in the tablets is <25% of that contained in a single **220 mg** daily zinc sulfate tablet.

This dose of zinc sulfate has been effectively used in combination with antimalarials in the early treatment of high-risk outpatients with COVID-19.[9]

ANTIMALARIES

Hydroxychloroquine (HCQ) is an anti-malarial/anti-inflammatory drug that alters the endosomal transfer of virions within human cells. HCQ is also a zinc ionophore that channels zinc intracellularly to block SARS-CoV-2 dependent RNA polymerase, the central enzyme of virus replication. [10]

[9] Carlucci PM, Ahuja T, Petrilli C, Rajagopalan H, Jones S, Rahimian J.
Zinc sulfate in combination with a zinc ionophore may improve outcomes in hospitalized COVID-19 patients.
J Med Microbiol. 2020 Oct;69(10):1228-1234. doi: 10.1099/jmm.0.001250. Epub 2020 Sep 15. PMID: 32930657.
https://www.microbiologyresearch.org/content/journal/jmm/10.1099/jmm.0.001250#tab2

Wessels I, Rolles B, Rink L.
The Potential Impact of Zinc Supplementation on COVID-19 Pathogenesis.
Front Immunol. 2020;11:1712. Published 2020 Jul 10. doi:10.3389/fimmu.2020.01712
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7365891/

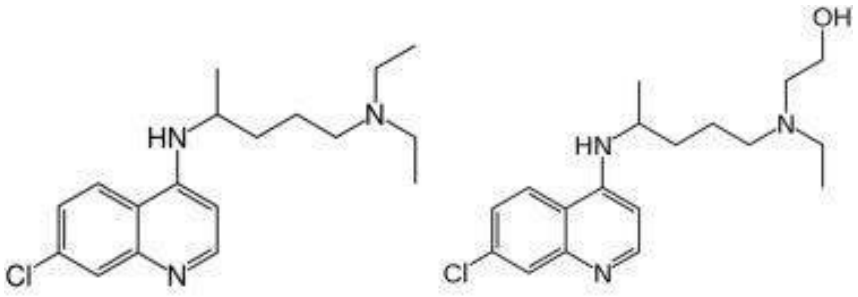
[10] Shittu MO, Afolami OI.
Improving the efficacy of Chloroquine and Hydroxychloroquine against SARS-CoV-2 may require Zinc additives - A better synergy for future COVID-19 clinical trials.
Infez Med. 2020 Ahead of print Jun 1;28(2):192-197. PMID: 32335560.
https://www.infezmed.it/media/journal/Vol_28_2_2020_9.pdf

Yadav, Vaishali & Dwivedi, Vasudha & Verma, Akanksha & Arya, Richa. (2020).
A threat that goes "viral" in the world: story of the COVID-19 (A popular science article). 10.13140/RG.2.2.31871.02725.
https://www.researchgate.net/publication/341579375_A_threat_that_goes_viral_in_the_world_story_of_the_COVID-19_A_popular_science_article

Cirino G, Ahluwalia A.
The many mechanisms of action of Chloroquine: to use or not to use (in COVID-19) that is the question.
Br J Pharmacol. 2020;177(15):3361-3362. doi:10.1111/bph.15177
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7348087/

Hashem AM, Alghamdi BS, Algaissi AA, et al.
Therapeutic use of chloroquine and hydroxychloroquine in COVID-19 and other viral infections: A narrative review.
Travel Med Infect Dis. 2020;35:101735. doi:10.1016/j.tmaid.2020.101735
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7202851/

Tripathy S, Dassarma B, Roy S, Chabalala H, Matsabisa MG.
A review on possible modes of action of chloroquine/hydroxychloroquine: repurposing against SAR-CoV-2 (COVID-19) pandemic.
Int J Antimicrob Agents. 2020;56(2):106028. doi:10.1016/j.ijantimicag.2020.106028
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7243790/



Chloroquine (CQ)
CAS number: 54-05-7
Formula: $C_{18}H_{26}ClN_3$
Mol.mass: 319.872 g/mol

Hydroxychloroquine (HCQ)
CAS number: 118-42-3
Formula: $C_{18}H_{26}ClN_3O$
Mol.mass: 335.872 g/mol

Retrospective studies and currently completed randomized trials have generally shown these results:

1) if started late in the hospital course and for short periods of time, antimalarials appear to be ineffective,

2) when started earlier in the hospital course, for progressively longer durations, and in outpatients, antimalarials can reduce disease progression, prevent hospitalization, and are associated with reduced mortality. [11]

In a retrospective hospital study of 2541 patients hospitalized with COVID-19, therapy associated with an adjusted reduction in mortality was observed with HCQ alone (hazard ratio [HR] = 0.34, 95% confidence interval [CI] 0.25-0.46, P < 0.001) and HCQ with azithromycin (HR = 0.29, 95% CI 0.22-0.40, P < 0.001) having a synergistic effect. [12]

HCQ was approved by the U.S. Food and Drug Administration in 1955, has since been used by hundreds of millions of people worldwide, and has a well-characterized safety profile. Recall that chloroquine (CQ) is used to prevent and treat malaria and amebiasis, while hydroxychloroquine (HCQ), a less toxic metabolite of chloroquine, is used

[11] Mikami T, Miyashita H, Yamada T, et al.
Risk Factors for Mortality in Patients with COVID-19 in New York City.
[published online ahead of print, 2020 Jun 30]. J Gen Intern Med. 2020;1-10. 10.1007/s11606-020-05983-z
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7325642/

Schrezenmeier E, Dörner T.
Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology.
Nat Rev Rheumatol. 2020;16(3):155-166. doi: 10.1038/s41584-020-0372-x.
https://www.nature.com/articles/s41584-020-0372-x

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https://pmj.bmj.com/content/postgradmedj/96/1139/550.full.pdf.

Prodromos C, Rumschlag T.
Hydroxychloroquine is effective, and consistently so when provided early, for COVID-19: a systematic review.
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7534595/

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Is hydroxychloroquine beneficial for COVID-19 patients?
Cell Death Dis 11, 512 (2020). https://doi.org/10.1038/s41419-020-2721-8
https://www.nature.com/articles/s41419-020-2721-8

[12] Arshad S, Kilgore P, Chaudhry ZS.
Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19.
Int J Infect Dis. 2020;97:396-403. doi: 10.1016/j.ijid.2020.06.099.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7330574/

Andreani J, Le Bideau M, Duflot I, et al.
In vitro testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect.
Microb Pathog. 2020;145:104228. doi:10.1016/j.micpath.2020.104228
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7182748/

to treat rheumatic diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), idiopathic juvenile arthritis (AIG), and Sjogren's syndrome.[13]

The most common known side effects of hydroxychloroquine and chloroquine include gastrointestinal symptoms, pruritus, and dermatological changes that can occur in up to 10% of patients.
More serious side effects have a low incidence and include neuromyopathy of proximal muscles, cardiotoxicity, and irreversible retinopathy due to accumulation of the drug in the eye that can lead to blindness. [14] The latter is well documented in long-term users with high doses. Asymptomatic QT prolongation[15] is also a well-known and rare (<1%) complication of HCQ, and symptomatic arrhythmias may develop in the context of acute disease. Because of systemic infection and comorbidities, patients with COVID-19 appear to have a higher a priori risk of cardiac arrhythmia, QT interval prolongation, and myocardial damage.
This could make the cardiotoxicity of CQ/HCQ of particular importance, especially when administered in combination with other QT-prolonging agents such as azithromycin .[16]

It follows that the administration of these drugs should be done in the context of a careful evaluation of the risk-benefit ratio in individual cases, carefully considering concomitant diseases (long QT syndrome, major arrhythmias, hepatic or renal failure, electrolyte disorders), drug combinations (especially for QT-increasing drugs), and history of favism (G6PD deficiency[17]), porphyria, and psoriasis. [18]

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[13] US Food and Drug Administration. FDA cautions against use of hydroxychloroquine or chloroquine for COVID-19 outside of the hospital setting or a clinical trial due to risk of heart rhythm problems.
Availableat: https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital- setting-or. Accessed July 3 2020.

[14] Gevers S, Kwa MSG, Wijnans E, van Nieuwkoop C.
Safety considerations for chloroquine and hydroxychloroquine in the treatment of COVID-19.
Clin Microbiol Infect. 2020;26(9):1276-1277. doi:10.1016/j.cmi.2020.05.006
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7228887/

[15] https://litfl.com/qt-interval-ecg-library/

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Cell Death Dis. 2020;11(7):512. Published 2020 Jul 8. doi:10.1038/s41419-020-2721-8
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7341710/

[16] Bonow RO, Fonarow GC, O'Gara PT, Yancy CW.
Association of Coronavirus Disease 2019 (COVID-19) With Myocardial Injury and Mortality.
JAMA Cardiol. 2020 Jul 1;5(7):751-753. doi: 10.1001/jamacardio.2020.1105. PMID: 32219362.
https://jamanetwork.com/journals/jamacardiology/fullarticle/10.1001/jamacardio.2020.1105

Mercuro NJ, Yen CF, Shim DJ, et al.
Risk of QT Interval Prolongation Associated With Use of Hydroxychloroquine With or Without Concomitant Azithromycin Among Hospitalized Patients Testing Positive for Coronavirus Disease 2019 (COVID-19).
JAMA Cardiol. 2020;5(9):1036-1041. doi:10.1001/jamacardio.2020.1834
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195692/

Stremmel C, Kellnar A, Massberg S, Kääb S.
Hydroxychloroquine in COVID-19 Therapy: Protection Versus Proarrhythmia.
J Cardiovasc Pharmacol Ther. 2020 Nov;25(6):497-502. doi: 10.1177/1074248420935740. Epub 2020 Jul 23. PMID: 32700555.
https://journals.sagepub.com/doi/pdf/10.1177/1074248420935740

[17] Sgherza N, Dalfino L, Palma A, et al.
"Hemolysis, or not Hemolysis, that is the Question." Use of Hydroxychloroquine in a Patient with COVID-19 Infection and G6PD Deficiency.
Mediterr J Hematol Infect Dis. 2020;12(1):e2020076. Published 2020 Nov 1. doi:10.4084/MJHID.2020.076
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7643778/

[18] DATA SHEET PLAQUENIL
https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_008055_013967_RCP.pdf&retry=0&sys=m0b1l3

https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/009768s037s045s047lbl.pdf

Zou L, Dai L, Zhang X, Zhang Z.
Hydroxychloroquine and chloroquine: a potential and controversial treatment for COVID-19.
Arch Pharm Res. 2020;43(8):765-772. doi:10.1007/s12272-020-01258-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7395211/

It should be noted that to date, drug regulatory agencies recommend the use of HCQ only in randomized clinical trials until the benefit/risk ratio is more clearly defined[19] . AIFA has suspended its use for hospital and home use, and therefore such use has been excluded from reimbursability.[20]

However, an international vote of 6227 physicians from 30 countries and regions released by the SERMO Global Medical Voting Society found that of the 15 treatment alternatives, 37% of physicians rated HCQ as "the most effective treatment for COVID-19."[21] .

It is worth noting again that this corroborates several clinical observations[22] , particularly that of Lammers et al[23] in which early treatment after hospitalization with low-dose HCQ (2400 mg total) is associated with a lower risk of intensive care unit admission and coincides with large observational studies showing lower mortality rates in patients treated with HCQ therapy compared with no or other treatment.

Note that in all of these studies, and in contrast to the RECOVERY study[24] , low doses of HCQ (<2.5 g total) were used, often immediately after admission[25] .

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[19] https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/public-health-advice-during-covid-19-pandemic#use-of-chloroquine-and-hydroxychloroquine-medicines-section
https://www.aifa.gov.it/web/guest/-/aifa-sospende-l-autorizzazione-all-utilizzo-di-idrossiclorochina-per-il-trattamento-del-covid-19-al-di-fuori-degli-studi-clinici
https://www.aifa.gov.it/documents/20142/1097058/2020.03.31_NII_clorochina_idrosssiclorochina_GP_consolidata+COVID-19.pdf/c928750d-dcb2-f38a-41a1-1fbf6af7a767
https://www.aifa.gov.it/raccomandazioni-sull-uso-dei-farmaci-nella-popolazione-esposta-al-virus
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https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital-setting-or
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Low-dose hydroxychloroquine therapy and mortality in hospitalized patients with COVID-19: a nationwide observational study of 8075 participants.
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Another recent large cohort study of patients on low-dose HCQ for inflammatory disorders reported an association between chronic HCQ use and reduced mortality following SARS -CoV-2 infection[26] .
Capucci et al in a recent letter to the editor of the Journal of the Italian Federation of Cardiology confirm the usefulness of the combination of hydroxychloroquine and azithromycin for the home treatment of COVID -19 patients: fifty-eight primary care physicians treated 350 patients with HCQ at the first flu symptoms; in 76 patients azithromycin w a s also associated. Of the 274 patients treated with HCQ alone, 16 patients required subsequent hospitalization (5.8%). Minor complications (mainly gastrointestinal, diarrhea) were found in eight patients (2.9%), none of whom had to discontinue treatment. No major cardiac complications were encountered. Of the 76 patients treated with the combination, 4 patients were hospitalized (5.2%). Minor complications occurred in only two patients (2.6%). There were no reports of major arrhythmias, syncope or sudden death. [27]

As highlighted by the results of Lammers et al, the timing of HCQ therapy (administration within 1 day of admission) could explain the discrepancies between the different studies. In the RECOVERY study, the median time between symptom onset and randomization was 9 days, and a substantial proportion of patients (16.7%) were already on mechanical ventilation at the time of randomization; in addition, the dosages used were higher than in the other studies that reported favorable results without reports of serious adverse reactions[28] .

It is important to note that CQ/HCQ have narrow therapeutic ranges and toxic effects are closely related to the ingested dose. A one-time dose of 20 mg/kg CQ has been described as toxic, and doses of 30 mg/kg CQ have resulted in deaths.[29] Therefore, long-term monitoring for adverse reactions is recommended.

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Relative frequencies (%)[a] of the most commonly reported suspected adverse drug reactions as registered in the WHO pharmacovigilance database (www.vigiaccess.org) for system organ classes considered relevant to patients with COVID-19 (access date April 9, 2020)

	Chloroquine	Hydroxychloroquine
Cardiac disorders	Tachycardia (1.6%), cardiomyopathy (0.7%), palpitations (0.6%), cardiac arrest 0.6%), atrioventricular block complete (0.5%)	Cardiomyopathy (0.7%), palpitations (0.6%), cardiac failure (0.4%), tachycardia (0.3%), cardiac failure congestive (0.3%)
Gastrointestinal disorders	Vomiting (10.5%), nausea (8.4%), diarrhoea (4.5%), abdominal pain (3.5%), abdominal pain upper (2.5%)	Nausea (5.3%), diarrhoea (3.6%), abdominal discomfort (2.4%), vomiting (2.3%), abdominal pain (1.3%)
Psychiatric disorders	Anxiety (2.1%), depression (2.0%), psychotic disorder (1.5%), hallucination (1.1%), insomnia (1.1%)	Insomnia (0.7%), depression (0.6%), anxiety (0.4%), completed suicide (0.3%), sleep disorder (0.3%)
Nervous system disorders	Headache (7.8%), dizziness (5.2%), seizure (2.8%), balance disorder (1.6%), neuropathy peripheral (1.2%)	Headache (2.8%), dizziness (2.1%), visual field defect (0.6%), paraesthesia (0.6%), hypaesthesia (0.6%)

[a]Relative frequencies (%) were calculated by dividing the absolute number of adverse reaction reports by the total number of adverse reaction reports for each drug. For chloroquine and hydroxychoroquine VigiAccessTM contains a total of 5797 and 22138 records, respectively.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7228887/

A typical regimen with HCQ is 200 mg/ 2 x die per os for 5 days and extended to 30 days for persistence of symptoms. A minimum sufficient dose of HCQ must be used, as at excessive doses the drug may interfere with the early immune response to the virus.

Mechanism of action of chloroquine/hydroxychloroquine

Savarino et al were the first to hypothesize that hydroxychloroquine and chloroquine might be useful in the treatment of SARS, as endocytosis may be involved in viral entry into the cell and there is a major immune response associated with clinical worsening, due to the release of inflammatory cytokines such as TNF-alpha and IL-6. [30]
Kayaerts et al later confirmed the inhibition of SARS-CoV by chloroquine in Vero E6 cells at different post-infection times,[31] and Vincent et al demonstrated that chloroquine is effective in preventing SARS-CoV infection in cell cultures if the drug is added to the cells 24 hours before infection. Furthermore, chloroquine was also significantly effective when the drug was added 3-5 hours after infection, suggesting an antiviral effect even after the onset of infection. Cells pretreated with chloroquine were resistant to virus due to impaired terminal glycosylation of the ACE2 receptor, which results in decreased viral receptor affinity and thus reduces the onset of infection.[32]

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Effects of chloroquine on viral infections: an old drug against today's diseases?
Lancet Infect Dis. 2003;3(11):722-727. doi:10.1016/s1473-3099(03)00806-5
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Biochem Biophys Res Commun. 2004;323(1):264-268. doi:10.1016/j.bbrc.2004.08.085
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Chloroquine is a potent inhibitor of SARS coronavirus infection and spread.
Virol J. 2005;2:69. Published 2005 Aug 22. doi:10.1186/1743-422X-2-69
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1232869/

In a recent publication, Wei-Yi Ong et al[33] report that antimalarials have unique properties that distinguish them from other anti-inflammatory drugs:

(A) They are very lipophilic, and this property increases their ability to cross the blood-brain barrier (BBB).
Thus, they have the potential to act not only peripherally but also in the CNS and to have a positive pharmacological effect in treating (or preventing) nerve tissue damage

(B) They are nonselective inhibitors of phospholipase A2 isoforms, including cytosolic phospholipase A2 (cPLA2).
The latter is not only activated by cytokines but itself generates arachidonic acid, which is metabolized by cyclooxygenase (COX) into pro-inflammatory eicosanoids. Free radicals are produced in this process, which can lead to oxidative damage to the CNS.

The authors discuss in detail 4 mechanisms by which antimalarials could be useful in combating COVID-19.

(1) Inhibition of PLA2.

(2) Inhibition of lysosomal enzyme activity.

(3) modification of expression and activity of the Fe symporter $^{2+}$ / H^{+} of iron transporters including bivalent metal transporter 1 (DMT1), leading to reduction of iron accumulation in tissues and iron-catalyzed free radical formation.

(4) action on viral replication, related to their effect on PLA2 isoform inhibition. Inhibition of cPLA2 is known to impair an early stage of coronavirus replication in cell culture, and a secretory PLA2 isoform (sPLA2), PLA2G2D, has been shown to be essential for SARS-CoV lethality in mice.

Following are summary tables from the article by Eugenia Quiros Roldan et al *"The possible mechanisms of action of 4-aminoquinolines (chloroquine/hydroxychloroquine) against Sars-Cov-2 infection (COVID-19): A role for iron homeostasis?*"[34] on the various mechanisms of action of hydroxychloroquine:

[33] Ong WY, Go ML, Wang DY, Cheah IK, Halliwell B.
Effects of Antimalarial Drugs on Neuroinflammation-Potential Use for Treatment of COVID-19-Related Neurologic Complications
[published online ahead of print, 2020 Sep 8]. Mol Neurobiol. 2020;1-12. doi:10.1007/s12035-020-02093-z
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The possible mechanisms of action of 4-aminoquinolines (chloroquine/hydroxychloroquine) against Sars-Cov-2 infection (COVID-19): A role for iron homeostasis?
Pharmacol Res. 2020;158:104904. doi:10.1016/j.phrs.2020.104904
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7217799/

Biological activity
Inhibition of viral attachment and entry in the host cell
Inhibition of the biosynthesis of sialic acids
• inhibition of the N-glycosylation of the cell surface viral receptor ACE2 • inhibition of the N-glycosylation of the viral spike (S) proteins • inhibition of the synthesis of cell membrane sialic acids
Inhibition of PICALM expression and CME
Endosomal alkalinization and inhibition of cellular endosomal protease (cathepsin and/or TMPRSS2)
Inhibition of new viral particle maturation and spread
Endosomal alkalinization and inhibition of endosome-lysosome membrane fusion
ERGIC and TGN vesicle alkalinization and inhibition of post-translational modifications of viral proteins
ERGIC vesicle alkalinization and inhibition of viral budding
Inhibition of p38 MAPK activation
Inhibition of phospholipase A2 and membranous structures essential for replication and transcription

Biological activity
Interference with platelet aggregation
Decrease of collagen activation
Decrease of alpha granule discharge
Inhibition of phospholipase A2 and of the release of tromboxanes
Increase of fibrinogen with decrease of plasmatic and blood viscosity
Decrease of rheological properties of RBCs
Inhibition of NETs
Interference with membrane binding of blood clotting proteins
Inhibition of the binding of aPL antibody- β2GPI complex to the phospholipid bilayer
Restoration of the AnxA5 anticoagulant shield
Improvement of biomarkers of endothelial dysfunction
Amelioration of NO bioavailability and decrease of oxidative stress
Improvement of endothelial relaxation
Increase of p-eNOS/eNOS ratio, with improvement of NO production
Inhibition of eNOX and NOX2
Improvement of lipid profile

Biological activity
Modulation of innate and adaptive immune cell activation, cytokine response and inflammation
Inhibition of antigen presentation by APCs
• Inhibition of PICALM expression, CME and pathogen internalization • Vesicle alkalinization and inhibition of endosomal/lysosomal antigen processing • Vesicle alkalinization and inhibition of MHC processing and MHC-antigen complex formation
Inhibition of Ca2+ signaling and T and B cell activation
Inhibition of Th17 proliferation and differentiation
Vesicle alkalinization and inhibition of the TLR signaling and MMPs
Inhibition of phospholipase A2 and of the release of prostaglandins
Inhibition of p38 MAPK activation and of the release of cytokines

Inhibition of TNF-α release
Inhibition of vasodilation, infiltration and adhesion of leukocytes at the site of inflammation
Inhibition of respiratory burst in polymorphonuclear leukocytes
Inhibition of IL- β release
Inhibition of neutrophil recruitment and Th17 differentiation
Inhibition of IL-6 release
Inhibition of Th17 differentiation
Induction of cytotoxic activity of CD8 + T cells
Activation of Treg cell functions
Reduction of tissue injury
Reduction of microorganism immune evasion strategy

Lysosomotropic and immunomodulatory action

These observations have now been confirmed for COVID-19, and most of the results initially evaluated for CQ have been adapted to HCQ because of better availability and lower toxicity. [35]

Chloroquine/HCQ, like azithromycin, are weakly basic compounds that accumulate in acidic organelles due to pH breakdown and interaction with negatively charged phospholipids.

These types of drugs are referred to as lysosomotropic (i.e., with tropism in lysosomes)[36] and are able, after entry into the cell, to accumulate in endosomes, lysosomes, and Golgi vesicles. By increasing endosomal pH in a dose-dependent manner, they inhibit spike protein-mediated viral entry, lysosomal activity, and autophagosome-lysosome fusion. [37]

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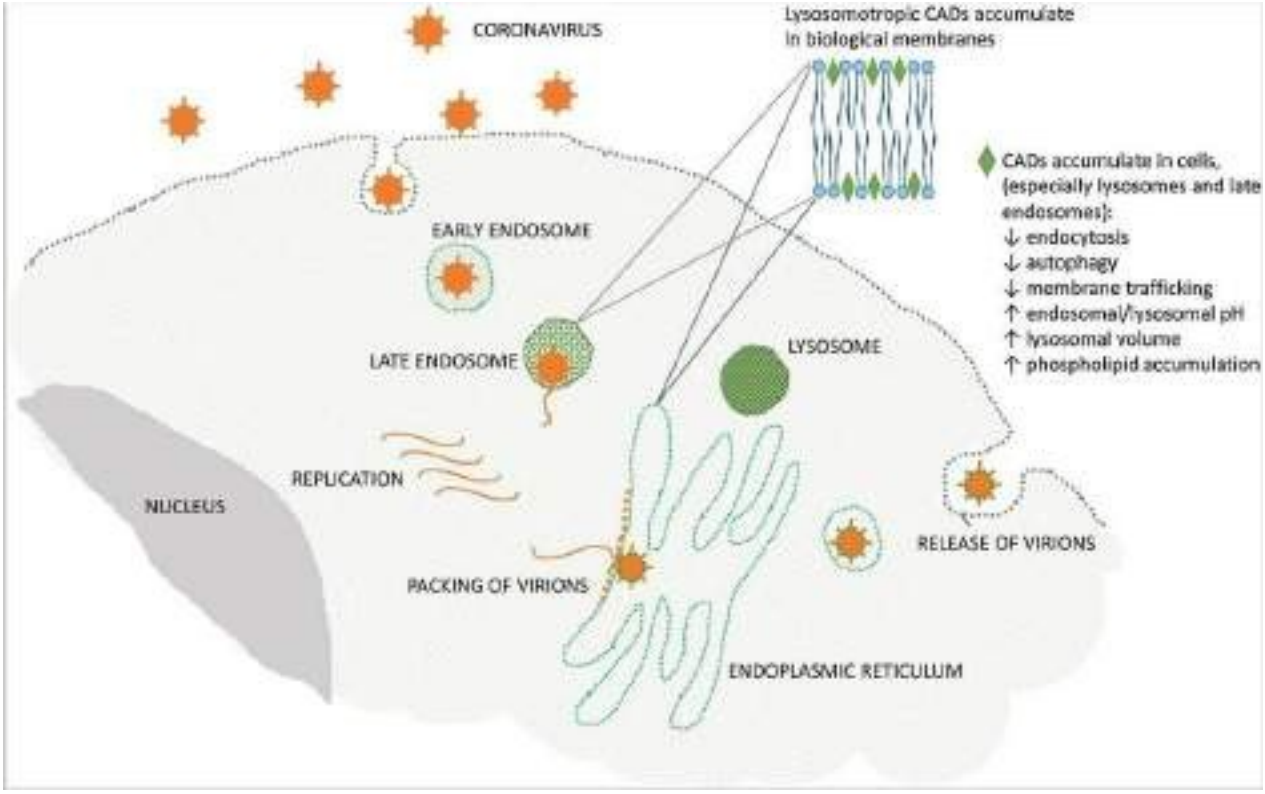
Korolenko TA, Johnston TP, Vetvicka V.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7392152/
Interaction of cationic lysosomotropic amphiphilic drugs (CAD) and coronavirus with membrane trafficking in the cell

Alkalinization of cell vesicles also modulates immune response (reduction of tumor necrosis factor α and interleukin 6 secretion[38]) and viral replication by altering protein translation and post-translational modifications in the Golgi apparatus. [39] Hydroxychloroquine, as seen above, alters the glycosylation of surface receptors used by Sars-CoV-2 for cellular infection such as the ACE2 receptor. [40] In addition, HCQ interferes with both Toll-like receptor (TLR) TLR7 and TLR9 signaling,

Lysosomotropic Features and Autophagy Modulators among Medical Drugs: Evaluation of Their Role in Pathologies.
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https://pubmed.ncbi.nlm.nih.gov/9002011/

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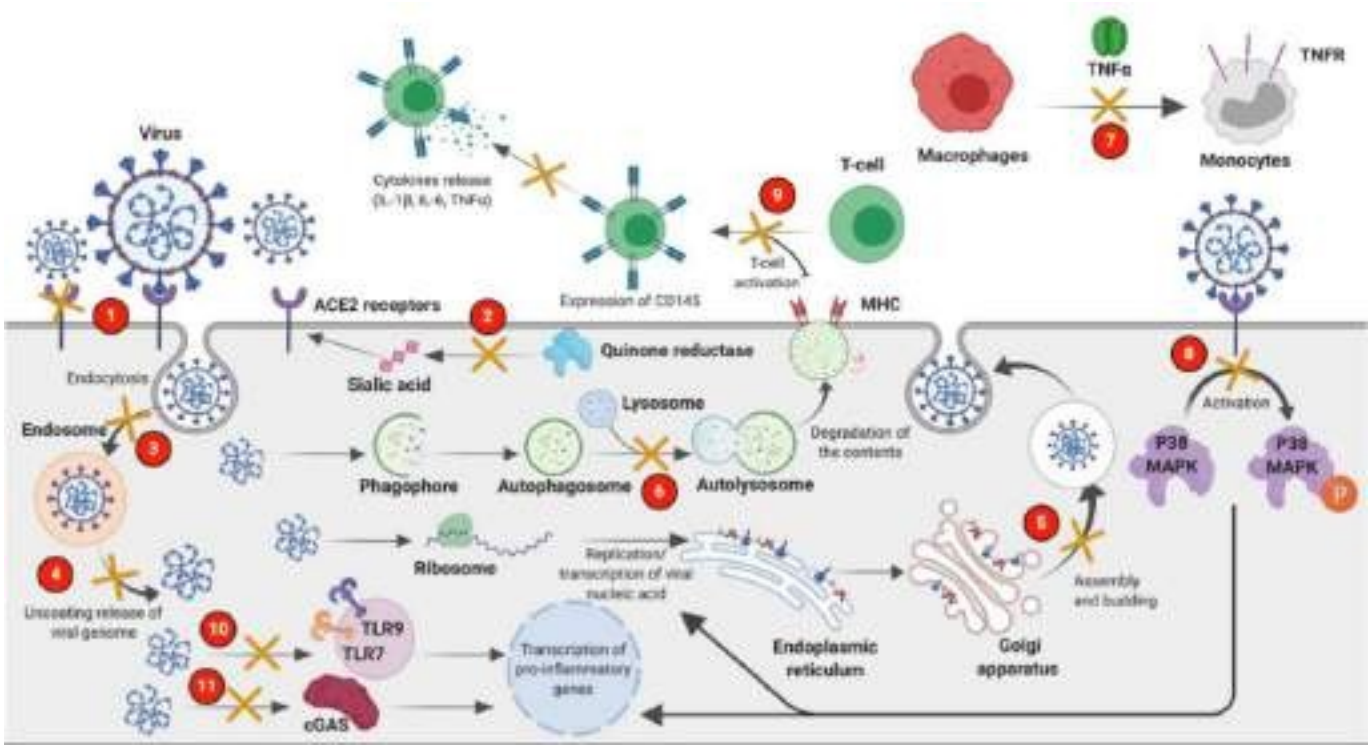
through pH-dependent changes and direct binding to nucleic acids, than by the cyclic GMP-AMP synthase signaling cascade; both of these immunomodulatory mechanisms are part of the signaling pathways that promote transcription of pro-inflammatory cytokines. [41]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7202851/
Possible cellular and molecular sites of action of CQ ± HCQ as antiviral agents. (X) Represents the site of inhibition by CQ ± HCQ. **(1)** CQ and HCQ inhibit the binding of virus to its receptor on the cell surface, **(2)** CQ inhibits sialic acid biosynthesis by suppressing the activity of quinone reductase 2, which influences the activity of the ACE2 receptor, **(3)** CQ and HCQ inhibit pH-dependent endocytosis of virus by increasing pH , (**4)** CQ interferes with virus envelope, **(5)** CQ interferes with assembly/gemming leading to accumulation of viral vesicles within the trans-Golgi network, **(6)** CQ interferes with lysosomal protein degradation and lysosomal fusion with autophagosomes. HCQ can interfere with lysosomal activity and prevent the expression of major histocompatibility complex (MHC) class II, **(7**) CQ interferes with the release and binding of TNF from macrophages and/or to monocytes, **(8**) CQ inhibits the phosphorylation of P38 MAPK and caspases in Th1 cells which in turn inhibits the production of pro-inflammatory cytokines and virus replication, **(9)** HCQ's blockade of MHC expression prevents T cell activation, CD145 expression and cytokine release, **(10)** HCQ alters TLR signaling t h r o u g h increasing endosomal pH and interfering with the binding of TLR7 and TLR9 to their DNA/ RNA ligands thus inhibiting transcription of pro-inflammatory genes, **(11)** HCQ inhibits DNA binding to cGAS and thus reduces transcription and cytokine production. ACE2: angiotensin-converting enzyme 2; MHC: major histocompatibility complex; TLRs: Toll-like receptors; cGAS: cyclic GMP-AMP synthase; MAPK: mitogen-activated protein kinase.

Chloroquine is a potent inhibitor of SARS coronavirus infection and spread.
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Anticoagulant action

Since the 1960s, anticoagulant activity of aminoquinoline drugs has been reported, and in particular, CQ inhibits the alternative pathway of the complement system and blocks plasma coagulation by calcium chloride and thrombin. However, these activities have been reported in vitro at concentrations of CQ higher than those that probably need to be achieved in human plasma at therapeutically acceptable dosages.

In 2019, Miranda et al. reported an inhibitory effect of CQ on coagulation in vivo through impairment of the extrinsic pathway, that is, by altering tissue factor (TF) release from the endothelium[42] . In this regard, the anticoagulant activity of HCQ can be seen as a by-product of its anti-inflammatory activity. This is in line with the anticoagulant effects of the drug reported in subjects with SLE[43] .

Thus, the anticoagulant activity of HCQ that primarily affects the extrinsic pathway may be complementary to that of low-molecular-weight heparin (EBPM), which acts, among other mechanisms, on the intrinsic pathway by inhibiting factor X activation by factor IXa. Since inhibition of the TF/factor VIIa pathway by HCQ also affects factor X activation, the HCQ/EBPM combination may exert synergistic inhibition of convergent coagulation on factor X and prevent thrombus formation both in the antiphospholipid syndrome[44] and during COVID-19. [45]

Antimalarials and iron homeostasis [46]

During infection and inflammation, anemia is frequently observed and caused by pro-inflammatory cytokines.

Some of them directly affect iron homeostasis, such as IL-1β, TNF-α, and IL-6, and the release of these cytokines, mainly IL-6, results in the upregulation of the iron-regulating hormone: hepcidin (HAMP), which is mainly produced by hepatocytes and released into the bloodstream to regulate systemic iron homeostasis.

Systemic HAMP blocks cellular iron export through ferroportin 1 (FPN1), resulting in reduced intestinal iron absorption, increased iron retention in hepatocytes and macrophages, and finally infection/inflammation anemia [4,95]. Various cells other than hepatocytes have been shown to produce and release HAMP, which can act as an autocrine and paracrine molecule, modulating local iron homeostasis [95,96]

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The possible mechanisms of action of 4-aminoquinolines (chloroquine/hydroxychloroquine) against Sars-Cov-2 infection (COVID-19): A role for iron homeostasis?
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Not only immune system cells such as lymphocytes, monocytes, and macrophages (including alveolar macrophages), but also airway epithelial cells have been shown to produce HAMP during infection and inflammation and potentially contribute to lung damage [[97], [98], [99]].

HAMP is also a peptide involved in antimicrobial innate immunity and is an acute phase protein [100]. Additional acute-phase iron-binding proteins such as transferrin (Tf), lactoferrin (LF), ferritin (FT), haptoglobin (HP), and hemopexin (HPX) are modulated by viral infections, further underscoring the crucial role of iron in host antiviral defense. The role of iron metabolism has been extensively studied in several human viral infections. [[2], [3], [4],101]

CQ/HCQ has been shown to modulate iron metabolism, impairing iron homeostasis at different levels [43,102] and decrease inflammatory cytokines such as IL-6, IL-1β, and TNF-α.
These mechanisms leading to iron deprivation could be responsible for the curative effects of CQ/HCQ in SARS-Cov-2 infection. In particular, as seen above, the alkalizing properties of CQ/HCQ have been widely used to impair endosome/lysosome fusion and inhibit autophagy * [5].
Ferritin (FT) is the main cellular protein that stores and compartmentalizes iron in an unreactive form within the cell until it is used. Iron release from FT occurs primarily through degradation of the protein by a selective autophagy pathway of the lysosome called ferritinophagy [101,111] inhibited by CQ [112].

> * **Macroautophagy**, commonly referred to as autophagy, is a process of self-degradation by which virtually all eukaryotic cells sequester cytoplasmic components (macromolecules, but also whole organelles or microorganisms) into *de novo* formed double-membrane vesicles (autophagosomes) and degrade them after lysosomal fusion. The degradation products released from lysosomes are recycled into metabolic and biosynthetic pathways. Autophagy, which occurs normally for organelle and protein turnover, can be induced under stress conditions as an adaptive and survival response to microenvironmental and intracellular noxia, including depletion of glucose, amino acids or growth factors, hypoxia, oxidative stress, mitochondrial or organelle dysfunction, infection and cytotoxic drugs. [47]

A second important point associated with CQ/HCQ-induced iron deprivation is their effect on immune cells, which are involved in innate and adaptive immune responses against the virus.

Like all cells in the body, immune cells require iron for their proper functioning and for their activation and proliferation. Resident macrophages can polarize under cytokine stimuli into classically activated pro-inflammatory macrophages (M1), by induction by interferon-γ (IFN-γ) and TNF-α, or into alternatively activated macrophages (M2) under the induction of interleukin-4 (IL-4) and 13 (IL- 13) and be involved in pathogen clearance, tissue repair, and reduction of inflammation.
While M2 macrophages have low levels of iron, M1 macrophages are characterized by iron retention, secrete high levels of pro-inflammatory cytokines, produce high amounts of radicals to kill pathogens, and produce HAMP, which acts in an autocrine manner to limit iron leakage.

Increased iron deposition in macrophages has been shown to induce M1 polarization and persistence of a pro-inflammatory state due to an incomplete transition to the M2 state [121].
Iron retention in macrophages could therefore promote the life cycle of intracellular virus in case of infection and further promote the inflammation process, while iron deprivation could have opposite effects. In addition, excess iron in macrophages promotes secondary infections with other microbes.
Legssyer et al [122,123] have shown that CQ decreases iron content in iron-laden alveolar macrophages, and their studies suggest that CQ could prevent infections, particularly those associated with diseases characterized by iron overload, by limiting iron availability in both infected cells and macrophages, reducing inflammation.

Thus, CQ/HCQ not only interfere with cellular iron metabolism by inducing iron deprivation in alveolar macrophages and resulting in a switch to the M2 anti-inflammatory state, but also inhibit the release of IL-6, IL-1β, and TNF-α, probably by reducing the local release of HAMP by macrophages. Inflammatory anemia caused by HAMP-mediated iron sequestration in the liver, spleen, and macrophages, as might occur in COVID-19 patients, can be considered functional iron deficiency (ID), and patients with this condition should be treated as patients with high risk of thrombosis [139].

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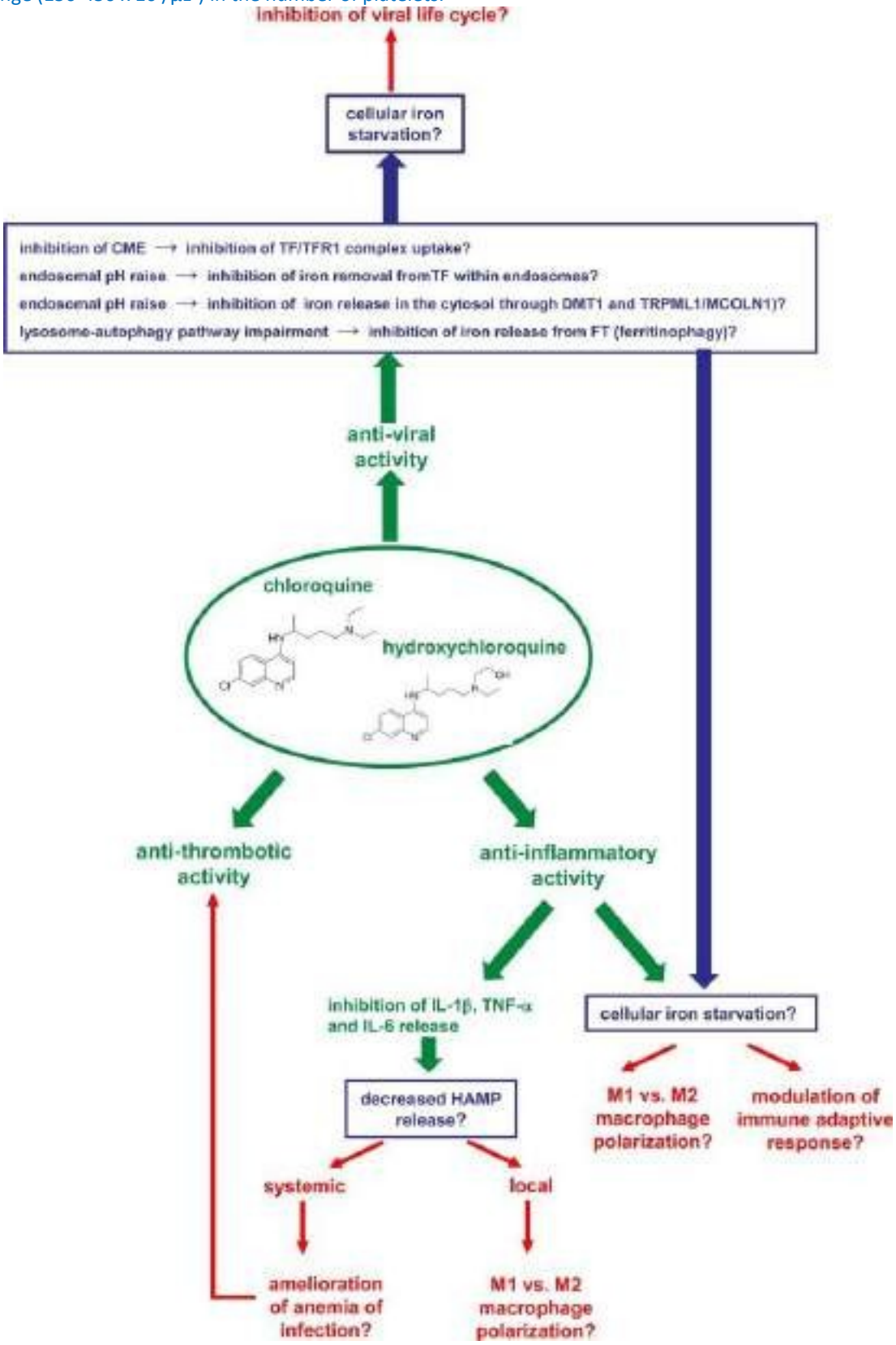
A recent study found that IDA (iron deficiency anemia) patients who manifested thrombocytosis* had a 2-fold increased risk of thrombosis compared with IDA patients with normal platelet counts [136]
. Interestingly, COVID-19 patients with severe pneumonia seem to have elevated platelet counts compared with non-COVID-19 patients with severe pneumonia [137] and this increase is more evident among non-survivors than among COVID- 19 surviving patients [138].

* **Thrombocytosis** or **thrombocytosis or thrombocythemia is** defined as an increase beyond the threshold of the normal range (150-450 x 10 /µL^3) in the number of platelets.



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Graphic representation of the possible pharmacological effects (in green) of chloroquine and hydroxychloroquine as antiviral, anti-inflammatory, and antithrombotic drugs and their possible links to systemic and cellular iron homeostasis. For each drug effect are in blue the hypothesized activities of the drugs on iron homeostasis, while in red are the possible consequences on the virus and host.

Medicinal plants that can be used as alternatives to hydroxychloroquine

NIGELLA SATIVA

N. sativa seeds contain unsaturated fatty acids (26% -38%), proteins, alkaloids, saponins (melanin), and essential oil (0.4% - 2,5%). A GC-MS analysis revealed a mixture of eight fatty acids and 32 volatile terpenes in the seed extract.
Thymoquinone, dithymoquinone (nigellone), thymohydroquinone and thymol are considered the main active constituents. Thymoquinone is the major component (28% -57%) of the volatile essential oil. The main alkaloids isolated from *N. sativa* seeds are nigellicin, nigellidin (indazole), nigellimin and nigellimin N-oxide (isoquinolines).
Other constituents include palmitic, glutamic, ascorbic, and stearic acids; arginine; methionine; lysine; glycine; leucine; and phytosterols.

It can be seen that a number of bioactive components such as nigellimine share structural similarities with chloroquine and hydroxychloroquine. [48]

With its wide range of bioactive components including thymoquinone and nigellimin, *N. sativa* could offer a number of advantages for the treatment of COVID-19 as it could

(i) Blocking virus entry into pneumocytes and

(ii) act as an ionophore for improved uptake of Zn^{2+} which in turn can enhance the host immune response against SARS-CoV-2 and inhibit its replication by blocking viral polymerase (RdRp: RNA-dependent RNA polymerase).

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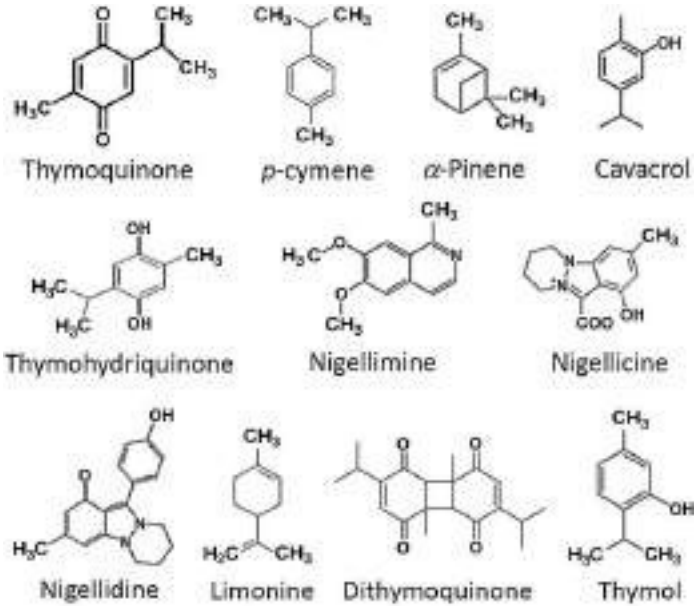
However, it is important to identify the right doses for both black seed or its derivatives such as oil and Zn.
Black seed oil has been used at doses between **40-80 mg/kg/day** as adjunctive therapy without side effects. On the other hand, intake of Zn above its recommended daily allowance (RDA), which varies according to age, sex, and other health conditions, should be avoided because it is potentially harmful.
For example, the RDA varies for children aged 1-8 years (3-5 mg), males 9-13 years (8 mg), males> 14 years (11 mg), females> 18 years (8 mg), and females 14-18 years (9 mg).
People with health conditions such as liver and kidney diseases and pregnant women should consult their doctor before
Of deciding to take any Zn-based supplement for self-care. [49]



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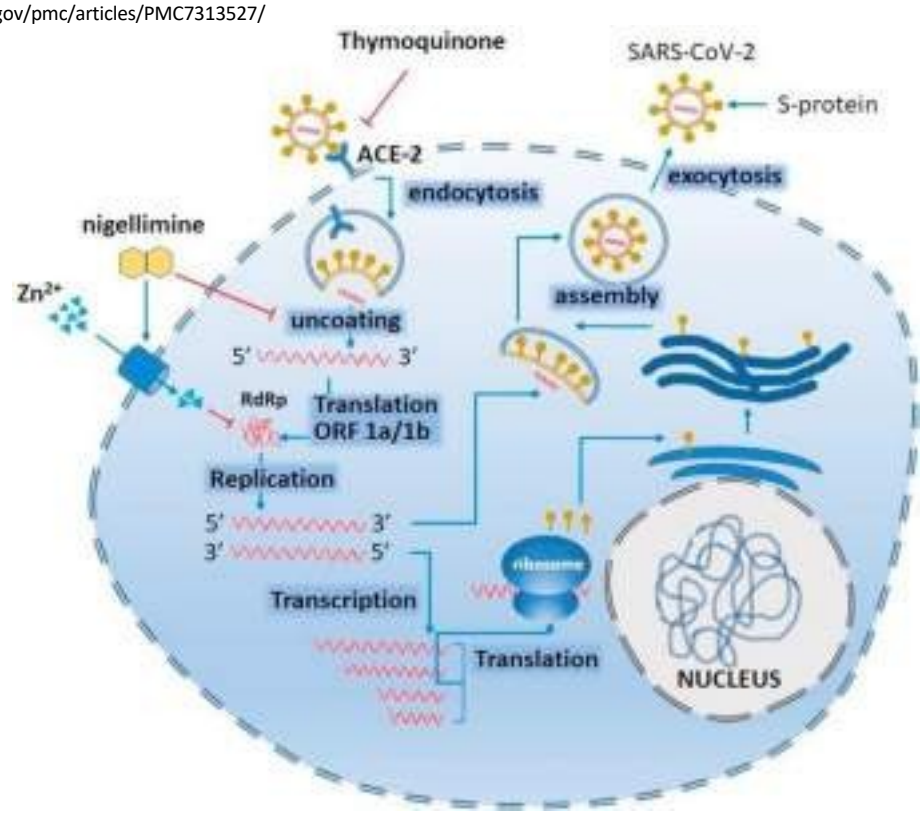

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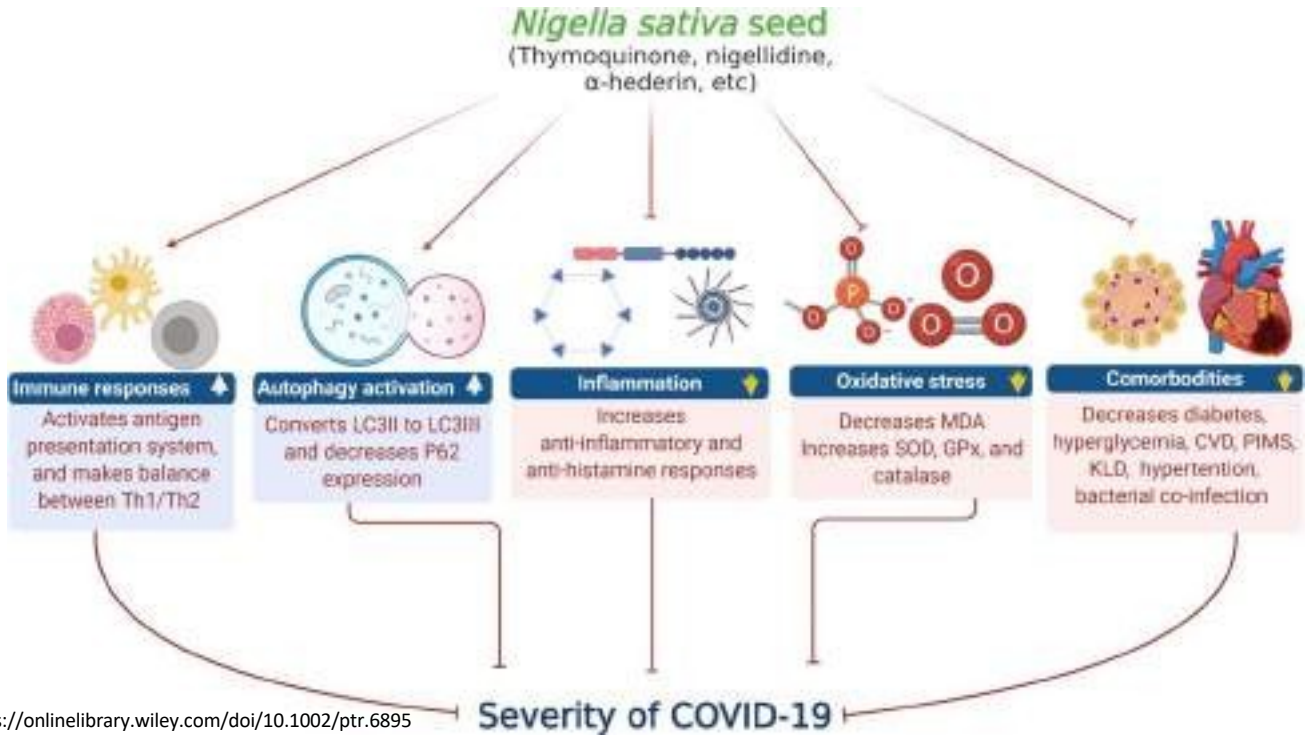
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7313527/

SARS-CoV binds its spike (S) proteins to the host's angiotensin-converting enzyme 2 (ACE2). After entry, viral RNA is released into the cytoplasm-a potential site of thymoquinone to stop release. ORF1a and ORF1ab are translated into RdRP that eventually synthesizes and mediates both replication and transcription. $Zn2^{+}$ can stop viral replication by deactivating (-|) RdRP. Another bioactive component of the black seed, i.e., nigellimin, could have a dual action acting as an ionophore (→) to enhance Zn entry into infected cells and to inhibit (-|) virus envelope formation within infected cells. However, if replication is allowed to continue, full-length (-)RNA copies of the genome are produced and used as molds for full-length (+)RNA genomes. During transcription, subgenomic RNAs are produced through discontinuous transcription which are eventually translated to synthesize viral proteins. Generally, viral nucleocapsids are assembled from genomic RNA and N protein in the cytoplasm, followed by budding in the lumen of the endoplasmic reticulum-Golgi intermediate compartment. The virions are then released from the infected cell through exocytosis. (→, activation or stimulation; -|, inhibition)



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MUGWORT

In 1820, the first antimalarial drug was quinine extracted from cinchona bark by French pharmacists Pelletier and Caventou. In the 1940s, limited by the raw materials for quinine extraction, German scientists synthesized chloroquine, which is similar to natural quinine in chemical structure. Its derivative, hydroxychloroquine, was also synthesized with greater efficacy and less toxicity. By the mid-20th century, malaria was gradually controlled in China.

However, a local epidemic broke out in the 1960s and quickly spread to Southeast Asia and South America.
In addition, Plasmodium falciparum developed strong resistance to chloroquine. Inspired by ancient TCM books, Youyou Tu, a Chinese scientist, successfully extracted artemisinin from *Artemisia annua*. With a 100% inhibition rate against plasmodium, artemisinin has since gradually replaced chloroquine as a life-saving anti-malarial drug.
Youyou Tu became the winner of the Nobel Prize in Physiology or Medicine in 2015 for this achievement. [50]

Artemisia is commonly known as "wormwood." Wormwood, however, strictly speaking, refers to *Artemisia absinthium L.*, which is one of the most common and well-known species of the genus. *Artemisia annua* commonly called "annual wormwood" is an annual herbaceous plant, hence the name "annual." The plant is cultivated in Asia, India, Central and Eastern Europe, temperate regions of America, Africa, Australia and tropical regions. It is widely used as a dietary spice, herbal tea and medicinal plant in the mild climates of Asia, such as China and Korea.

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Artemisia annua has been used for many years in traditional medicine in Asia and Africa for the treatment of malaria and fever, in the form of tea or pressed juice.
The current Pharmacopoeia of the People's Republic of China officially lists the dried herb of *Artemisia annua* as a remedy for fever and malaria, at a **daily dose of 4.5-9 g of dried herb prepared as an infusion.** This is the medicinal plant preparation that has been used for clinical trials.[51]
The chemical composition and biological properties of aqueous or alcoholic extracts of *Artemisia annua* can vary greatly depending on its geographical origin, the plant material used, and the way it is processed, unlike those of the essential oil, which vary only slightly. [52]

Since its discovery, *Artemisia annua* has been the subject of extensive research into its chemical composition.
More than 600 secondary metabolites have been identified throughout the plant, including several sesquiterpenoids, triterpenoids, monoterpenoids, steroids, flavonoids, coumarins, alkaloids and benzenoids[53] .

In addition to artemisinin, *Artemisia annua* also has an interesting nutritional profile with the presence of amino acids, vitamins and minerals, and elements essential for health.[54]

Due to this richness, *Artemisia annua* possesses a large number of biological properties such as hepatoprotective, antifungal, antitumor, antioxidant, anti-inflammatory, and antiasthmatic activities.[55]
Artemisinins are a group of artemisinin-related drugs developed for the treatment of malaria[56] and have been reported to have multiple pharmacological activities, including antitumor, antiviral, and immunomodulatory.

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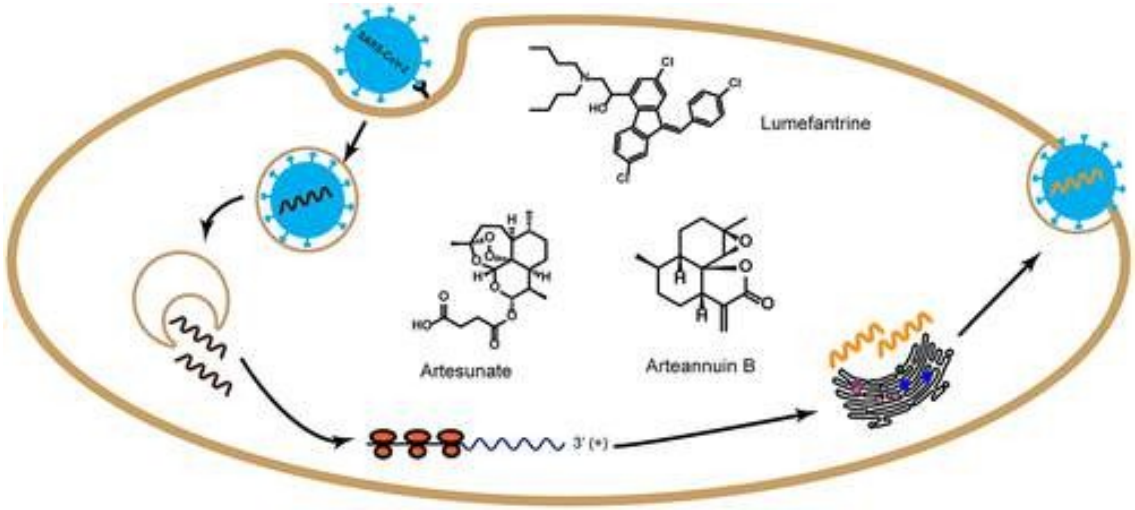
Considerations on the mechanism of action of artemisinin antimalarials: part 1--the 'carbon radical' and 'heme' hypotheses.

Among the reported artemisinins, artemisinin, dihydroartemisinin, artemetere-lumefantrine, artesunate, arteether and artemisone are approved drugs derived from artemisinin. For a more in-depth discussion of the constituents of *Artemisia annua* and its biological actions, we suggest reading the article "*Artemisia annua, a Traditional Plant Brought to Light.*" [57]
Considering the reported broad-spectrum antiviral potential of artemisinins, researchers investigated whether they could be used to combat COVID-19. Various in vitro studies have already demonstrated the efficacy of *Artemisia Annua* extracts and its active ingredients artemisinin, artenusate and artemeter against SARS-Cov-2. [58]

The pharmacological mechanism is mainly the inhibition of the enzymatic activity of CLPro (chymotrypsin-like protease), an enzyme produced by SARS-CoV-2 during infection, increased production of proinflammatory cytokines prostaglandins E2 (PGE2), IL-6, TNF-a , IFN-γ and $CD4^+$ and CD8 T-cell populations$^+$. [59]



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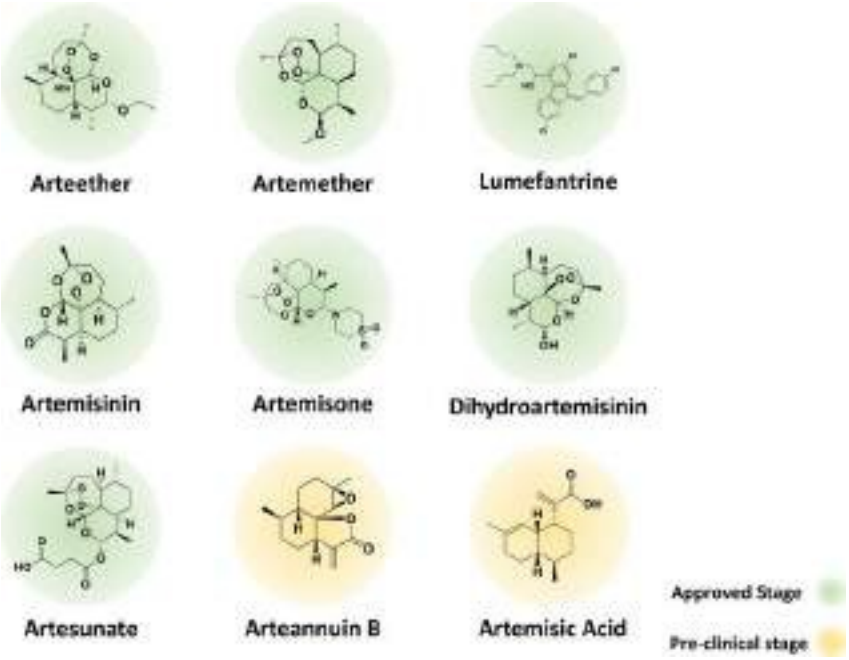
More clinical trials are currently underway to evaluate the in vivo efficacy against COVID-19,[60] however, it should be mentioned that in China decoctions and powders containing *Artemisia annua (*Haoqin Qingdan, Qinghao Bieija and Jinhua Qinggan)[61] and combination therapies between active ingredients of Artemisia annua and conventional drugs[62] have already been tested on patients with COVID-19 successfully, and some patented preparations are included in hospital therapy protocols.
The precautionary notes for use seen for Chloroquine and hydroxychloroquine also apply to Artemisia and its active ingredients, particularly the potential cardiac and neurological adverse reactions, although artemisinin has a better safety profile than the chemical derivatives and therefore can be used at higher therapeutic concentrations. [63]

In addition, it is important to keep in mind the risk of resistance to the active ingredients, which could occur with the massive use of the plant for COVID-19 therapy against malaria plasmodium in countries where this infection is still a major health problem.



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THE CHINESE

Another plant useful for its antiviral, antimalarial and febrifuge action is *Chincona officinalis* (or China in dialect terms). Chinchona are trees that, in the wild, grow to a large size with natural habitat in South America (particularly Peru). The part used for medicinal purposes is the bark of the branches, which is harvested when the plants are 15 to 25 years old (Cinchonae cortex F.U.), from which various alkaloids have been extracted including quinine (quinine salts), the precursor from which chloroquine and hydroxychloroquine (H-CQN) have been synthesized.[64]
Known as an authorized aromatic agent, quinine is added to bitter lemon and tonic water. According to EC Guideline No. 1334/2008[65] , quinine hypochloride (CAS-Nr. 130-89-2), quinine sulfate (CAS-Nr. 804-63-7) and quinine monohydrochloride dihydrate (CAS-Nr. 6119-47-7) can be added to food or beverages.

According to the well-developed pharmacokinetics for this drug, 50-100 mg of quinine, present in 1 Lt of e.g., tonic water, could result in a plasma concentration of ~0.5 µg/mL, and a molarity of ~1.5 µM[66] , which would achieve effective values in the in vitro systems tested[67] and thus could have utility in preventing infection.

Another comparative study showed that chloroquine, hydroxychloroquine and quinine can interact with amino acid residues in the peptidase domain of the ACE2 receptor. According to the results, quinine showed a higher affinity for the ACE2 receptor (-4.89 kcal/mol) followed by hydroxychloroquine (-3.87 kcal/mol) and chloroquine (-3.17 kcal/mol), respectively, and this suggests that quinine, chloroquine and hydroxychloroquine can prevent SARS-CoV-2 virus infection by interacting with the Lys353 residue in the peptidase region of the ACE2 receptor. [68]

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[65] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC (Text with EEA relevance)
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2752626/

[67] Maximilian Große, et al
Evidence That Quinine Exhibits Antiviral Activity against SARS-CoV-2 Infection In Vitro
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https://www.preprints.org/manuscript/202007.0102/v1/download

[68] Lestari, K., Sitorus, T., Megantara, S. and Levita, J
Molecular Docking of Quinine, Chloroquine and Hydroxychloroquine to Angiotensin Converting Enzyme 2 (ACE2) Receptor for Discovering New Potential COVID-19 Antidote,
J Adv Pharm Edu Res, (2020) 10(2): 1-4.
https://japer.in/storage/models/article/35pnDAguYFOxpT6iPhlybXNg64ULBZMQIxvX4aD2WzEEyhkSwVdOsU92f9OQ/molecular-docking-of-quinine-chloroquine-and-hydroxychloroquine-to-angiotensin-converting-enzyme-2.pdf

It follows that because quinine appears to exert a higher antiviral action in vitro against SARS-CoV-2 with a significantly better toxicity profile and has a higher plasma bioavailability than its chemical derivatives (chronic therapeutic doses for quinine result in a plasma concentration twenty times higher than H-CQN[69]), this natural product could pave the way for a more tolerable treatment of SARS-CoV-2 infection and also usable as a preventive measure[70] .

The available drug is quinine hydrochloride or quinine sulfate licensed for the treatment of malaria either orally or by injection, which is referred to for dosage, for off-label use for COVID-19. [71]
With regard to toxicology, it should be kept in mind that prolonged administration of quinine may give rise to symptoms of intolerance (cinchonism) manifested by headache, nausea and other phenomena affecting the digestive system, auditory and visual disturbances, cardiovascular disorders, and skin manifestations.

Quinine and China bark preparations are contraindicated in cases of favism (G6PD deficiency)[72] and can cause allergic phenomena with skin manifestations such as urticaria, eczematous allergic dermatitis, phenomena that, in particularly sensitive individuals, can also occur with the use of common bitters and aperitifs containing quinine. [73]

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[71] https://www.torrinomedica.it/schede-farmaci/chinina_solfato_nova_argentia_250_mg_compresse_rivestite/
https://www.torrinomedica.it/schede-farmaci/chinina_clagenzia_id_10_f500/

[72] https://www.pharmgkb.org/labelAnnotation/PA166105232

[73] https://www.torrinomedica.it/piante-medicinali/china/

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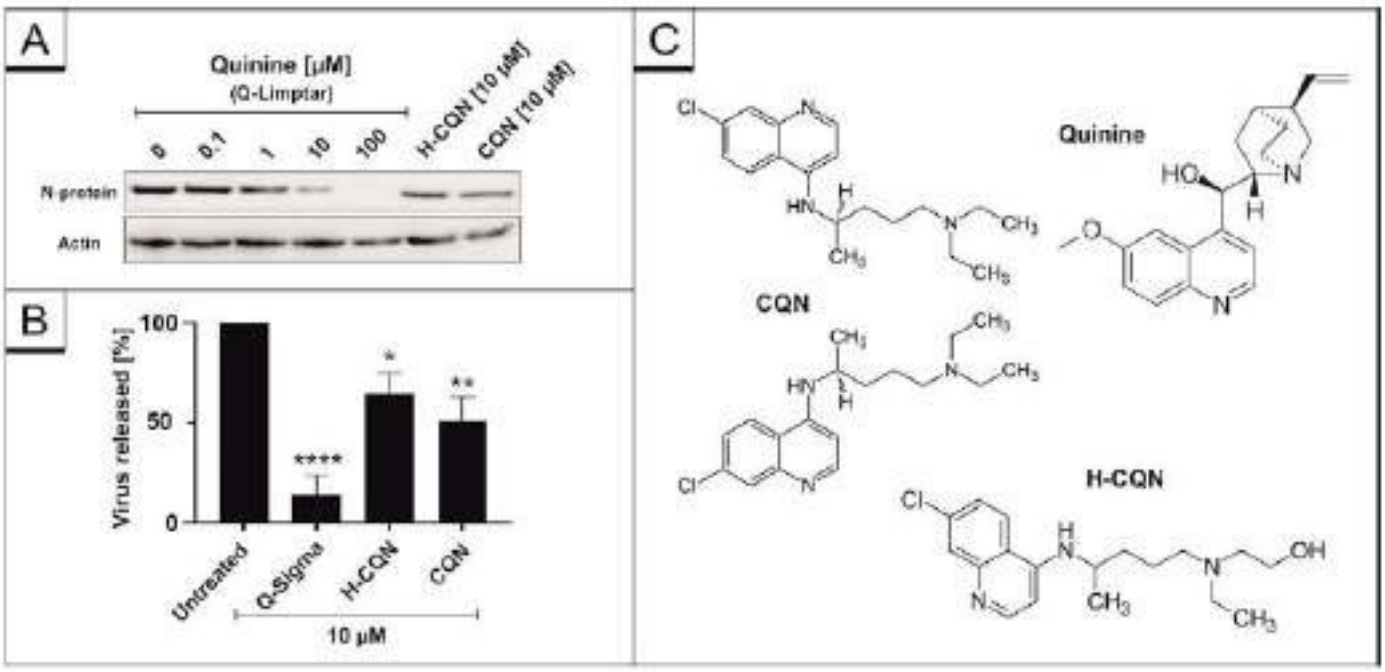

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Influence of quinine, H-CQN and CQN on SARS-CoV-2 replication in Vero B4 cells. (A) Western blot analysis of viral fractions (N-protein = Nucleoprotein). Cell fractions were stained with ß-actin antibody. Quinine sulfate was used as Q-Limptar (A) or Q-Sigma (B). Cell culture supernatants were harvested at 3 dpi. Virions were purified and analyzed by Western blot using a SARS-CoV-2 convalescent serum.(B) Densitometric analysis of Western blot analysis was performed using AIDA®. Analysis of five independent experiments ± standard deviation (SD) (Q-Sigma p
<0.001), three independent experiments ± SD (H-CQN p = 0.0270) or four independent experiments ± SD (CQN p = 0.0039). (C) Molecular structures of quinine, H- C Q N and CQN.

AZITHROMYCIN

Azithromycin is a macrolide antibiotic* commonly used for antiviral properties mainly attributed to reduced endosomal virion transfer as well as known anti-inflammatory effects.[74]

[74] Pani A, Lauriola M, Romandini A, Scaglione F.
Macrolides and viral infections: focus on azithromycin in COVID-19 pathology.
Int J Antimicrobial Agents. 2020;56(2) doi: 10.1016/j.ijantimicag.2020.106053.
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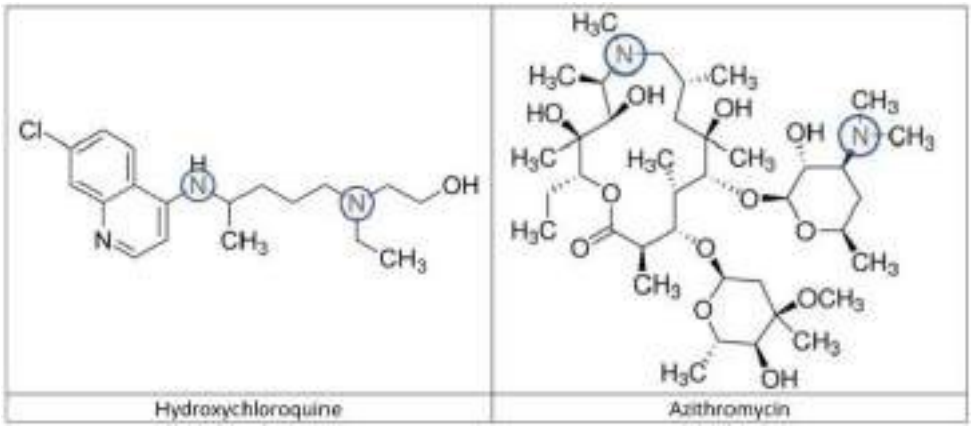
Firth A, Prathapan P.

The ability of azithromycin to localize to macrophages and increase type I interferon expression during viral infection indicates a potential to promote viral clearance and reduce the development of CRS (cytokine release syndrome) and MAS (macrophage activation syndrome).
However, this effect is strictly dependent on timing: prophylactic or therapeutic administration at an early stage may prevent viral entry and therapeutic intervention, whereas at an advanced stage it may cause a deleterious effect due to worsening immunopathology[75] .

* Macrolides are a class of naturally occurring compounds that consist of a 14-, 15-, or 16-unit macrocyclic lactone ring to which one or more deoxy sugars may be attached. Macrolides are bacteriostatic, a property achieved through reversible binding to the P site on the 50S subunit of the bacterial ribosome. Erythromycin, the first macrolide discovered, has been widely used as a penicillin substitute for patients with a penicillin-resistant disease or allergy. Azithromycin, a derivative of erythromycin, is designed to be more easily absorbed with fewer side effects and shows bacteriostatic activity against both Gram-positive and Gram-negative bacteria, including *Bordetella pertussis* and *Legionella* species.

As an endocytosis inhibitor*, azithromycin offers a second antiviral strategy against SARS-CoV-2. Since its first administration as malaria prophylaxis in 1998, azithromycin has been found to cause greater impairment of lysosomal acidification than chloroquine and not only can it proteolytically inhibit endocytosis in this way, but its accumulation within vacuoles increases their osmotic pressure with stronger vacuolation of late endocytic compartments than chloroquine.

* Endocytosis is a key pathway for protein recovery and recycling and plays a key role in viral infection. After binding to ACE2 or CD147, the SARS-CoV-2 S protein is proteolytically cleaved into two subunits that mediate viral entry and replication through the endocytic pathway. There are currently three different groups of endocytic pathway inhibitors being tested against COVID-19: the first being lysosomotropic agents such as hydroxychloroquine; the second being direct inhibitors of endosomal-lysosomal protease such as E64d; and the third being inhibitors of clathrin-mediated endocytosis such as chlorpromazine.[76]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7204663/
Chemical structures of hydroxychloroquine and azithromycin. Circles indicate pH-sensitive basic nitrogen groups.

Azithromycin: The First Broad-spectrum Therapeutic.
Eur J Med Chem. 2020;207:112739. doi:10.1016/j.ejmech.2020.112739
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Sultana J, Cutroneo PM, Crisafulli S, Puglisi G, Caramori G, Trifirò G.
Azithromycin in COVID-19 Patients: Pharmacological Mechanism, Clinical Evidence and Prescribing Guidelines.
Drug Saf. 2020;43(8):691-698. doi:10.1007/s40264-020-00976-7

[75] Channappanavar R, Fehr AR, Vijay R, et al.
Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice.
Cell Host Microbe. 2016;19(2):181-193. doi:10.1016/j.chom.2016.01.007
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4752723/

[76] Yang N, Shen HM.
Targeting the Endocytic Pathway and Autophagy Process as a Novel Therapeutic Strategy in COVID-19.
Int J Biol Sci. 2020;16(10):1724-1731. Published 2020 Mar 15. doi:10.7150/ijbs.45498
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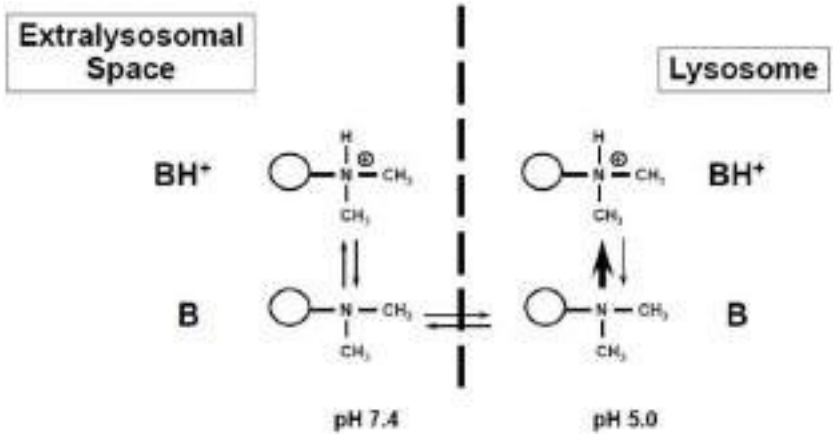
Although the two compounds are from two chemically distinct classes, they have structural similarity that is pharmacokinetically relevant. Both compounds are multibasic amines with pKa values susceptible to protonation in the physiological pH range.
Azithromycin has two nitrogen groups with pKa values of 8.1 and 8.8. Hydroxychloroquine has three nitrogen groups with pKa values of 4.0, 8.3 and 9.7. However, only the two nitrogen groups with the highest values (shown in circles) are protonated under physiological conditions.

If the pH of the environment in which the molecules are located is lower (more acidic), more nitrogen groups are protonated, and this prevents the now charged parts from crossing membranes. This is particularly important for the intracellular distribution of basic drugs between the cytosol (pH about 7.4) and the lysosomal acid space (pH about 5.0).
Basic compounds are in equilibrium between a less polar nonionized form (B) that can cross membranes and a protonated polar form (BH^+) that cannot cross membranes easily. When the nonionized drug enters the acidic environment of the lysosome, it will be protonated and "trapped" in the lysosome because the protonated BH form$^+$ cannot diffuse into the cytosol. As a result, high concentrations of the compound can accumulate in lysosomes.

This phenomenon is called "ion trapping." The extent of this accumulation depends on the mathematical relationship of the pKa of the compound of interest, its permeability, and the pH gradient between the two environments (e.g., cytosol [pH 7.4] and lysosome [pH 5.0]). The accumulation of up to 250-fold higher concentrations in lysosomes can be explained by the mechanism described. [77]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7204663/
Concept of lysosomal ion trapping. Basic compounds are in an equilibrium between a less polar nonionized form (B) that can easily cross membranes and a protonated polar form (BH^+) that cannot easily cross membranes. When the nonionized drug enters the acidic environment of a lysosome, it will be protonated and "trapped" in the lysosome because the protonated BH form$^+$ cannot easily diffuse into the cytosol. As a result, high concentrations of the compound can accumulate in lysosomes.

Azithromycin was commonly used in the COVID-19 studies initially based on French reports showing significantly reduced duration of virus spread, fewer hospitalizations, and reduced mortality in combination with HCQ compared with untreated.[78]
This agent is well tolerated but, similarly to HCQ, can prolong QTc in <1% of patients. The same safety precautions for HCQ listed above should be extended to azithromycin with or without HCQ.

[77] Derendorf H.
Excessive lysosomal ion-trapping of hydroxychloroquine and azithromycin.
Int J Antimicrob Agents. 2020;55(6):106007. doi:10.1016/j.ijantimicag.2020.106007
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[78] Lagier JC, Million M, Gautret P.
Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: a retrospective analysis.
Travel Med Infect Dis. 2020;36 doi: 10.1016/j.tmaid.2020.101791.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7315163/

Million M, Lagier JC, Gautret P.
Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: A retrospective analysis of 1061 cases in Marseille, France.
Travel Med Infect Dis. 2020;35 doi: 10.1016/j.tmaid.2020.101738.
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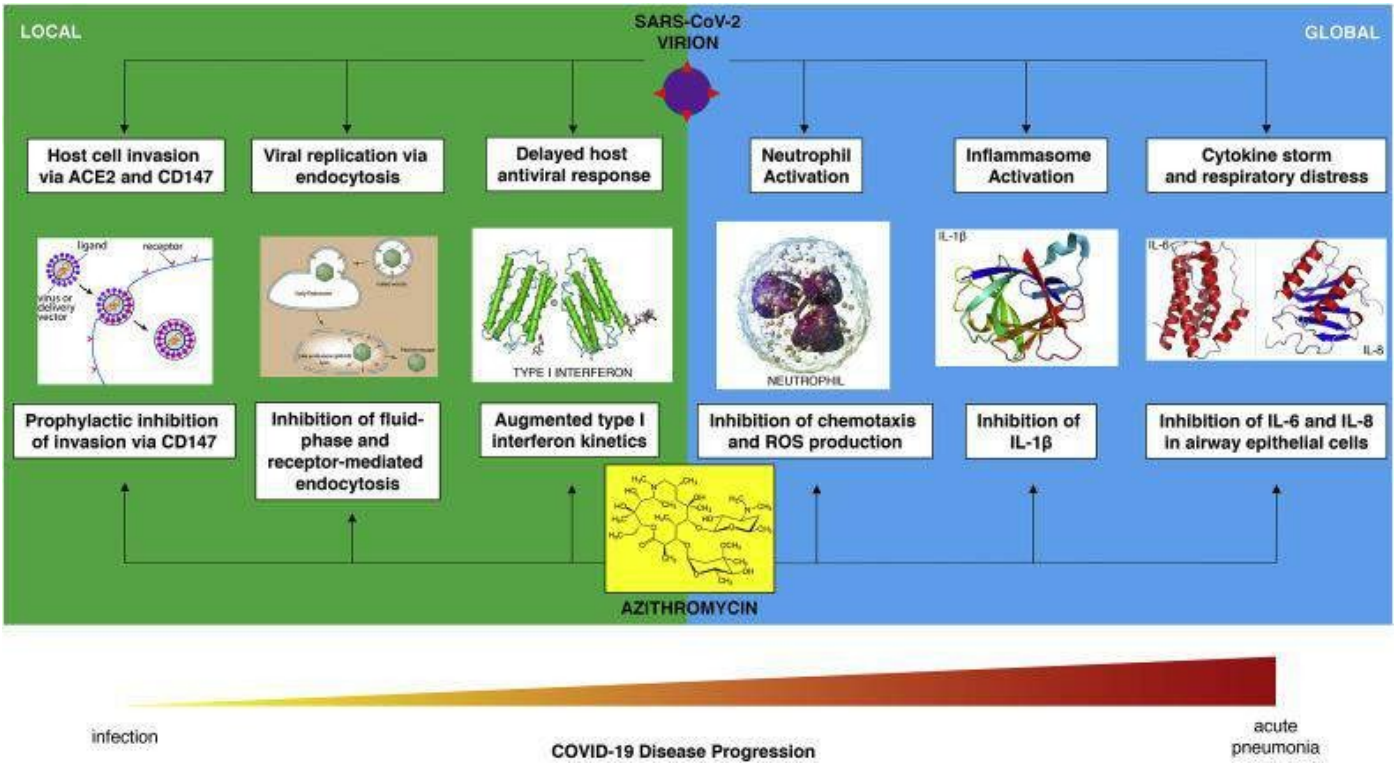
Because both HCQ and azithromycin have modest but potentially additive risks of QTc interval prolongation, patients with known or suspected arrhythmias or those taking contraindicated drugs should undergo a more thorough examination (e.g., baseline electrocardiogram reassessment, imaging studies, etc.) before receiving the two drugs together.
Azithromycin for its antibiotic action provides additional coverage against pathogenic upper respiratory tract bacteria that could potentially play a role in concomitant or secondary infections. Therefore, this agent may protect patients with COVID-19 from the bacterial component of community-acquired pneumonia.[79]

One of many dosing schedules is **250 mg per os / 2 x die for 5 days** and can extend to 30 days for persistent symptoms or evidence of bacterial superinfection.

To summarize, the in vivo properties of azithromycin can be classified into those that locally interface with the initial SARS-CoV-2 infection and those that globally modulate the subsequent host immune response in COVID-19 disease.
After administration, azithromycin localizes rapidly and at high concentrations in phagocytes and polarizes macrophage subpopulations toward the activated M2 phenotype, facilitating the host's innate response to infection.
Azithromycin upregulates both IFNβ, which potentiates the type I interferon signaling pathway, and MDA5 and RIG-I, which restore the host viral recognition system.
Access to host endocytic processes, a key mechanism of SARS-CoV-2 viral replication, is inhibited at the level of endolysosomal and receptor-mediated endocytosis, probably CD147. After initial infection, cytokine storm and hyperinflammation cause COVID-19 pneumonia; fifty years of use of macrolides for respiratory disease confirms their ability to reduce global inflammation, and azithromycin is particularly known for its more potent immunomodulatory effects and fewer side effects than other macrolides.

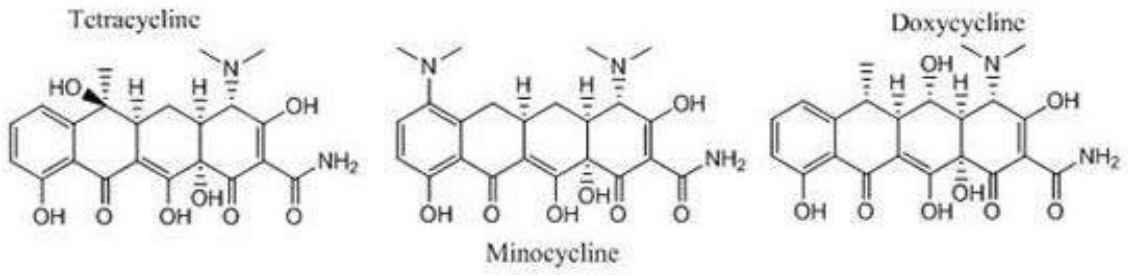


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7434625/
Pharmacological profile of azithromycin during the pathogenesis of COVID-19 pneumonia. A) Azithromycin preemptively inhibits viral invasion via CD147. B) As a lysosomotropic agent, azithromycin accumulates and increases the pH of endosomes and lysosomes, preventing viral replication.
C) Azithromycin increases host type I interferon (IFN) kinetics during viral infection. D) Dysregulation of the phagocyte compartment.

[79] Eljaaly K, Alshehri S, Aljabri A.
Clinical failure with and without empiric atypical bacteria coverage in hospitalized adults with community-acquired pneumonia: a systematic review and meta-analysis.
BMC Infect Dis. 2017;17(1):385. doi: 10.1186/s12879-017-2495-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5457549/

mononuclear (MNP)-associated COVID-19, lymphopenia, neutrophil activation, and blood hypercoagulation can be ameliorated by azithromycin. E) SARS- CoV-2 activates the inflammasome leading to aberrant release of cytokines such as IL-1β. Azithromycin, as a macrolide, reduces inflammasome activity and lowers IL-1β levels. F) By reducing IL-6, IL-8 and TNF-alpha, azithromycin can antagonize cytokine release syndrome (CRS) associated with COVID-19 and acute respiratory distress syndrome (ARDS).

DOXYCYCLINE AND MINOCYCLINE



https://pharmafactz.com/medicinal-chemistry-of-antibacterial-drugs/

Doxycycline is an antibiotic belonging to the tetracycline group, with multiple intracellular effects leading to reduction of viral replication, cellular damage, and expression of inflammatory factors.
This drug has no effect on cardiac conduction and has gastrointestinal disorders and esophagitis.
Like azithromycin, doxycycline has the advantage of providing antibacterial coverage for co-infections in the upper respiratory tract with a high degree of activity against many common respiratory pathogens including *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis*, anaerobes such as *Bacteroides* and anaerobic/microaerophilic streptococci, and atypical agents such as Legionella, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*.

One of many dosages is **200 mg per os followed by 100 mg per os /2 times a day for 5 days** and may extend to 30 days for persistent symptoms or evidence of bacterial superinfection. Doxycycline may be useful with HCQ for patients in whom HCQ-azithromycin combination is not desired. [80]

[80] Sodhi M, Etminan M.
Therapeutic Potential for Tetracyclines in the Treatment of COVID-19.
Pharmacotherapy. 2020;40(5):487-488. doi:10.1002/phar.2395
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Doxycycline is a cost-effective therapy for hospitalized patients with community-acquired pneumonia.
Arch Intern Med. 1999 Feb 8;159(3):266-70. doi: 10.1001/archinte.159.3.266. PMID: 9989538.
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC99026/

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Doxycycline treatment of high-risk COVID-19-positive patients with comorbid pulmonary disease.
Ther Adv Respir Dis. 2020;14:1753466620951053. doi:10.1177/1753466620951053
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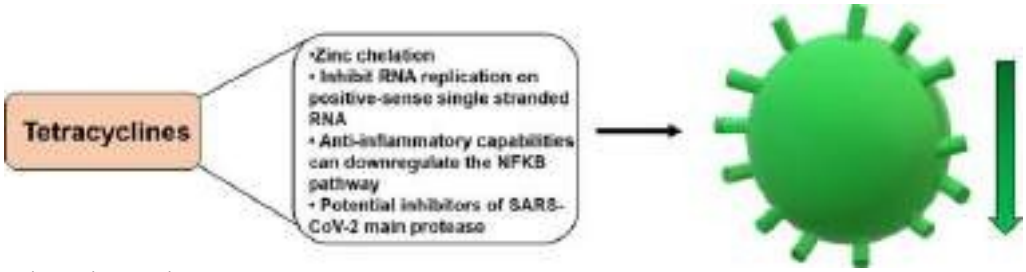

https://link.springer.com/article/10.1007/s00705-020-04860-8
Potential effects of tetracyclines on SARS-CoV-2. Several properties of tetracyclines may potentially reduce the ability of SARS-CoV-2 to enter and replicate in the cell

Minocycline is another antibiotic in the tetracycline group and possesses broad-spectrum activity against gram-positive and gram-negative bacteria, *Rickettsia*, *Mycoplasma pneumoniae*, *Chlamydia, Plasmodium spp.* and many spirochetes.
The drug has been used since 1972, and its range of clinical applications includes infections with susceptible microorganisms and rheumatoid arthritis. In more recent years, several studies have demonstrated its nonantibiotic activities, including anti-inflammatory, antioxidant, anti-apoptotic, neuroprotective, and antitumor properties.

The significant anti-inflammatory and immunomodulatory effects of minocycline may offer potential advantages in the management of COVID-19 patients, particularly for its respiratory complications-ARDS and multi-organ damage.
Minocycline has been shown to significantly reduce the secretion of cytokines such as IL-6, IL-2, and TNF-α, thus offering
A potential role in ameliorating the cytokine release syndrome associated with COVID-19.

Minocycline is known to concentrate in normal and ischemic myocardium and has demonstrated remarkable cardiac cytoprotective properties in several preclinical studies by virtue of its anti-inflammatory, antiapoptotic, antioxidant, and matrix metalloproteinase inhibitory actions.
Although used chronically at doses up to 200 mg/day, the highest approved dosage, the drug is generally safe and well tolerated by patients. Compared with other members of the tetracycline class, it has a better pharmacokinetic profile for rapid and complete absorption and excellent oral bioavailability, long half-life, marked tissue penetration, and safety even in elderly populations.

Common adverse effects, including nausea, dizziness, and mild dizziness, generally appear immediately after administration and are short-lived after discontinuation. Considering the fact that most COVID-19 patients will require treatment for only a few days, the use of minocycline in these patients is unlikely to result in significant tolerability problems. However, because of the potential risk of teratogenicity, its use in pregnant women is not recommended.
Minocycline also has cost advantages because it is an off-patent drug and is relatively inexpensive compared with other currently studied alternatives such as lopinavir/ritonavir, remdesivir, tocilizumab, and other biologic drugs.
Given the magnitude of the COVID-19 pandemic so far and based on future projections, such cost benefits may be crucial to support overburdened national health systems around the world, especially in low- and middle-income countries with limited resources. The drug is also readily available compared with alternatives such as chloroquine and hydroxychloroquine. [81]

It has no major safety concerns or potential life-threatening drug interactions, particularly additive cardiotoxicity with HCQ that can occur with concomitant use of HCQ and azithromycin.

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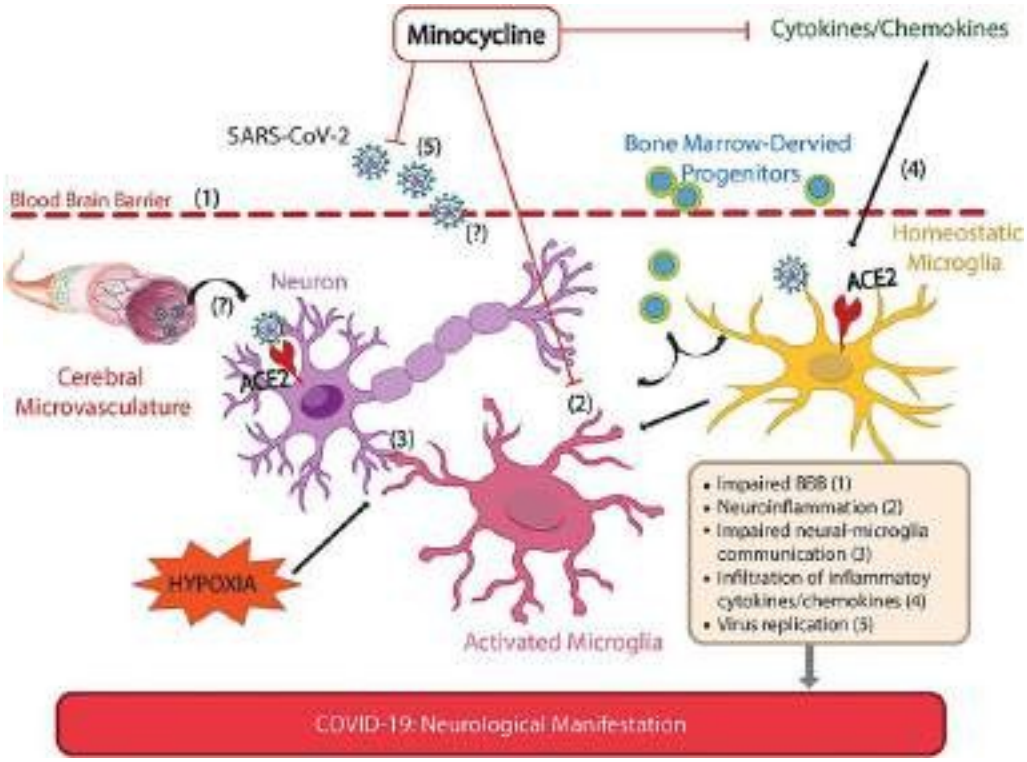
Thus, minocycline may be an ideal candidate for combination with HCQ for better outcomes in moderate to severe patients with COVID-19 infection.[82]

Apart from the role for treatment of COVID-19 based on immunomodulatory properties, recent in silico screening studies have shown great antiviral potential of minocycline against SARS-CoV-2, particularly the potent inhibitory action toward M^{pro} (the protease of SARS-Cov-2). [83]
Another very important effect of minocycline is its protective effect on nerve tissue through inhibition of the mediators of inflammation and microglia activation.
Experimental and clinical data have confirmed the preclinical and clinical potential of minocycline in the treatment of stroke due to its anti-inflammatory, antioxidant, and antiapoptotic properties.
These neuroprotective effects make it a good candidate for the prevention and treatment of neurological complications due to COVID-19. [84]



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Possible sites of action of minocycline to alleviate neurological manifestations of Covid-19. Neurological manifestations have been widely reported in patients with COVID-19 and are associated with more severe symptoms. These manifestations may result from direct and/or indirect mechanisms following SARS-CoV-2 infection. The SARS-CoV-2 receptor, ACE2, is expressed in neurons, glial and endothelial cells allowing the virus to become infected and spread to the brain, impairing cellular communication. SARS-CoV-2 has been detected in brain parenchyma, and possible pathways for SARS-CoV-2 infiltration such as the compromised blood-brain barrier are shown. Anosmia, a common sign of SARS-CoV-2 infection reflecting damage to olfactory nerves, may illustrate another possible pathway for virus infiltration, via peripheral nerves to the CNS. Indirect effects on the CNS include those of Covid- 19-induced hypoxia such as neuroinflammation, altered neuron-microglia communication, autonomic imbalance, altered BBB, increased inflammatory cytokines, and increased release of BM-derived progenitor cells. This is consistent with rodent experiments that directly investigated brain damage due to hypoxia. Minocycline, an anti-inflammatory antibiotic that readily penetrates the CNS, counteracts neuroinflammation, virus replication and attenuates the increase in pro-inflammatory cytokines. Together, these actions of minocycline alleviate hypoxia-induced neuroinflammation and impaired neural-microglia communication that can precipitate neuronal and glial injury, thereby preventing potential long-term neurological consequences of COVID-19.

For the treatment of COVID-19, it is of great importance to consider the interaction between SARS-Cov-2 and the gastrointestinal microbiota. It is known from a large body of scientific literature that SARS-Cov-2 RNA can be detected in the stool of COVID-19 patients for prolonged times beyond negativity in oropharyngeal or bronchoalveolar samples, and that infection is associated with both a state of dysbiosis and a worse prognosis in cases of pre-existing dysbiosis. [85]

In a recent preprint article, Petrillo et al[86] tested the in vitro replication capacity of SARS-Cov-2 in stool samples obtained from a COVID-19 patient and a healthy control in the presence of specific antibiotics (metronidazole clindamycin, lincomycin, piperacillin+tazobactam, vancomycin, amoxicillin, ampicillin, cefixime, ceftriaxone, meropenem, rifaximin, azithromycin, erithromycin, gentamicin, ciprofloxacin, colistin, levofloxacin, and teicoplanin). The bacterial growth and metabolic activity in the infected specimen were analyzed and monitored over time using SANIST Biotyper according to the method described by Cristoni et al.[87] The bacteria most commonly implicated were found to be of the genus *Clostridium* and *Escherichia coli*.

Three days after the addition of the different antibiotics, the SARS-CoV-2 RNA load was found to be affected by their presence in several ways:

- SARS-CoV-2 RNA load was reduced to negligible levels in the aliquots treated with metronidazole, vancomycin, amoxicillin and azithromycin;
- SARS-CoV-2 RNA load decreased from 20% to 85% in aliquots treated with piperallicin + tazobactam, ampicillin, cefixime, ceftriaxone, meropenem, gentamicin, ciprofloxacin, and teicoplanin. For example, cefixime induced a decrease in viral RNA load by 85%, ciprofloxacin by 61% and teicoplanin by 56%;
- SARS-CoV-2 RNA load did not decrease substantially in the aliquots treated with clindamycin, lincomycin, rifaximin, erythromycin, colistin and levofloxacin.

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Predicting and preventing intestinal dysbiosis on the basis of pharmacological gut microbiota metabolism.
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These preliminary results highlight that SARS-CoV-2 replicates efficiently in a bacterial growth medium and that replication follows bacterial growth, leading to the hypothesis that SARS-Cov-2 is able to colonize intestinal bacteria with a mechanism of action similar to Bacteriophages[88] , confirming the antiviral efficacy on SARS-Cov-2 of some types of antibiotics.

In a further study by the same group,[89] the production of specific toxin-like peptides * associated with virus replication in intestinal bacteria and potentially responsible for extrapulmonary manifestations peculiar to COVID- 19 was found.
The types of toxic peptides found resemble known conotoxins, phospholipase A2, metalloproteinases, prothrombin activators, and coagulation factors usually found in animal venoms known to have high specificity and affinity toward human ion channels, receptors, and nervous system transporters such as the nicotinic acetylcholine receptor.

Preliminary evidence indicates that they are completely reduced to negligible levels in fecal infected sample aliquots treated with metronidazole and vancomycin (data not shown, manuscript in preparation).

* Toxin-like peptides detected in urine and plasma of patients with extrapulmonary manifestations, particularly neurological, by COVID-19:

- *conotoxins*: many of these peptides have been shown to modulate the activity of several receptors, including ion channels, nicotinic acetylcholine receptors (nAChRs) and enzymes (acetylcholine-esterases) that degrade acetylcholine causing altered acetylcholine levels and cholinergic transmission. The presence of conotoxin-like peptides, could explain the occurrence of many symptoms (such as hyposmia, hypogeusia and typical signs of Guillain-Barre syndrome) observed in some patients with COVID-19.

- *phospholipase A2*: These enzymes hydrolyze phospholipids and lead to the release of phospholipidic acid and arachidonic acid, the precursor of many pro-inflammatory mediators such as leukotrienes, thromboxanes, and prostaglandins; as a consequence, an out-of-range presence of active PLA2 can induce severe inflammation. In animal venoms, PL A2 acts as a neurotoxin resulting in a severe inflammatory response leading to nerve terminal and skeletal muscle degeneration. The drug dexamethasone is able to inhibit prostaglandin synthesis and leukotriene formation, so it is plausible that the positive effect of this drug on patients with COVID-19 is also due to the reduction of the identified PLA2-like peptide.

- metallo-proteinases: metallo-proteinases present in animal venoms are zinc-dependent enzymes that cause hemorrhage, local myo- necrosis, skin damage, and inflammatory reactions. It has been reported that symptomatic COVID-19 patients have significantly lower zinc levels than controls and that zinc is deficient in patients who develop more complications. The presence of this specific group of toxin-like peptides, which sequester zinc, may be the reason for such significantly low zinc levels in symptomatic COVID-19 patients.

Based on this knowledge, isopathicand immunobiological medicines* (e.g., SANUM line products) may find the rationale f o r use to inhibit SARS-Cov-2

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* Isopathic and immunobiological therapy is a treatment method belonging to biological medicine, which aims to rebalance the symbiosis between our cells and symbiotic microorganisms. Since this symbiosis and the environment where the cells live are key factors for staying healthy, isopathic therapy is suitable for many disorders and diseases including those caused by COVID-19. [90]

ANTIVIRAL AGENTS

The following is a summary table with treatment patterns of off-label and investigational chemical drugs for antiviral action in the treatment of COVID-19, taken from the article "*COVID-19: A review of the proposed pharmacological treatments.* " [91]
This is followed by chemical structures and a figure representing the mechanism of action of individual drugs.
For adverse reactions and precautions for use, please refer to the data sheets of individual drug products.

[90] https://vdocuments.site/dr-flavio-tonello-associazione-omeopatica-dulcamara-faculty-of-homeopathy-of-united-kingdom-introduzione-alla-terapia-immunoisopathic-first-part-1.html

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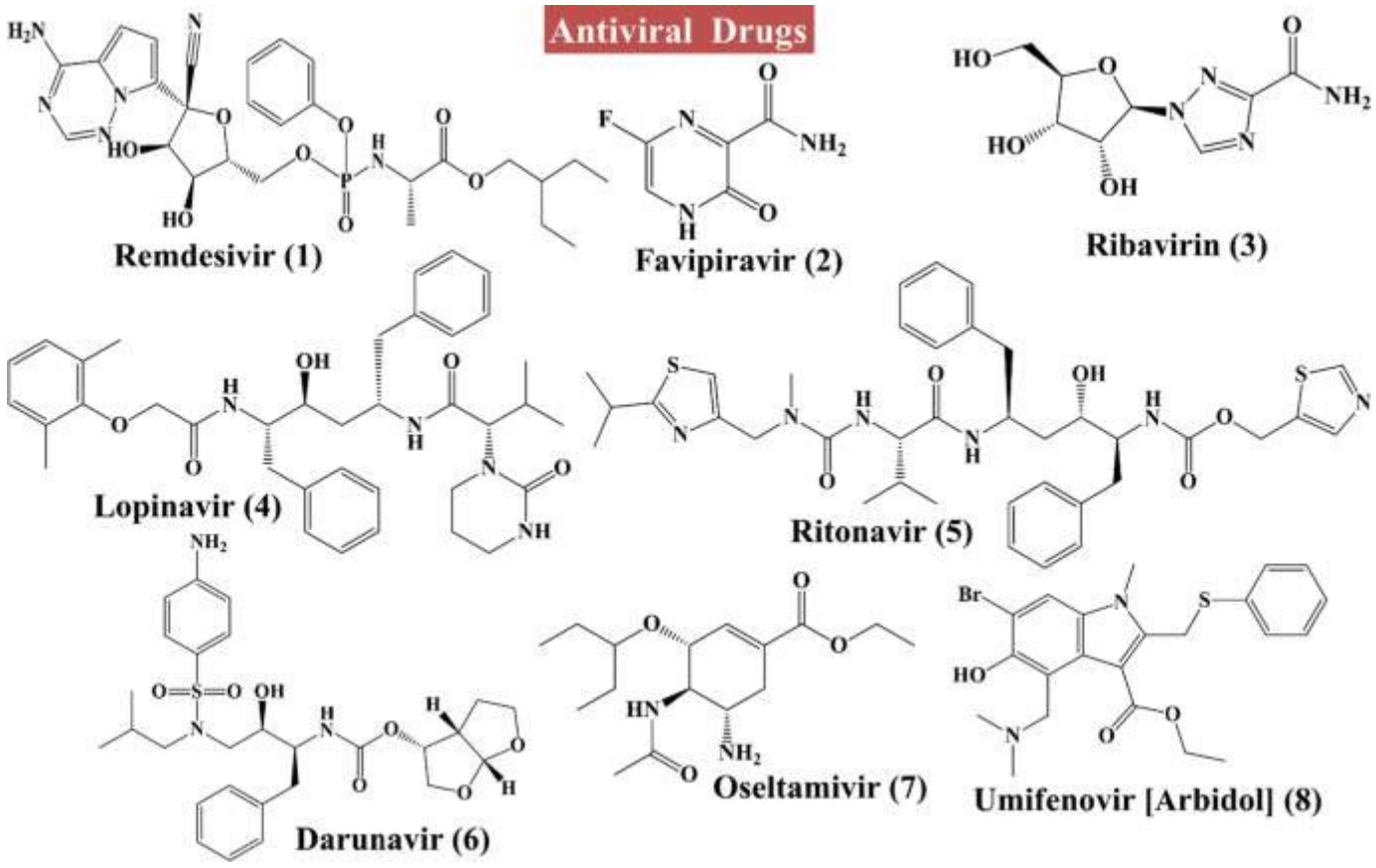
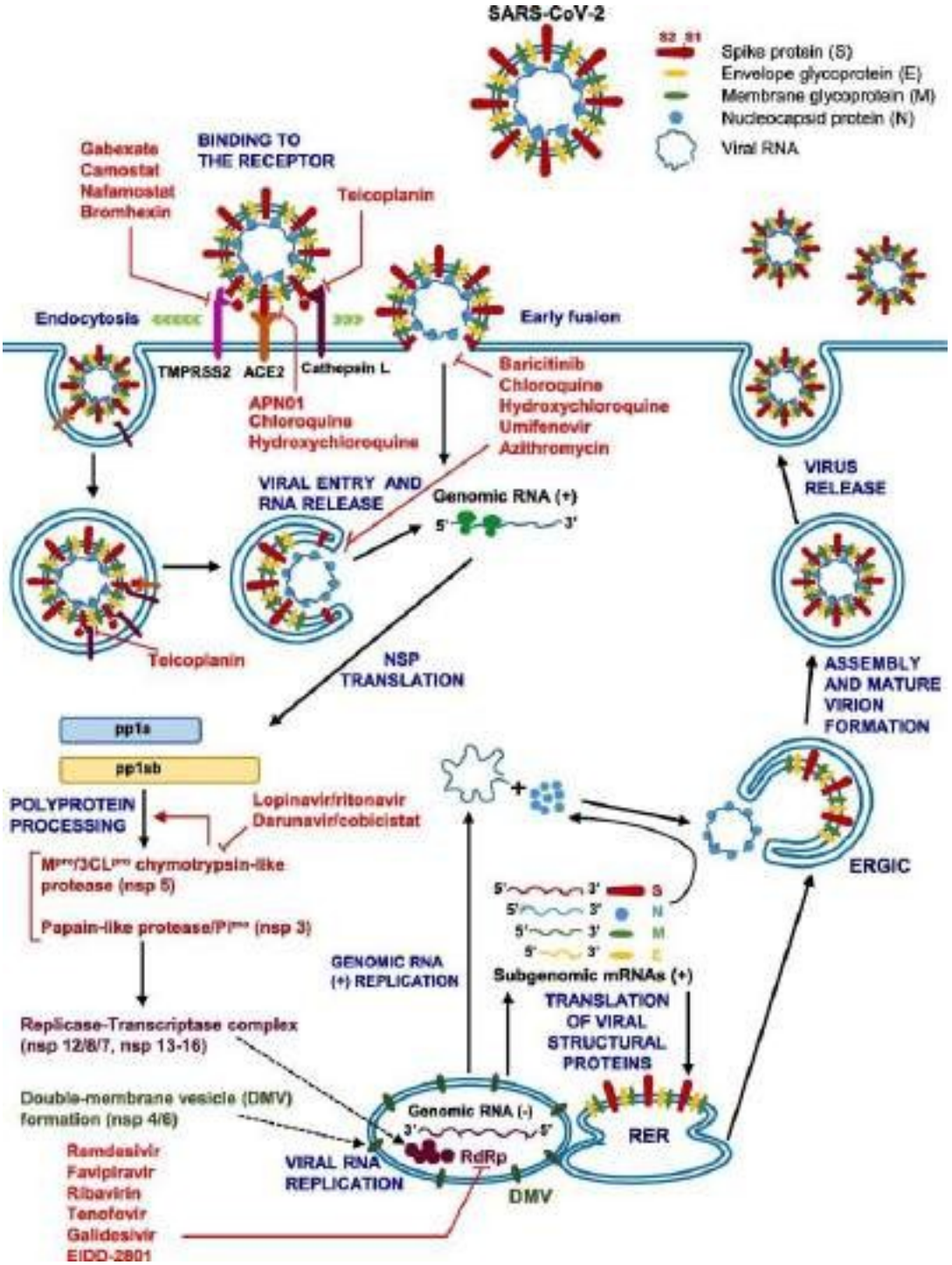
COVID-19 Treatments: Antiviral and Anti-inflammation
https://www.medchemexpress.com/literature/article/COVID-19.html

Drug	Administration	Dosage	Approved indication(s)
Remdesivir	IV	10 day administration; day 1 200 mg QD loading dose, followed by 100 mg QD	None
Ribavirin	Oral	500 mg BID or TID in combination with IFN-α or lopinavir/ritonavir	RSV infection, hepatitis C, bunyavirus, herpesvirus, adenovirus, poxvirus, and some viral hemorrhagic fevers
Lopinavir-ritonavir (Kaletra)	Oral	400mg/100 mg BID for up to 14 days	HIV
Favipiravir	Oral	600 mg BID	Influenza A and B, Ebola virus, Norovirus
Chloroquine (Aralen)	Oral	500 mg orally QD or BID for 5–10 days	Systemic lupus erythematosus (SLW), rheumatoid arthritis (RA), malaria
Hydroxychloroquine (Plaquenil)	Oral	Day 1 400 mg BID, followed by 200 mg BID for 5–10 days Alternative: 200 mg TID for 10 days or 400 mg QD for 5 days	Systemic lupus erythematosus (SLW), rheumatoid arthritis (RA), malaria
Oseltamivir	Oral	75 mg QD	Influenza A and B
Umifenovir (Arbidol)	Oral	200 mg TID for 7–14 days	Influenza A and B

https://www.medchemexpress.com/literature/article/COVID-19.html



https://link.springer.com/article/10.1007/s11030-020-10134-x



https://europepmc.org/article/med/32710969

Schematic diagram of the SARS-CoV-2 replication cycle in human cells and potential viral targets of repurposed drugs that have been used and empirically tested in clinical trials for COVID-19 treatment. During the viral replication cycle, the SARS-CoV-2 spike protein (S) binds to ACE2 in host cells, and after the attachment phase, the entry process requires priming of the S protein by cellular proteases (i.e., TMPRSS2, cathepsin L, furin). Fusion of the virus and cell membranes probably occurs either at the plasma membrane (early fusion) or at the endosomal level (endocytosis), after which nucleocapsid release into the cytoplasm occurs. Most of the viral genome sequence is translated directly to produce the pp1a and pp1ab polyproteins, which are processed by viral proteases (3CLpro / Mpro, PLpro) into 16 nonstructural proteins (nsps), including the RNA-dependent RNA polymerase (RdRp) and other proteins to form the replication-transcription complex, which is anchored to double-membrane vesicles (DMVs) embedded in a reticulo- vesicular network of modified endoplasmic reticulum membranes. The viral RdRp synthesizes a complementary full-length negative-strand RNA as a template for the production of the positive-strand genome of the virus progeny and a set of subgenomic mRNAs derived from negative-sense RNA intermediates (not shown). Subgenomic mRNAs are translated into structural proteins in the rough endoplasmic reticulum (RER) [spike protein (S), membrane (M), envelope (E)] or cytosol [nucleocapsid protein (N)]. The S, E and M move along the intermediate compartment of the endoplasmic reticulum-Golgi (ERGIC). The viral genomic RNA is encapsulated by the nucleocapsid protein N and subsequently sprouts in the ERGIC and acquires a membrane containing the structural proteins S, E and M. Finally, the virus is released by exocytosis. The blunt red arrows indicate the potential drug targets listed. See text for further details.

For phytotherapeutic and functional medicine antiviral treatments, please refer to previously disclosed documents On the topic. [92]

3) IMMUNOMODULATION

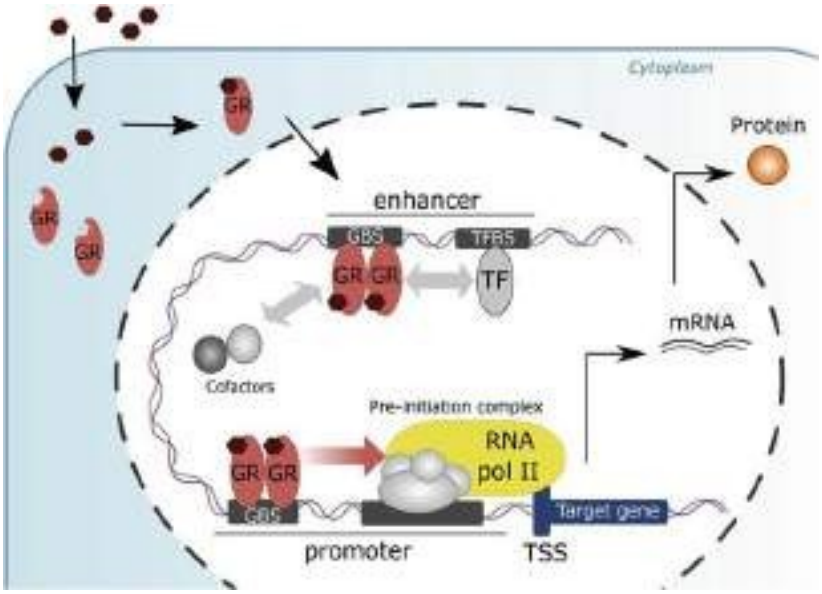
CORTICOSTEROIDS

Mechanism of action

Corticosteroids are endogenous hormones produced in the adrenal cortex or obtained by chemical synthesis.

> Corticosteroids, also called corticosurrenal hormones or corticoids, are a group of steroid hormones synthesized in the cortex of the adrenal gland. Based on their physiological function, they are divided into three families, glucocorticoids-so named because of their importance in glucose metabolism-mineralcorticoids-active in mineral salt balance, particularly sodium and potassium-and ***sex hormones***.

Glucocorticoids, after entering cells, bind to intracellular receptors (GR) and translocate to the nucleus where they interact with GR binding sites (GBS) in the promoter and/or enhancer regions. Genome-bound GR, together with cofactors and other transcription factor binding sites (TFBS), influences the recruitment and activity of RNA polymerase II to regulate the expression of its target gene



https://www.researchgate.net/figure/Signaling-pathway-of-the-glucocorticoid-receptor-Unbound-glucocorticoid-receptor-GR_fig1_311075263

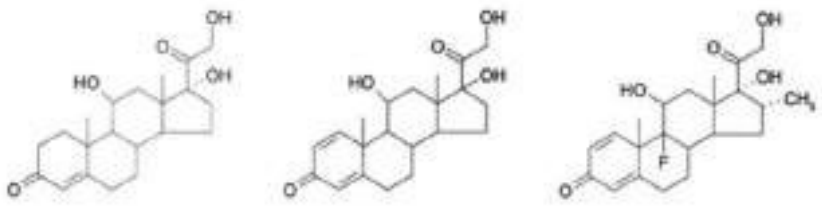
Glucocorticoids increase the production of anti-inflammatory compounds, such as annexin-1, SLP1, MOP-1, IκB-α, GILZ, and nitric oxide synthase, and at high doses reduce the production of pro-inflammatory compounds, including cytokines, chemokines, adhesion molecules, and pro-inflammatory enzymes, such as phospholipase A2 and cyclooxygenase.

The consequences of glucocorticoid action are very broad and include: mobilization of glucose through increased gluconeogenesis, which causes hyperglycemia and thus increased insulin secretion and glycogen storage; redistribution of body fat; protein breakdown; neutrophilia, lymphopenia, and immunosuppression.

Immunosuppression results partly from inhibition of kinases responsible for cytokine production and partly from inhibition of the nuclear transcription factor NF-κB, which stimulates transcription of cytokines, chemokines and other molecules in the

[92] https://rinascimentoitalia.it/approfondimenti-tematici/
https://www.studiesalute.it/salute

inflammatory pathway. Glucocorticoids also induce histone deacetylase (HDAC2) by activating transcription factors such as CREB, AP-1 and NF-κB. This activates chromatin, which increases gene transcription.
Glucocorticoids increase apoptosis in inflammatory cells such as eosinophils, T lymphocytes, mast cells, and macrophages, reducing cellular immune responses and cytokine production.[93]



Corticosteroids and COVID-19

Manifestations of COVID-19 that require hospitalization and could lead to multiorgan damage are attributed to a cytokine storm. The characteristic profile of a severely ill COVID-19 patient includes leukocytosis with relative neutropenia.
These patients have higher serum levels of cytokines (i.e., TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6 and IL-10) and C-reactive protein than control individuals. Among patients with COVID-19, serum levels of IL-6 and IL-10 appear even higher in critically ill patients. [94]

As with any acute inflammatory state, early treatment with immunomodulators is expected to provide greater benefit. [95] In COVID-19, some of the early respiratory symptoms are nasal congestion, coughing and wheezing, features due to excessive inflammation and cytokine activation.

Early use of corticosteroids is a rational intervention for patients with COVID-19 with these features, as they would be in acute asthma or reactive airway disease.[96]

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https://www.cebm.net/covid-19/dexamethasone/

[94] Han H, Ma Q, Li C, et al.
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The RECOVERY trial randomized 6425 patients hospitalized with COVID-19 in a 2: 1 ratio with dexamethasone 6 mg PO/EV daily for up to 10 days and found a reduction in mortality with dexamethasone (HR = 0.65, 95% CI 0.51- 0.82, P < 0.001). [97]

A potential dosing schedule for outpatients starting on day 5 or at the onset of respiration symptoms are **prednisone 1 mg/kg administered daily for 5 days** with or without subsequent gradual reduction.

Treatment with corticosteroids is a double-edged sword, which can exacerbate an excessive immune response. Based on this mechanism, the clinical use of corticosteroids should be very cautious.
Lymphopenia could be a critical factor associated with disease severity and mortality. [98] Guo and his colleagues demonstrated that in viral pneumonia, the absolute counts of CD3 T lymphocytes$^+$, CD3 T lymphocytes$^+$ CD4$^+$ and CD3 T lymphocytes$^+$ CD8$^+$ in deceased patients were significantly lower than in survivors[99] , suggesting that the cellular immune function of individuals with severe viral pneumonia was significantly inhibited.

In fatal COVID-19, autopsy found that CD4 and CD8 T lymphocyte counts were substantially reduced and hyperactivated. Further research showed that the concentration of highly pro-inflammatory CCR6$^+$ Th17 in CD4 T cells was increased, and high concentrations of cytotoxic granules were found in CD8 T cells.
These results imply that excess T-cell activation, with elevation of Th17 and high cytotoxicity of CD8 T cells may explain the severe immune damage.
For these patients, corticosteroid use may inhibit T-cell immunity and lead to persistent viral replication and subsequent delays in clearance.[100]

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Respir Physiol Neurobiol. 2020;280:103492. doi:10.1016/j.resp.2020.103492
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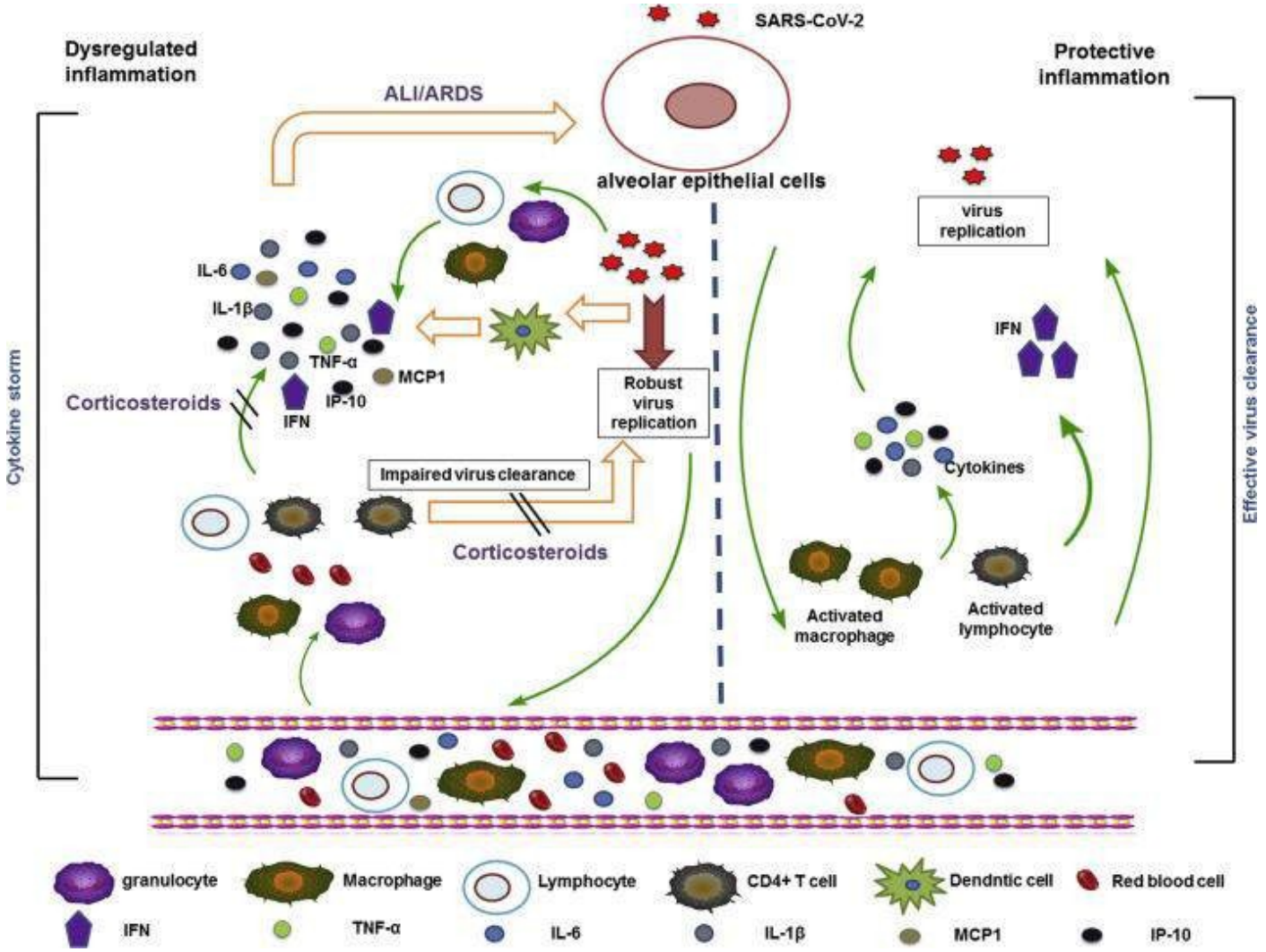


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7320691/

For the use of corticosteroids, please refer to the guideline recently published by WHO on 02.09.2020 [101]

COLCHICINE

Mechanism of action

Colchicine is a tricyclic alkaloid that is extracted from the plant *Colchicum autumnale*.
Colchicine acts as a potent inhibitor of tubulin polymerization due to its high affinity to the β-tubulin subunit. Microtubules are the key element of the cytoskeleton and are involved in multiple cellular processes such as maintenance of cell shape, intracellular substance transfer, secretion of cytokines and chemokines, cell migration, regulation of ion channels, and cell division.

Colchicine is also an antimitotic substance that blocks cell division during metaphase: when colchicine binds to tubulin, the straight conformation of αβ-tubulin heterodimeric subunits is lost resulting in curved tubulin heterodimers. The lateral contacts between adjacent αβ subunits that are necessary to maintain the interaction between them are lost, and when the lateral contacts decrease, the microtubules disassemble.

[101] Corticosteroids for COVID-19
https://www.who.int/publications/i/item/WHO-2019-nCoV-Corticosteroids-2020.1

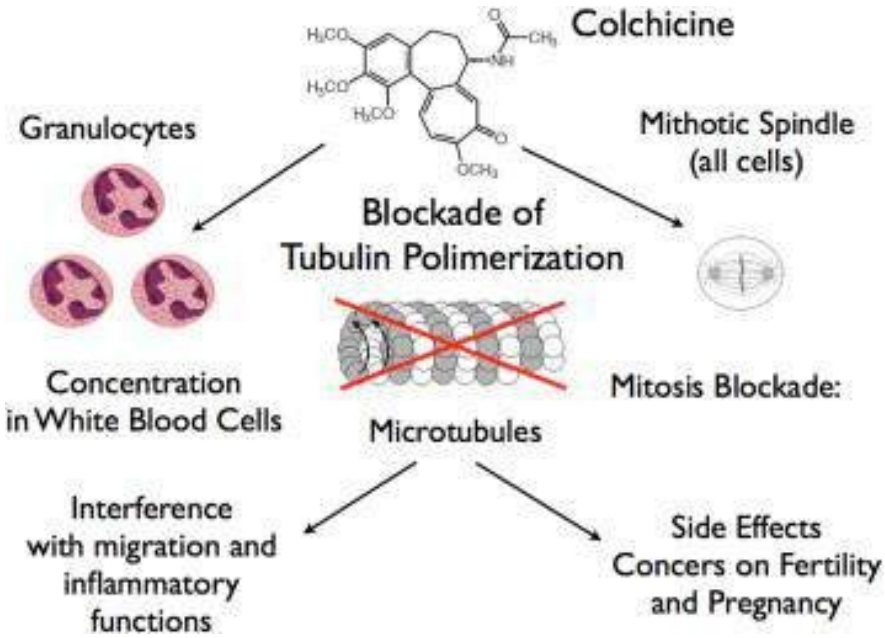
Colchicine can also modify the voltage-dependent anion channels of mitochondrial membranes, thereby limiting mitochondrial metabolism, and has an additional effect on the chemotaxis of inflammatory cells such as neutrophils and monocytes and on the intracellular transport of vesicles such as endosomes and exosomes.

It also inhibits the expression of E-selectin, an adhesion molecule important for binding leukocytes to endothelial cells and the recruitment of monocytes and neutrophils to the inflamed tissue.

Finally, colchicine reduces the production of free radicals such as superoxide in neutrophils. It has also been associated with inflammasome inhibition, thereby suppressing caspase-1 activation and subsequent release of IL-1β and IL-18.

The most common side effects are gastrointestinal (nausea, vomiting, and especially dose-related diarrhea) occurring in 5-10% of patients. Toxicity of colchicine is due to its antimitotic properties and may cause multiorgan dysfunction; however, it is safe when used according to established therapeutic guidelines and toxicity is rare if recommended doses are not exceeded.[102]

In the blood about 40% of colchicine binds to albumin. Peak plasma concentrations occur 1h after administration, and maximal anti-inflammatory effects develop over the 24-48h period based on intra-leukocyte accumulation. Colchicine reaches much higher concentrations in leukocytes than in plasma and persists there for several days after ingestion. This partly explains its rapid blockade of acute inflammatory diseases such as gout and in idiopathic recurrent pericarditis.



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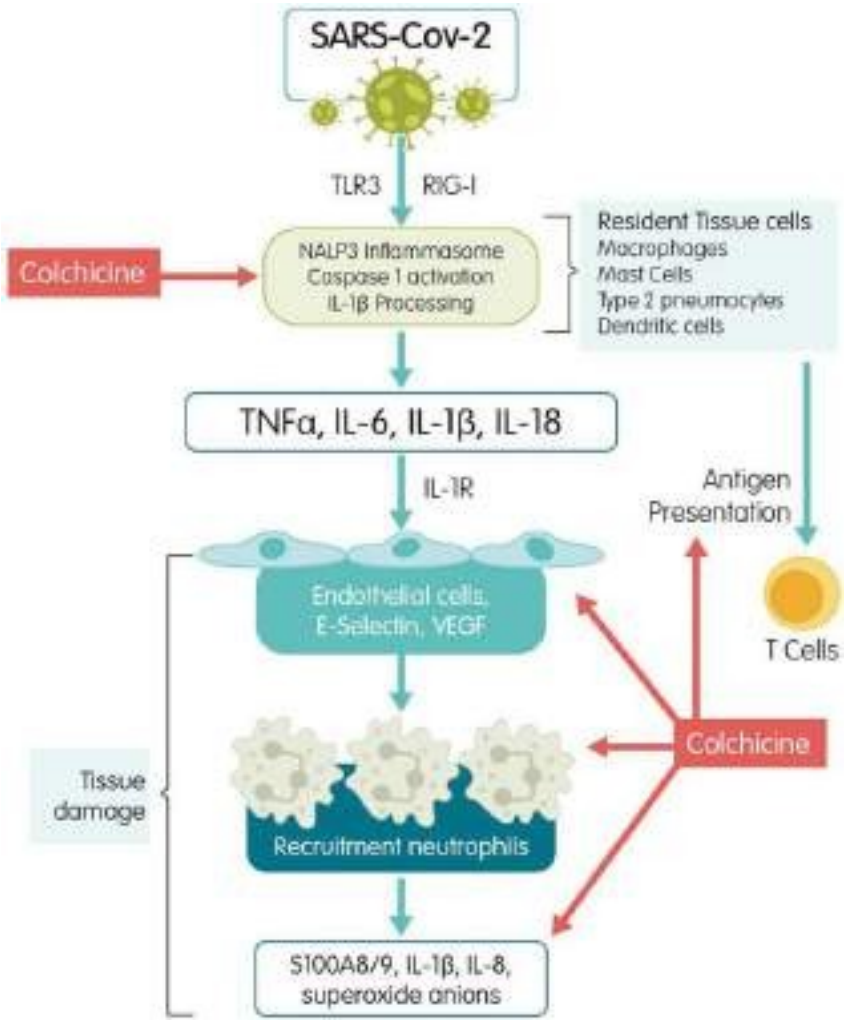
https://www.semanticscholar.org/paper/Colchicine-for-pericarditis.-Imazio/5e211189c353e3aa4b2699bbad7ed0ad44a277de.

Colchicine and COVID-19

The GRECCO-19 randomized open-label trial of 105 patients hospitalized with COVID-19 found that colchicine was associated with reduced D-dimer levels and improved clinical outcomes. [103]

Since the short-term safety profile is well known, this agent can be kept in mind along with corticosteroids in an attempt to reduce the effects of the cytokine storm.

A dosing schedule of **1.2 mg per os followed by 0.6 mg per os/2 times a day for 3 weeks** may be considered.



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https://www.eurekaselect.com/159288/article

CANNABINOIDS AS IMMUNOMODULATORS [104]

In a recent article,[105] it was reported that *Cannabis sativa* extracts high in cannabidiol (CBD) upregulate angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) enzyme, crucial receptors in oral, lung, and intestinal epithelia, important SARS-CoV2 invasion pathways.

Because high-cannabidiol products are able to reduce ACE2 and TMPRSS2 enzymes, the authors suggest their use as mouthwashes as a preventive strategy in COVID-19 infection to limit SARS-CoV2 infection in susceptible hosts.
Although this article advances the concept that cannabinoid-containing products can serve as a preventive treatment for topical use, there is evidence to support that the immunomodulatory activities of cannabidiol also play a role in later stages of disease and post-infectious sequelae.

Cannabidiol is a nonpsychotropic phytocannabinoid, and it is considered one of the most interesting molecules being studied in the field of pharmacology, as it exerts a wide range of therapeutic effects, and can act as an anticonvulsant, sedative, hypnotic, antipsychotic, antitumor, anti-inflammatory, neuroprotective, antioxidant, and immunomodulatory[106] .

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Hemp (Cannabis sativa L.) Protein Hydrolysates Promote Anti-Inflammatory Response in Primary Human Monocytes.

A formulation based on CBD and 30 terpenes (NT-BRL™) extracted from *Cannabis sativa was* tested in vitro to evaluate its inhibitory effects on the release of inflammatory cytokines and was found to exert a dose-dependent inhibition of LPS-induced cytokine secretion, twice as effective as dexamethasone, confirming the potent anti-inflammatory effect.
[107]

Such pleiotropic pharmacological activity has been tested in various pathological conditions, including respiratory diseases similar to COVID-19-induced respiratory distress.
Acute lung injury refers to a characteristic form of parenchymal lung disease, characterized by bilateral lung infiltrates, alveoli-capillary vasculitis with neutrophil infiltration and proinflammatory cytokine release, comparable to COVID-19.
By acting on adenosine A2A receptors, cannabidiol causes a marked improvement in reduced lung function as a consequence of a significant reduction in leukocyte migration into the lung, accompanied by a marked inhibition of both pro-inflammatory cytokines (TNF-α and IL-6) and chemokines (MCP-1/ MIP-2/CXCL2). [108]

Cannabidiol and other cannabinoids exert their activity through interaction with nuclear PPARs[109] . PPARs belong to the nuclear hormone receptor family and their activity is regulated by steroids and lipid metabolites. Three different PPAR isoforms (PPARα, PPARβ, also called δ and PPARγ) have been identified and shown to regulate the expression of genes related to lipid and glucose homeostasis and inflammatory responses.
Agonism on PPARγ in resident alveolar macrophages significantly limits lung inflammation and promotes host recovery following respiratory viral infection[110] . As has been shown during acute pneumonia, alveolar macrophages widely express PPARγ. Activation of PPARγ is also responsible for controlling excessive cytokine secretion resulting in amelioration of tissue damage.

It is therefore likely that in addition to directly causing an improvement in lung dynamics, cannabidiol may significantly counteract the initiation of the cytokine storm by resident macrophages.

Biomolecules. 2020;10(5):803. Published 2020 May 22. doi:10.3390/biom10050803
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6777801/

Recent reports show that a subgroup of COVID-19 survivors may develop post-infectious sequelae with persistently impaired lung function and pulmonary fibrosis[111] .
PPARγ receptors represent a potential therapeutic target in fibrotic lung disease, given their ability to regulate fibroblast/myofibroblast activation and collagen secretion in mouse models[112] .
In particular, cannabidiol has been shown to reduce lung inflammation and fibrosis in animal models of asthma[113] .

It is therefore conceivable that cannabidiol, being a PPARγ receptor agonist, could potentially limit the onset of the Late-onset pulmonary fibrosis in patients cured by COVID-19.

Although cannabidiol appears to be a relatively safe molecule for humans, as demonstrated in several studies conducted[114] or ongoing mainly for the treatment of neurological disorders, there are currently no data on the efficacy and relative toxicity of cannabidiol in COVID-19.
According to a precautionary principle, one possible strategy would be to test the therapeutic potential of cannabidiol in COVID-19 patients (aged 18 years or older) at an early stage of the disease to stop the cytokine storm and the development of respiratory distress, or alternatively, to evaluate its efficacy in cured COVID-19 patients to prevent pulmonary fibrosis.

The effects of cannabidiol in vivo depend largely on its dose and the bioavailability of its receptor targets under various pathological conditions.
Different plasma concentrations of cannabidiol may be required to activate the distinct pathways responsible for its multifaceted activity.

Indeed, subtherapeutic dosing (0.3 mg/kg/day) has been suggested to explain the lack of efficacy of cannabidiol in Crohn's disease . In humans, cannabidiol has been tested over a wide dose range, ranging from <1 to 50 mg/kg/day depending on the trials and the disease condition explored, with both in vitro and in vivo studies suggesting immunosuppressive action at higher concentrations or doses.
In both human immunodeficiency virus (HIV) and post-Ebola syndrome, cannabidiol has been proposed as a therapeutic agent to control immune activation at doses of 10-20 mg/kg/day and 1.7-10 mg/kg/day (100 mg/day with titration up to 600 mg/day), respectively[115] .

It is suggested that cannabidiol be administered orally, starting with **100 mg per day** titrating up to **300 mg per day** (2.5 mg/kg/day), as this dosage produced no relevant adverse effects even after prolonged dosing (up to 18 weeks) in human clinical trials.

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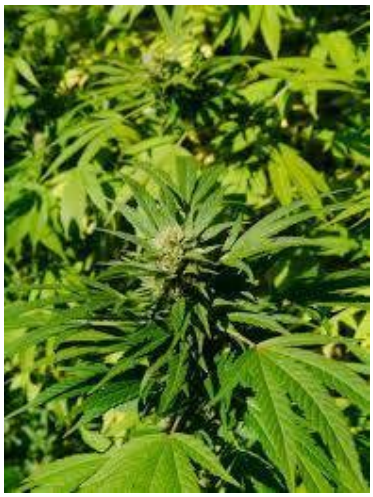
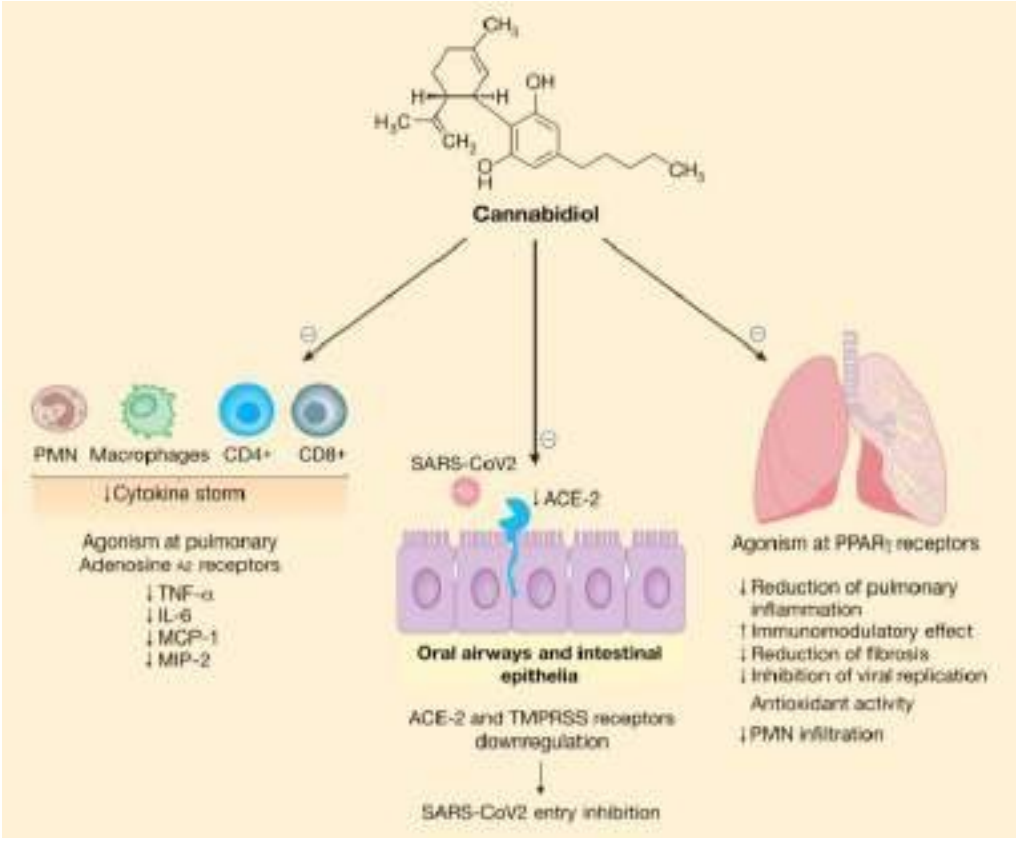
However, it is worth mentioning that most published clinical studies lack data on the actual plasma concentrations achieved by orally administered cannabidiol in vivo. This also has implications for its safety profile, because cannabidiol acts as an in vitro inhibitor of several CYP450 isoforms[116] .

As pointed out earlier, there is a lack of drug-drug interaction studies between cannabidiol and anti-COVID-19 treatments, so patients would need to be monitored for potential drug interactions.
Likewise, CYP inhibitors are expected to increase plasma concentrations of cannabidiol, so patients should be closely monitored for adverse effects.
However, no serious adverse side effects are expected, as the proposed dose of cannabidiol is generally well tolerated in humans and the concentration (IC50) required to inhibit CYP450 is significantly higher than the plasma concentration of cannabidiol obtained after oral administration.

Finally, regarding possible concerns about immunosuppression during acute infections, we think it is important to highlight the observation that cannabidiol did not cause increased mortality in acutely infected animals, rather in pneumococcal meningitis animal survival increased and TNF-α concentrations decreased with the doses of 2.5, 5, and 10 mg/kg[117] .





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THERAPY WITH HIGH DOSE ANTIOXIDANTS (VITAMIN C + N-ACETYLCISTEIN) please refer to chapter

UNCTIONAL MEDICINE'S APPROACH TO COVID-19

QUERCITIN.

Quercetin is a flavonoid found in fruits and vegetables with unique biological properties that can improve mental/physical performance and reduce the risk of infection.

These properties form the basis for potential benefits for overall health and disease resistance, including anti-carcinogenic, anti-inflammatory, antiviral, antioxidant and psychostimulant activities, as well as the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability and to stimulate mitochondrial biogenesis.[118] Quercetin derivatives (mainly as quercetin glycosides) are the most abundant flavonoid molecules found in plants. They are found in a wide variety of fruits and vegetables and in medicinal botanicals, including *Ginkgo biloba*, *Hypericum perforatum*, and *Sambucus canadensis*. [119]

Bhutto et al.[120] identified molecular and functional similarities between dexamethasone and quercetin, and in particular that they share a role in modulating permeability glycoprotein (P-gp)* and its functional activity.

> * P-gp, is the main member of the ABC membrane transporters responsible for the efflux of many xenobiotics and plays a central role in the absorption, distribution, and excretion of therapeutic agents.

It has been observed that pro-inflammatory cytokines released during infections inhibit P-gp expression and activity[121] , and that both dexamethasone and quercetin, by inducing P-gp expression, can inhibit the consequences due to cytokine storm in COVID-19 patients.

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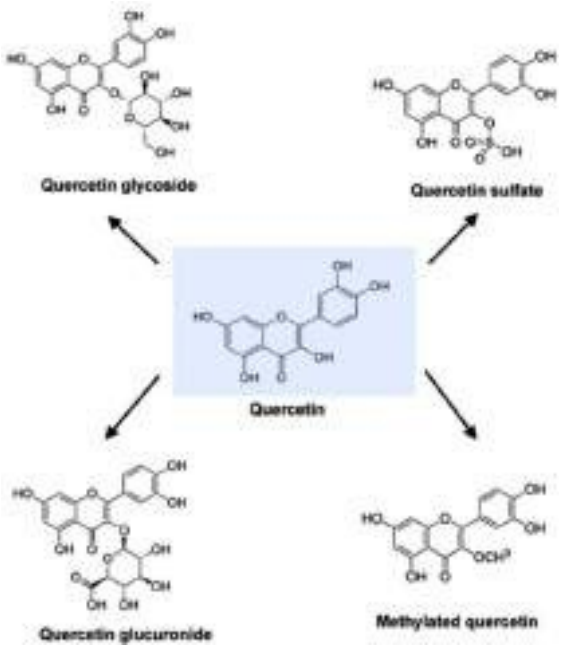

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4808895/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5849680/

The molecular similarity of quercetin (chemical structure on the left and thin green lines on the right) to P-gp inducers (thick gray lines) (A) myricetin, (B) rhodamine 123 and (C) dexamethasone. All molecules are shown in the stick representation colored according to the CPK scheme except for the carbon atoms of quercetin colored in green. Hydrogen atoms are not shown for clarity.

Flavonoids can inhibit both transmembrane peptidase serine 2 (TMPRSS2) and furin, enzymes that cleave the Spike protein of SARS - CoV - 2 allowing infection into cells[122] . Quercetin in particular also appears to prevent virus entry by blocking the expression of angiotensin-converting enzyme 2 (ACE2)[123] and altering the folding of viral proteins[124] .
The in vitro study by Abian et al.[125] confirmed the efficacy of quercetin as a 3CL protease inhibitor[pro] of SARS-Cov-2. Other in vitro studies revealed its immunomodulatory effects through inhibition of tumor necrosis factor-α production in macrophages[126] and interleukin-8 production in lung cells[127] .

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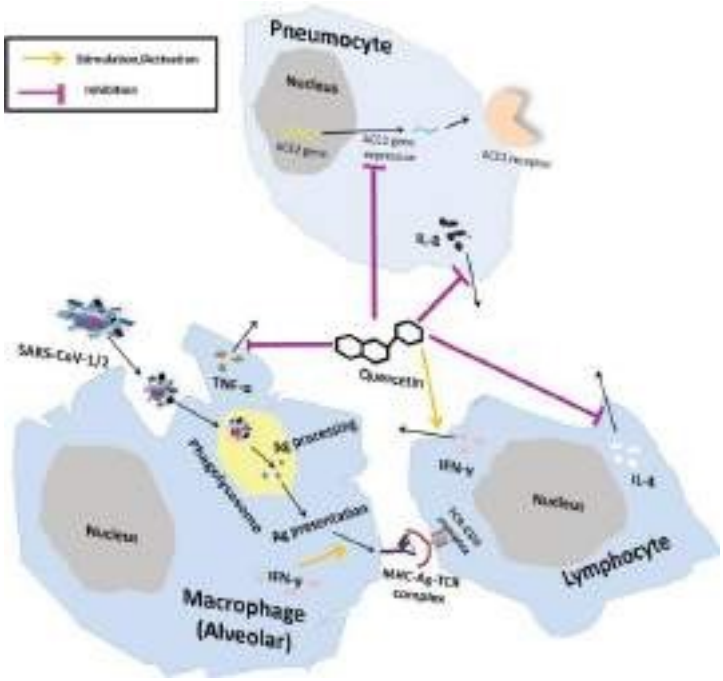
It is important to point out that production of interferon-γ (Th-1-derived cytokine) and inhibition of IL-4 (Th-2-derived cytokine) may be responsible for the beneficial immunostimulatory effects of quercetin.

In addition, the researchers argue that due to its properties as a zinc ionophore, the quercetin/zinc combination may be a potential therapeutic/prophylactic option for Covid-19 subjects. Specifically, zinc has been shown to inhibit SARS virus-dependent RNA polymerase RNA polymerase activity in vitro in a dose-dependent manner [128]



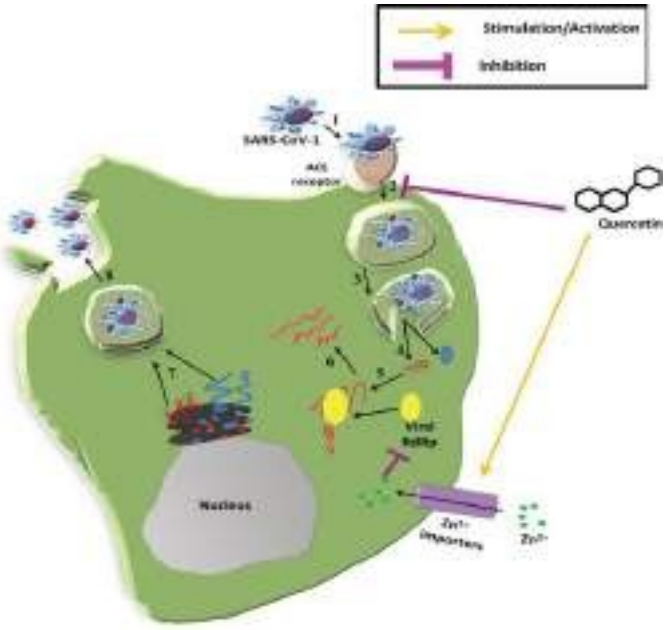


Fig.1Fig . 2

Fig.1 Schematic representation of the possible anti-inflammatory effects of quercetin against SARS-CoV-1/2. Quercetin has been shown to inhibit the entry of SARS-CoV-1/2 inside cells by interfering with the expression of ACE2. In addition, quercetin initiates IFN-γ production by T lymphocytes, which enhances antiviral activity and antigen presentation through phagocytocytosis and expression of MHC-I and MHC-II molecules. Reduction of inflammation and blood clotting in the lungs is achieved by inhibiting IL-8 production by quercetin. Importantly, quercetin also inhibits the production of IL-4 and TNF-α, which helps improve inflammation. ACE2, angiotensin-converting enzyme 2; IL, interleukin; IFN-γ, Interferon-gamma; TNF-α, tumor necrosis factor-alpha; SARS-CoV, severe acute respiratory syndrome coronavirus

Fig. 2 Schematic representation of the different steps of the SARS-CoV-1 replication cycle inhibited by quercetin and zinc. Viral attachment (1), viral entry (2), preparation for viral envelope removal (3), viral envelope removal, release of viral genome and viral protein (4), viral transcription (5), viral protein translation (6), viral replication (5), virus assembly and maturation (7), and finally release of mature viral particles (8). Quercetin has been shown to inhibit the entry phase (2) of SARS-CoV-1, while zinc inhibits SARS-CoV-1 genome transcription by inhibiting viral RdRp in a dose-dependent manner (passage 5). ACE2, angiotensin-converting enzyme 2; RdRp, RNA-dependent RNA polymerase; SARS - CoV, severe acute respiratory syndrome coronavirus; Zn, zinc

The bioavailability of oral quercetin is extremely variable, reaching values from 0 to 50 percent. The distribution in rat tissue of long-term (12 weeks) orally administered quercetin shows the highest concentration in the lungs, while pigs show the highest concentrations in the liver and kidneys.
In contrast, short-term administration does not show marked distribution, implying that the beneficial effects of quercetin in preventing respiratory pulmonary viral infection could be maximized by long-term administration.

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Oral quercetin supplementation up to 1 g/day for 3 months produced no significant adverse effects. In a randomized placebo-controlled trial, 30 patients with chronic prostatitis were supplemented with oral quercetin (1 g/day) and reported only two mild adverse reactions (headache and temporary peripheral paresthesia).
Intravenous administration of quercetin in a phase I clinical trial of cancer patients resulted in nausea, vomiting, sweating, flushing, and dyspnea at doses> 10.5 mg/kg (756 mg per 70-kg individual). Only higher doses administered intravenously up to 51.3 mg/kg (about 3,591 mg per individual) have been associated with renal toxicity. The safety of oral quercetin supplementation during pregnancy and lactation has not been established. [129]

Since quercitin synergizes with vitamin C[130] the proposal for a multi-drug approach for both prophylaxis for the high-risk population and treatment of mild and severe cases is as follows

	Quercetin	Vitamin C
Prophylaxis	250–500 mg BID	500 mg BID
Mild cases	250–500 mg BID	500 mg BID
Severe Cases*	500 mg BID	3 gr q6 for 7 days

*ARDS-like presentation, require assisted ventilation/intubation, ICU hospitalization.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7318306/

Quercetin spontaneously oxidizes to form O-semiquinone and O-quinone/quinone methide (QQ), which can bind protein thiols to form toxic compounds.

This process with both antioxidant and pro-oxidant effects has been called the "quercetin paradox."
However, QQ can be recycled into quercetin by electron donors such as NADH or ascorbate, or form together with glutathione the 6-glutathionyl-quercetin or 8-glutathionyl-quercetin (GSQ).

Importantly, if ascorbate or glutathione levels are insufficient, quercetin can be diverted to QQ and exert prooxidant effects. Therefore, the importance of its co-administration with vitamin C should be emphasized. [131]

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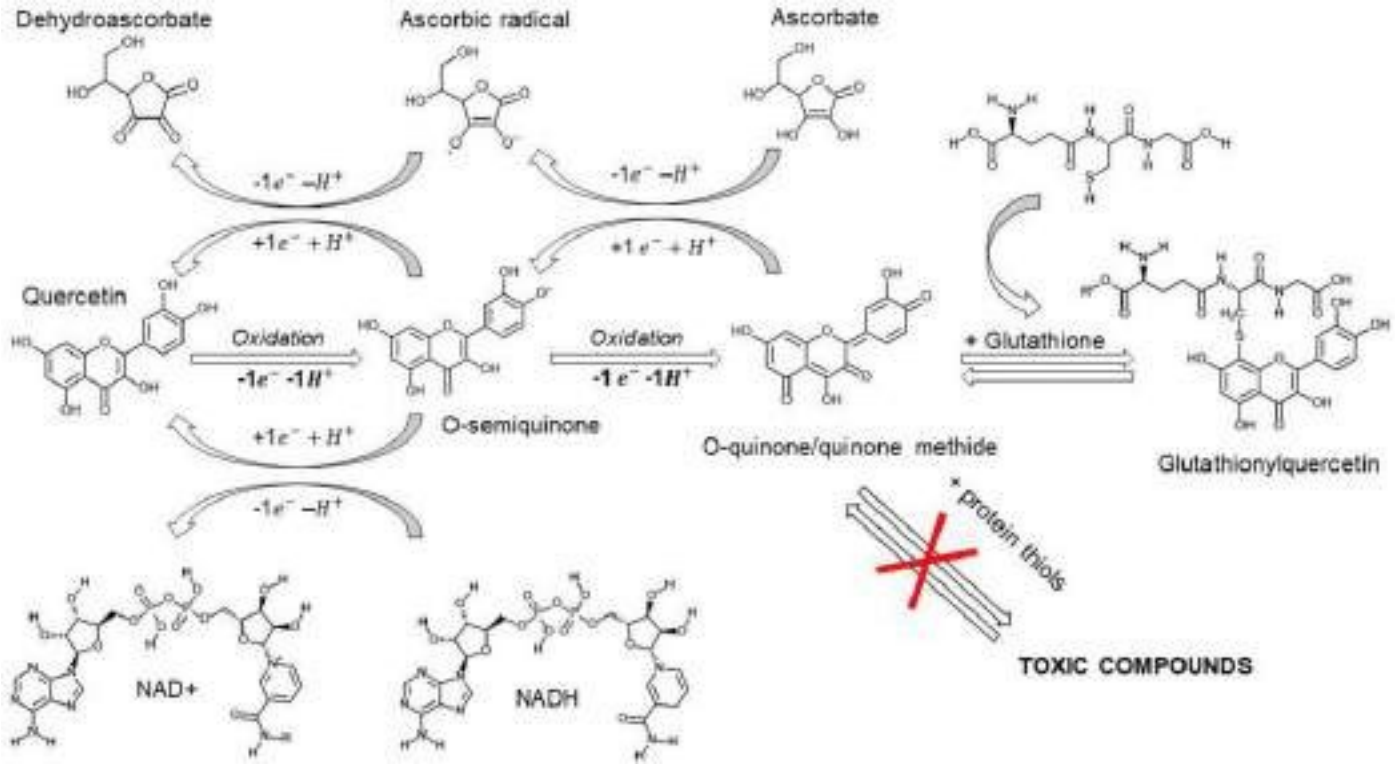

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7318306/

Supraphysiological concentrations of ascorbate obtained by intravenous administration (i.v.3 gr/every 6 hours) are able to scavenge free radicals and donate electrons, preventing oxidation of quercetin or glutathione.

In this circumstance, ascorbate can exert antioxidant and immunoprotective effects; quercetin and its metabolites exert a simultaneous antiviral response; and, if oxidized compounds are formed from quercetin, they can be partially recycled by ascorbate and transported by glutathione, thus preventing possible toxicity. [132]

4) ANTIPLATELET / ANTITHROMBOTIC THERAPY [133]

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Several studies have described increased rates of pathologic macro- and micro-thrombosis during complication of infection,[134] and observed that some patients with COVID-19 reported chest heaviness associated with desaturation suggesting the possibility of pulmonary thrombosis. [135]

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Multiple reports have described elevated levels of D-dimer in acute conditions in COVID-19 patients associated with an increased risk of deep vein thrombosis and pulmonary embolism, and[136] in autopsy studies of deceased COVID-19 patients, pulmonary microthrombosis has been confirmed.[137]

These observations support the idea that endothelial damage and thrombosis play a role in oxygen desaturation, a pivotal symptom of hospitalization and supportive care.

Based on this pathophysiologic rationale, **aspirin** can be administered as an initial antiplatelet and anti-inflammatory agent in amounts of **81 mg per day**. [138]

Outpatients can be treated with the addition of **low-molecular-weight heparin** or new short-acting anticoagulant drugs with the dosing schedule similar to that used in home thromboprophylaxis.

In a retrospective study of 2773 patients admitted with COVID-19, 28% had received anticoagulant therapy within 2 days of admission, which was associated with reduced mortality (HR = 0.86 per day of therapy, 95% CI: 0.82-0.89; P < 0.001).
Additional supportive data have been reported on the use of anticoagulants associated with reduced mortality in hospitalized patients with elevated D-dimer levels and higher comorbidity scores. [139] Many outpatients with acute disease also have general indications for venous thromboembolism prophylaxis applicable to COVID-19. [140]

Giuseppe Patty et al in the recent article "*Questions and Answers on Practical Thrombotic Issues in SARS-CoV-2 Infection: A Guidance Document from the Italian Working Group on Atherosclerosis, Thrombosis and Vascular* Biology" [141] pose ten

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Questions and answers on risk stratification and antiplatelet/anticoagulant treatments in patients at risk of/with severe acute respiratory syndrome SARS-CoV-2 infection based on scientific evidence gathered during the pandemic.
The following are the main proposed directions, referring further discussion to the text of the article:

1) Patients on chronic anticoagulation therapy at risk of SARS-CoV-2 infection or with mild COVID-19 maintained at home:

Patients on VKAs at risk of SARS-CoV-2 infection
If INR values are stable (i.e., time in therapeutic range > 60%), a prolongation of the INR control intervals may be considered (every 4–8 weeks)
The use of portable coagulometer devices with self-measurement of INR is encouraged
Switching from VKAs to DOACs must be considered
In the case of unstable INR values, switching from VKAs to DOACs is recommended
Patients on VKAs with mild COVID-19 maintained at home
The use of portable coagulometer devices with self-measurement of INR is encouraged
Switching from VKAs to DOACs must be considered, taking into account possible drug interactions
In the case of unstable INR values, switching from VKAs to DOACs is recommended
Patients not on oral anticoagulant therapy with asymptomatic SARS-CoV-2 infection
No thromboprophylaxis is indicated
Patients not on oral anticoagulant therapy with mild COVID-19
Thromboprophylaxis with LMWH is indicated if multiple risk factors for VTE are present and bleeding risk is low

COVID-19 coronavirus disease 2019, *DOAC* direct oral anticoagulant, *INR* international normalized ratio, *LMWH* low-molecular weight heparin, *SARS-CoV-2* severe acute respiratory syndrome coronavirus 2, *VKA* vitamin K antagonist anticoagulant, *VTE* venous thromboembolism

2) Patients receiving chronic antithrombotic treatment hospitalized for COVID-19

Patients on chronic antiplatelet therapy
Aspirin therapy for primary cardiovascular prevention should be continued, unless contraindications have arisen or there is need for venous thromboprophylaxis
Antiplatelet therapy for secondary cardiovascular prevention must be continued, considering possible drug interactions
Dual antiplatelet therapy in patients who have undergone PCI within ≤ 3 months must be continued unless hemorrhagic events are reported
In patients on aspirin plus clopidogrel/ticagrelor who have undergone a recent PCI (≤ 3 months) for ACS requiring treatment with lopinavir/ritonavir or atazanavir, switching from clopidogrel/ticagrelor to prasugrel is indicated. If prasugrel is contraindicated, therapy with clopidogrel/ticagrelor is continued, monitoring blood cell count and ischemic/bleeding events
In patients on aspirin plus clopidogrel who have undergone a recent PCI (≤ 3 months) for stable coronary syndrome requiring treatment with lopinavir/ritonavir or atazanavir, clopidogrel is continued, monitoring blood cell count and ischemic events
No significant interaction between clopidogrel/prasugrel/ticagrelor and the other agents used for COVID-19 are present
Patients on chronic OAC
If indication for OAC is adequate and no contraindication exists, short-term switching from OAC to LMWH is reasonable

ACS acute coronary syndrome, *COVID-19* coronavirus disease 2019, *LMWH* low-molecular weight heparin, *OAC* oral anticoagulant therapy, *PCI* percutaneous coronary intervention

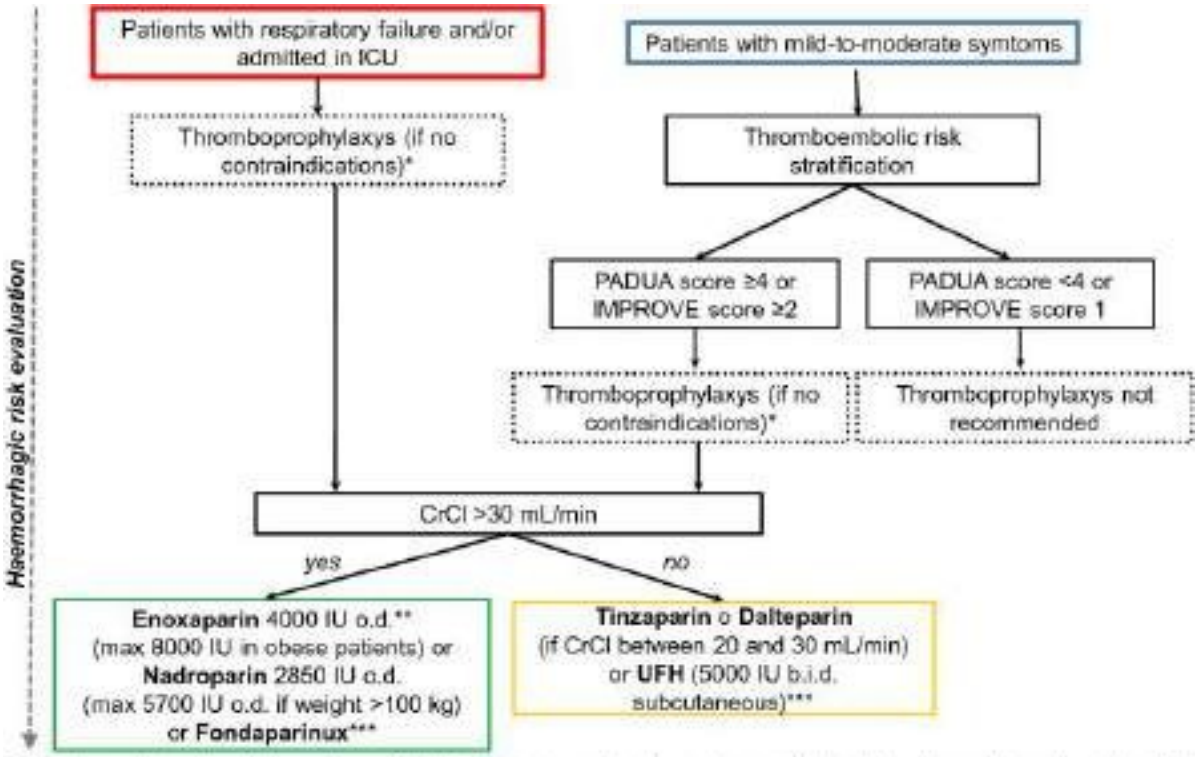
3) Relevant interactions between anticoagulant drugs and anti-SARS-CoV-2 agents

Interactions with drugs used to treat COVID-19 should be considered when choosing anticoagulant therapy:

Interactions between antithrombotic drugs and agents used for COVID-19. White: no data; green: no interaction; yellow: minor interaction, which may require dose reduction of DOACs, additional INR checks (if VKA therapy) or functional monitoring of antiplatelet activity (in the case of P_{2Y12} treatment); red: coadministration is contraindicated due to significant interactions. Upward arrows: increase in antithrombotic drug activity, proportional to the number of arrows; downward arrows: decrease in antithrombotic drug activity, proportional to the number of arrows. Adapted from ESC Guidance for the Diagnosis and Management of CV Disease during the COVID-19 Pandemic (https://www.escardio.org/Education/COVID-19-and-Cardiology/ESC- COVID-19-Guidance). COVID-19 coronavirus disease 2019, cardiovascular CV, direct oral anticoagulant DOAC, ESC European Society of Cardiology, international normalized INR ratio, unfractionated heparin UFH, vitamin K antagonist anticoagulant VKA

		DOACs				VKAs		Heparins				ANTIPLATELET				
		Dabigatran	Apixaban	Edoxaban	Rivaroxaban	Warfarin	Acenocoumarol	Enoxaparin	Fondaparinux	Dalteparin	UFH	Aspirin	Clopidogrel	Prasugrel	Ticagrelor	Cangrelor
Anti-COVID-19 agents	(Hydroxy)chloroquine	↑		↑												
	Azithromycin	↑	↑	↑↑	↑	↑↑↑					↑↑↑					
	Lopinavir/ritonavir	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↓↓	↓↓						↓↓	↓	↑↑	
	Atazanavir	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑							↓↓	↓	↑↑	
	Ribavirin					↓↓										
	Remdesivir															
	Tocilizumab		↓		↓	↓	↓						↓	↓	↓	
	Interferon-β															
	Methylprednisolone					↓↓					↓					
	Paracetamol					↑↑	↑↑									

4) Thromboprophylaxis in patients with COVID-19



* If pharmacological prophylaxys is contraindicated, it is possible to perform mechanical thromboprophylaxys by intermittent mechanical elasto-compression

** Due to recent reports showing in COVID-19 a pro-thrombotic milieu and high rates of venous thromboembolism, the use of higher-than-prophylactic doses of enoxaparin (e.g. 4000 IU BID) has been recently encouraged

*** In patients at high thrombotic risk (PADUA score ≥4 or IMPROVE score ≥4) and low bleeding risk, consider thromboprophylaxys up to 45 days after discharge

CrCl creatinine clearance, BID/b.i.d. twice daily; ICU; international IU, o.d. daily; unfractionated heparin UFH

5) Approach for the diagnosis of venous thromboembolism in patients with COVID-19

In patients with a worsening clinical status, especially in those without anticoagulant treatment, a diagnosis of VTE must always be suspected
In patients with suspected VTE, the diagnostic and therapeutic workup must integrate clinical data, laboratory findings, and imaging test results
Measurement of D-dimer for diagnosing VTE must be performed only if a clinical suspect exists
Vascular/cardiac ultrasound imaging for diagnosing VTE should precede radiological imaging
Patients undergoing a *CT scan* for worsening respiratory status should receive angio-CT sequences to exclude PE
The use of LMWH for treating a VTE episode is preferred. UFH should be limited to patients with CrCl < 30 mL/min
An invasive "catheter"-based therapy for PE is indicated in selected cases with contraindication to anticoagulant drugs, recurrent events despite adequate anticoagulation, or when systemic fibrinolysis cannot be performed
For the risk stratification of patients with VTE, monitoring of the following parameters is useful: troponin, BNP, D-dimer, blood cell count, fibrinogen, prothrombin time, activated partial thromboplastin time, and degradation products of fibrin
After the initial approach, DOACs may represent an option for in-hospital treatment of a VTE episode in patients with clinical stability and decreasing inflammation
After a VTE episode, DOACs should represent the therapy of choice at discharge

BNP brain natriuretic peptide, *COVID-19* coronavirus disease 2019, *CrCl* creatinine clearance, *CT* computed tomography, *DOAC* direct oral anticoagulant, *LMWH* low-molecular weight heparin, *PE* pulmonary embolism, *UFH* unfractionated heparin, *VTE* venous thromboembolism

The use of imaging techniques in the diagnosis of a VTE episode is complex because of the risk of viral transmission to other patients and health care providers, and must be governed by specific hospital protocols designed to limit that risk.
Each imaging test must follow an integrated assessment of clinical and laboratory data. In particular, vascular or cardiac ultrasound examination usually precedes radiologic imaging and requires appropriate personal protective equipment and specific methods for sanitizing instruments.
In view of the high rates of DVT in ICU patients, extensive use of vascular ultrasound to evaluate the diagnosis of DVT is recommended.
Because of the higher risk of EP, patients with COVID-19 undergoing pulmonary computed tomography (CT) for worsening respiratory function should receive angio-CT sequences to optimize the diagnostic process.
In patients with acute respiratory distress syndrome (ARDS) and suspected EP, performing radiological tests is difficult because of the patient's prone position and unstable clinical condition.
In this case, echocardiographic evidence of deteriorating right ventricular function or (more rarely) thrombus transit is a relevant finding that warrants further diagnostic steps and on this basis the initiation of specific treatments.

6) Stratification of the prognosis of patients with COVID-19

A higher inflammatory state (identified by increased C-reactive protein levels or neutrophil/lymphocyte ratio) was correlated with a worse outcome, including lower survival.
Measurement of troponin and brain natriuretic peptide (BNP) is also useful, as an increase in these parameters indicates acute myocardial damage and hemodynamic overload.
Various abnormalities in hemostatic parameters were associated with a higher risk of cardiovascular complications, mechanical ventilation and mortality, especially, thrombocytopenia (especially <50,000 × 109/L) and increased D-dimer (> 3 µg/mL), fibrinogen (> 1 g/L) or fibrin degradation products.
Recent studies have suggested a relationship between severity in the clinical course of COVID-19 and spontaneous prolongation of prothrombin time (> 3 s) or prolongation of activated partial thromboplastin time (> 5 s). Accordingly, all of the above hemostatic markers should be routinely monitored in these patients, with the aim of achieving more accurate prognostic stratification.

7) Management of antithrombotic treatment in patients with COVID-19 complicated by venous thromboembolism

In COVID-19 patients with VTE, indications for treatment are based on preexisting guidelines and should be supplemented with specific assessments related to SARS-CoV-2 infection[142] .

[142] Zhou F, Yu T, Du R, et al.
Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study

As described above, the choice of treatment regimen must also take into account considerations regarding the severity of clinical presentation, coexistence of renal disease, liver dysfunction and/or thrombocytopenia, and possible drug interactions with antiviral drugs.
The use of parenteral anticoagulants is the best initial option because of its greater manageability and lower risk of drug interference.
EBPM at anticoagulant dosages (i.e., subcutaneous enoxaparin 1 mg/kg/ 2 times a day or nadroparin 86 IU/kg/ 2 times a day) should be preferred to UFH, which exposes health care workers to an increased risk of infection because of frequent blood draws for dose adjustment.
UFH should be used only in patients with creatinine clearance <30 ml/min. Invasive catheter therapy for PE (locoregional thrombolytic therapy or embolectomy) is indicated in selected cases with contraindication to anticoagulant drugs, in those who experience recurrent events despite adequate anticoagulation, or when systemic fibrinolysis cannot be performed.
After the initial approach with EBPM, DOACs may be an option for in-hospital treatment of a VTE episode only in patients with clinical stability and decreasing inflammation.
Unless contraindicated, DOACs should be preferred over AVKs, as they are easier to manage during hospital stay and transition to the subsequent home regimen. However, as mentioned earlier, the choice of OAC drug should consider possible interactions with anti-SARS-CoV-2 drugs.[143]

8) Management of anticoagulation therapy in patients hospitalized for COVID-19 who develop atrial fibrillation (AF)

In patients with COVID-19, the onset or recurrence of AF is promoted by fever, hypoxia, and adrenergic activation due to respiratory failure. In this case, anticoagulant treatment for the prevention of thromboembolic events should be guided by the CHA2DS2-VASc score rather than the characteristics of arrhythmic episodes (i.e., duration of episodes, number of recurrences).
It is reasonable to start anticoagulation with EBPM and then switch to OAC during the course of hospitalization, preferably with a DOAC, taking into account possible drug interference.

9) Management of patients with COVID-19 and arterial thrombosis

Hospitalizations for SCA (acute coronary syndrome) apparently decreased during the pandemic.
However, it has been observed that 20-30% of patients hospitalized for COVID-19 have a history of cardiovascular disease.
As demonstrated in other viral inflammatory syndromes, an SCA due to coronary thrombosis may result from the destabilization of preexisting lesions as a result of the cytokine storm.
Given the paucity of specific data, pharmacological and interventional management of patients with COVID-19 and ACS should follow pre-existing guidelines[144] , using specific hospital protocols and appropriate measures to prevent infection of healthcare workers[145] .
As indicated earlier, antiplatelet strategies play a crucial role in this context.

[published correction appears in Lancet. 2020 Mar 28;395(10229):1038] [published correction appears in Lancet. 2020 Mar 28;395(10229):1038]. Lancet. 2020;395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270627/

[143] Bikdeli B, Madhavan MV, Jimenez D, et al.
COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review.
J Am Coll Cardiol. 2020;75(23):2950-2973. doi:10.1016/j.jacc.2020.04.031
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164881/

Paranjpe I, Fuster V, Lala A, et al.
Association of Treatment Dose Anticoagulation With In-Hospital Survival Among Hospitalized Patients With COVID-19.
J Am Coll Cardiol. 2020;76(1):122-124. doi:10.1016/j.jacc.2020.05.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7202841/

[144] Neumann FJ, et al; ESC Scientific Document Group.
2018 ESC/EACTS Guidelines on myocardial revascularization.
Eur Heart J. 2019 Jan 7;40(2):87-165. doi: 10.1093/eurheartj/ehy394. Erratum in: Eur Heart J. 2019 Oct 1;40(37):3096.
https://academic.oup.com/eurheartj/article/40/2/87/5079120

[145] Scotto Di Uccio F, et al
ANMCO Position paper: Network organization for management of patients with acute coronary syndrome during COVID-19 pandemic emergency
[ANMCO Position paper: The network organization for the management of patients with acute coronary syndrome during the COVID-19 pandemic].
G Ital Cardiol (Rome). 2020 May;21(5):332-335. Italian. doi: 10.1714/3343.33129.
https://www.giornaledicardiologia.it/archivio/3343/articoli/33129/.

Aspirin can be used without specific additional concerns. In patients with COVID-19 and ACS being treated with Lopinavir/Ritonavir or Atazanavir, coadministration of aspirin and Prasugrel after PCI should be preferred.
If prasugrel is contraindicated, the use of clopidogrel or ticagrelor may be considered, possibly with testing of their antiplatelet efficacy. Cangrelor metabolism is independent of liver function. No drug interactions are expected between this agent and all drugs used in COVID-19.
It should be noted that in patients with COVID-19, a high incidence of SCA has been reported, with a presentation similar to a "ST-segment elevation myocardial infarction (STEMI)" and without coronary lesions.
Notably, recent data showed that about 40% of patients with COVID-19 and STEMI had no *culprit* lesions on coronary angiography. Possible pathogenetic mechanisms for this finding are acute myocarditis, myocardial infarction type 2 (due to mismatch between oxygen supply and demand) or "Takotsubo-like" myocardiopathy. Therefore, in patients with confirmed (or suspected) SARS-CoV-2 infection and "STEMI-like" presentation, fibrinolytic therapy should be considered only in selected cases when coronary angiography and possible percutaneous revascularization cannot be performed promptly and safely.
Regarding arterial thrombosis in non-coronary vessels, a recent observational survey reported a 1.6% incidence of ischemic stroke among consecutive patients hospitalized for COVID-19.
In addition, there is evidence that patients with SARS-CoV-2 infection may have a more severe stroke presentation than uninfected patients. Finally, cases of ischemic stroke associated with acute bilateral lower extremity ischemia have been described in the context of antiphospholipid syndrome.

10) Management of patients hospitalized for COVID-19 developing disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) is a possible complication in all patients admitted to the ICU with critical infectious diseases. In patients with COVID-19, a severe clinical course was associated with DIC in> 50% of cases.

Evaluation of the risk of DIC	Does the patient present a clinical condition consistent with DIC? No: do not proceed with the score Yes: proceed with the score
ISTH score	
Coagulation parameters	Prothrombin time, platelet count, D-dimer, degradation products of fibrin, fibrinogen
Diagnostic score	*Prolongation of prothrombin time:* ≤ 3 s = 0 points; > 3–≤ 6 s = 1 point; > 6 s = 2 points *Platelets:* ≥ 100×10^9/L = 0 points; < 100×10^9/L = 1 point; < 50×10^9/L = 2 points *D-DIMER, DEGRADATION PRODUCTS OF FIBRIN:* normal = 0 points; moderately increased = 2 points; markedly increased = 3 points *Fibrinogen:* > 1 g/L = 0 points; ≤ 1 g/L = 1 point
ISTH score calculation	≥ 5 points: compatible with DIC < 5 points: non-suggestive for DIC

DIC disseminated intravascular coagulation, *ISTH* International Society on Thrombosis and Haemostasis

Mechanism of action of anticoagulants in COVID-19 Heparins

146

As discussed above, hospitalized COVID-19 patients often suffer from significant infection-related coagulopathy and high risks of microvascular thrombosis, [1,14,17,20], and anticoagulants may have positive effects, reducing the severity of thrombotic disease and hyperactivity of coagulation, and may also have beneficial direct anti-inflammatory effects against sepsis and the development of ARDS.

Heparins, including unfractionated heparin (UFH) and low-molecular-weight heparin (EBPM), have several nonanticoagulant properties and may exert anti-inflammatory effects.

[146] Reference for bibliography in superscript in red brackets
Godino C, Scotti A, Maugeri N, et al.
Antithrombotic therapy in patients with COVID-19? -Rationale and Evidence
[published online ahead of print, 2020 Sep 28]. Int J Cardiol. 2020;S0167-5273(20)33894-8. doi:10.1016/j.ijcard.2020.09.064
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7521414/

In fact, heparins block P-selectin, the protein responsible for the interaction between platelets and neutrophils [21], inhibit the neutrophil response and NETosis [22] and reduce the release of IL-1β, IL-6, E-selectin and ICAM-1 [23,24].

Cytokines and in particular the interleukin (IL) family are known to play an important role in inflammation and have a direct effect on plasma molecules, erythrocytes, and platelets.
Hypercoagulability and impaired fibrinolysis are usually the markers of various inflammatory conditions. It was found that IL-1β, IL-6 and IL-8 could cause hypercoagulation, leading to scattered fibrin clots [25].
Patients with severe COVID-19 had higher levels of IL-6, thus suggesting that the hypercoagulable state might be related to elevated cytokine levels [[26], [27], [28]].

In addition to anticoagulant and anti-inflammatory properties, other mechanisms may explain the favorable effect of heparins on COVID-19 patients. In fact, they are being investigated for potential use as direct antiviral agents for their inhibitory effects on pathogen adhesion to cell surfaces.
The direct antiviral effect of heparins involves heparan sulfate, a family of polysaccharides, ubiquitous components found on the cell surface and in the extracellular matrix of all animals [29].
Heparan sulfate is known as an initial contact point between target cells and several human viruses (i.e. herpesvirus, influenza A virus, hepatitis C virus, human immunodeficiency virus, dengue virus) [[30], [31], [32], [33], [34]], including SARS-CoV-2 [35].

Heparins have been shown to effectively compete with heparan sulfate and thus attenuate viral attack and cellular infection.
In addition, it is known that SARS-CoV2 is characterized by the presence of several Spike (S) proteins that project from the virion surface. Each S protein consists of two subunits (S1 and S2), the S1 subunit has the binding domain that interacts with the host cell's main receptor, ACE2.

Recently, it has been reported that the SARS-CoV-2 S1 receptor binding domain binds to heparin and a significant structural change is induced upon binding, providing clear evidence for a direct antiviral effect of EBPM in patients with COVID-19. [35]
Entry of SARS-CoV-2 into the human cell requires cleavage of S1-S2 subunits to expose S2 for adhesion to the cell membrane [36,37]. TMPRSS cellular proteases including cathepsins, factor Xa, furin, and trypsin have been shown to proteolytically process the spike protein and that factor Xa facilitates SARS-CoV entry into cells [39]. Therefore, combining this knowledge with the mechanisms of action of UFH and LMWH, which are all inhibitors of different proteases such as factor Xa, thrombin, furin, and cathepsin-L, [38][40] it was hypothesized that this may be another direct mechanism of heparins to prevent virus entry.

Antiplatelet therapy (or antiplatelets) [147]

Antiplatelet agents are antithrombotic drugs that act by interfering with specific steps in the process of platelet activation.
Based on their mechanism of action, they can be classified into drugs that:

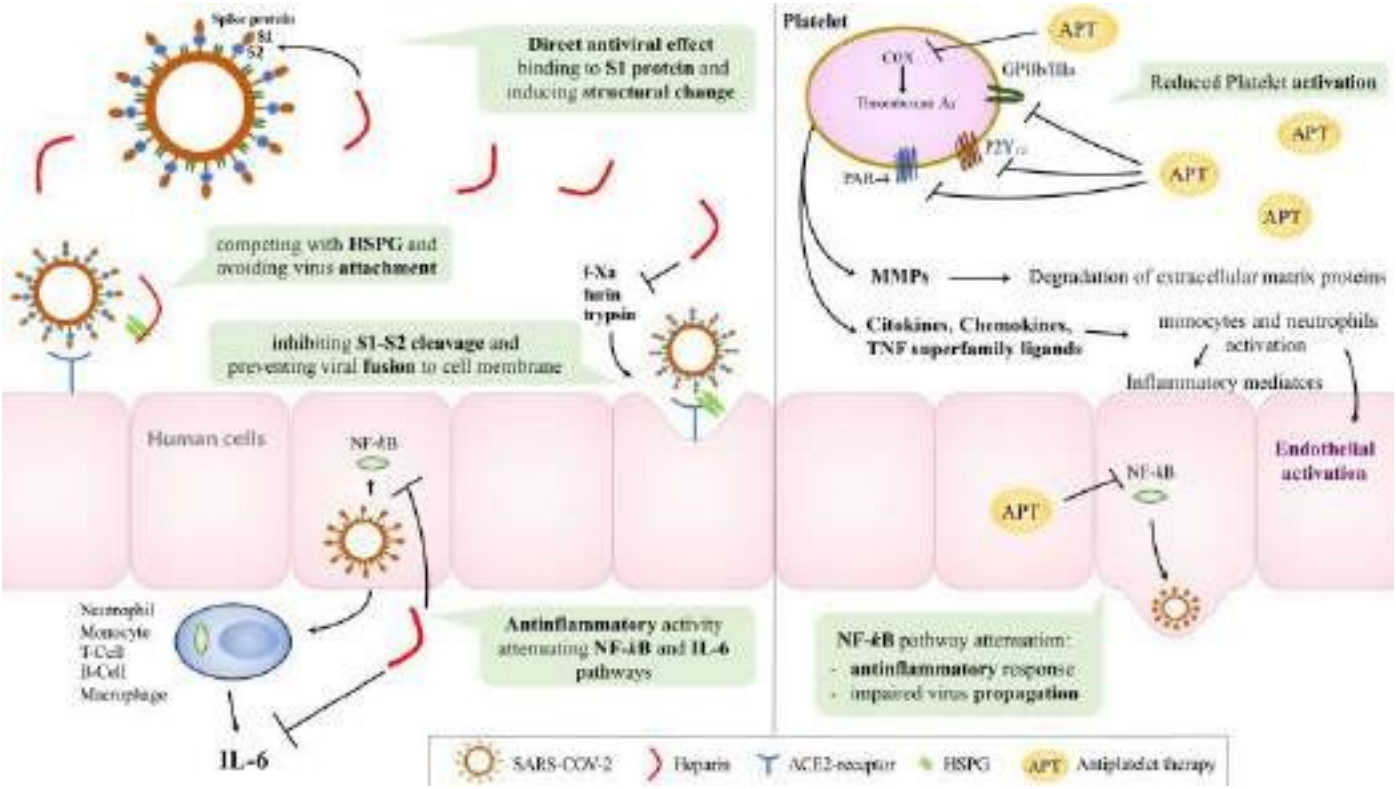
1. Modulate arachidonic acid (AA) metabolism: *aspirin, indobufene, triflusal, picotamide*;
2. Inhibit the platelet adenosine diphosphate (ADP) P2Y12 receptor: *ticlopidine, clopidogrel, prasugrel, ticagrelor, cangrelor, elinogrel;*
3. increase cyclic adenosine monophosphate (cAMP) levels: *dipyridamole, cilostazol*;
4. Inhibit platelet glycoprotein GpIIb/IIIa: *abciximab, eptifibatide, tirofiban*;
5. Inhibit the platelet thrombin receptor PAR (Proteinase activated Receptor)-1: *vorapaxar*.

Unlike anticoagulant therapy, no clinical observations have been reported on the possible protective or therapeutic effects of antiplatelet therapy in COVID-19.

However, there is a pathophysiological rationale for the theoretical benefits. SARS-CoV-2 infects endothelial cells using ACE-2 receptors, which are widely expressed on the vascular tissues of several organs (kidney, heart, brain, intestine, and liver) [12,13]. Postmortem histology revealed typical lymphocytic endotheliitis as a direct consequence of SARS-CoV-2 infection, with widespread inflammation and endothelial dysfunction [5].

[147] https://anticoagulazione.it/index.php/le-terapie/terapia-antiaggregante-piastrinica

Endothelial dysfunction promotes a shift in the hemostatic balance toward the procoagulant state, triggering platelet adhesion and aggregation [41] and thereby initiating a thromboinflammatory process.
In several models of tissue damage during virus infection (influenza A virus, dengue, HIV-1, SARS), unsuppressed platelet activation results in destructive inflammation [42].
Activated platelets release considerable amounts of proinflammatory molecules (cytokines, chemokines, high-mobility group box 1, metalloproteinases, and P-selectin), which induce neutrophil rolling, adhesion, and recruitment, and NETosis [43].
In addition, the physical interaction between activated platelets and neutrophils further contributes to neutrophil retention and activation, degradation of extracellular matrix proteins, and further activation of endothelium and thrombin generation [[43], [44], [45], [46], [47], [48], ~~[49]~~].
Furthermore, in an elevated inflammatory scenario, as in COVID-19, increased plasma concentration of TNF-alpha could contribute to platelet activation and expression of a highly thrombotic platelet phenotype because this stimulus induces expression of biologically active tissue factor on the platelet surface [50].



https://www.internationaljournalofcardiology.com/article/S0167-5273(20)33894-8/fulltext
COX = cyclooxygenase; HSPG = proteoglycan heparan sulfate; MMP = matrix metalloproteinase; NF-kb = nuclear enhancer factor kappa light chain of activated B lymphocytes; SARS-COV-2 = severe acute respiratory syndrome coronavirus 2; TNF = Tumor Necrosis Factor.

For more in-depth discussion of the mechanism of COVID-19-associated coagulopathy, please refer to the dedicated chapter CHAPTER-2-COMPLICATIONS-RESPIRATORY-PART-PRIME-presentation-clinical

INTRAVENOUS IMMUNOGLOBULIN INFUSION THERAPY

Intravenous immunoglobulin (IVIG) is a blood product consisting of aggregated IgG fractions obtained from about 3,000 to 60,000 plasma samples from blood donors.
Since its discovery 60 years ago, IVIG has been conventionally used as a therapeutic treatment for immunocompromised individuals suffering from immunodeficiency diseases such as hypogammaglobulinemia.

Hyperimmune plasma, on the other hand, is derived from individuals with high antibody titers to specific pathogens (convalescent people cured of disease) and has been used successfully in the treatment of infections, such as cytomegalovirus and H1N1 influenza. [148]

Comparison of Intravenous Immunoglobulin (IVIG) vs. Hyperimmune Sera.

	Intravenous immunoglobulin (IVIG)	Hyperimmune sera
Preparation	- Pooled human plasma	- Pooled human plasma
Donors	- General population	- Individuals seropositive for specific pathogen(s) with sufficient neutralizing antibody titer(s)
Usage	- Ig replacement in primary and secondary immunodeficiency - Immune modulation	- Treatment of specific pathogen(s)
Benefits	- Provides widespread protection against common infections - Treatment of hyper-inflammatory states - Large donor pool - Commercial availability	- Targeted therapy in specific infection(s), especially novel infections without herd immunity
Limitations	- Absent or variable specific neutralizing antibody titer(s) against novel pathogen(s)	- Limited donor availability, must be previously exposed - Variable antibody titer among donors, limited timeframe for donation - May aggravate disease
Rationale for use in COVID-19	- May provide immunomodulatory effect in hyperinflammation state (limited/inconclusive data) - Competitively bind Fcγ receptor to prevent antibody-dependent enhancement triggered by virus-antibody immune complexes[19]	- Has demonstrated effectiveness in SARS and MERS corona virus infections[16,17,18]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211658/

Apheresis

Apheresis is the recommended procedure for obtaining plasma and is based on continuous centrifugation of whole blood with plasma separation and collection. [149] The effectiveness of this technique is to obtain approximately 400-800 mL of plasma from a single apheretic donation.

[148] Nguyen AA, Habiballah SB, Platt CD, Geha RS, Chou JS, McDonald DR. Immunoglobulins in the treatment of COVID-19 infection: Proceed with caution! Clin Immunol. 2020;216:108459. doi:10.1016/j.clim.2020.108459 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211658/

[149] Reference for bibliography cited in superscript in red curly brackets
Annamaria P, Eugenia Q, Paul S.

The Transfusion Service has premises suitable for its intended use in order to limit the risk of errors, as well as cleaning and maintenance operations to minimize the risk of contamination.
For apheresis activities, cell separators with performance characteristics that ensure the highest degree of safety for the donor as well as the quality of the final products must be used, meeting the requirements for apheresis blood component collection as stipulated in current regulations.
For the sealing of the connection circuits of the collection systems, the centers have suitable sealing systems to prevent the risk of microbial contamination during the collection and production of blood components, and have a device capable of freezing the plasma produced, to ensure compliance with the specifications defined by current regulations.
The collected plasma units are then subjected to the biological qualification tests required by DM 2/11/2015 and therefore must be negative for the following viral markers: - HBV- HIV-HCV serological and molecular, Syphilis serological. [150]
Since this is convalescent plasma from nonperiodic donors, it must also be performed:
-the HAV and Parvovirus B19-DNA tests (<105 copies/mL).
-the content of immunoglobulins (IgG, IgM, IgA),
-The titer of the specific antibody (according to EIA must be> 160 or equivalent by another method). The efficacy of this therapy was associated with the concentration of neutralizing Ab in donor plasma.

Volume and posology
The volume and dosage of hyperimmune plasma administration are variable and anecdotal because they are based on clinical experience from previous outbreaks and comparison with Chinese clinical trials of Sars-Cov-2 patients [32]

Based on the Sars-Cov-1 studies, hyperimmune therapeutic plasma was used at a dosage of 5 mL/kg with an antibody neutralization titer of 1: 160 [16].
It should also be considered that 3.125 / mL / kg of plasma has an antibody titer> 1.64, so 5 mL / kg of plasma corresponds to a hyperimmune globulin titer 1: 160. If we consider a typical patient of about 80 kg, the volume of plasma to be infused will be 250 mL (3,125 mL / kg x 80 kg = 250 mL> 1.64 [31]).

The timing and therapeutic dosage to be used according to different clinical experiences are as follows: 250-300 mL of hyperimmune plasma administered to each of the admitted patients for a maximum of 3 times within 5 days [32].

The maximum volume administered should not exceed 600 ml as the use of larger plasma volumes would be contraindicated due to the risk of overloading [41].

Timing of administration
Early administration is strongly recommended, is optimal in the first 7 days, efficacy remains good within 14 days, certainly not indicated beyond three weeks after disease onset. It is important to perform therapy during the viral replication phase [31], [32], [33], [34]

Adverse effects and contraindications
Passive immunotherapy, according to studies conducted on Ebola and Mers-Cov, results in serious adverse events in 2% of cases in the form of transfusion-related acute lung injury (TRALI) and mild adverse events in 8% of treated patients, 5% of whom had a febrile reaction and 4% had a urticarial reaction. [28] --[35]

Studies in animal models (rhesus macaques) have shown the risk of antibody-dependent enhancement (ADE), as will be discussed below. This phenomenon is related to early seroconversion prior to lung clearance of the virus with increased macrophage-dependent inflammatory damage by these nonneutralizing antibodies[151] . Finally, this product is subject to all the precautions regarding side effects and contraindications common to human plasma therapy; these include in particular: the absolute contraindication to its administration in complete IgA deficiency, (which is why IgA dosing is recommended prior to initiation of therapy), and caution against circulatory overload (TACO: a complication that occurs during or immediately after transfusion, due to the excessive rate of

Anti-SARS-CoV-2 hyperimmune plasma workflow.
Transfus Apher Sci. 2020;59(5):102850. doi:10.1016/j.transci.2020.102850
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7283061/
[150] Official Gazette I.T. 2015. Provisions on quality and safety requirements for blood and blood components. [Accessed December 28, 2015]
https://www.gazzettaufficiale.it/eli/id/2015/12/28/15A09709/sg

[151] Dzik S.
COVID-19 Convalescent Plasma: Now Is the Time for Better Science.
Transfus Med Rev. 2020;34(3):141-144. doi:10.1016/j.tmrv.2020.04.002
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7177063/

transfusion in relation to cardiovascular reserve. Symptoms are given by tachycardia, dyspnea, pulmonary congestion, and headache) [152].

Clinical research

It should be kept in mind that according to WHO, the use of plasma therapy is allowed in the face of "serious diseases for which there is no effective drug treatment."[153] . On Aug. 23, the Food and Drug Administration (FDA), approved the use of CP for compassionate use in the treatment of patients with critical COVID-19 infection,[154] and the European Commission recently funded a research project (SUPPORT-E: SUPporting high quality evaluation of COVID-19 convalescent Plasma thrOughouT Europe) to determine whether transfusion of COVID-19 convalescent plasma, is an effective and safe treatment. [155]

The efficacy and safety of convalescent plasma immunotherapy is mainly tested in Asia with 5 types of clinical trials:

- for post-exposure prophylactic use
- In patients with mild disease
- In patients with moderate disease
- as rescue therapy
- In high-risk pediatric patients

Clinical research results still remain inconclusive regarding the efficacy of hyperimmune or convalescent plasma therapy in COVID-19,[156] although positive findings published especially by Italian teams are particularly encouraging. [157]

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[152] SIMTI /SIDEM; 2020. SIMTI /SIDEM "Position paper" on the production of hyperimmune plasma for use in the therapy of SARS- CoV2 disease.https://aiceonline.org/wp-content/uploads/2020/04/SIMIT-SIDEM-convalescent-plasma.pdf [Accessed March 26, 2020]

[153] World Health Organization. (2020). Guidance on maintaining a safe and adequate blood supply during the coronavirus disease 2019 (COVID-19) pandemic and on the collection of COVID-19 convalescent plasma: interim guidance, July 10, 2020. World Health Organization. https://apps.who.int/iris/handle/10665/333182

[154] https://www.fda.gov/news-events/press-announcements/fda-issues-emergency-use-authorization-convalescent-plasma-potential-promising-covid-19-treatment

[155] https://ec.europa.eu/info/news/commission-supports-crucial-research-convalescent-plasma-treat-covid-19-2020-sep-11_en

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Treatment of COVID-19 with convalescent plasma: lessons from past coronavirus outbreaks. Clin Microbiol Infect. 2020;26(10):1436-1446. doi:10.1016/j.cmi.2020.08.005
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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7641519/

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Covid-19 plasma task force. Mortality reduction in 46 severe Covid-19 patients treated with hyperimmune plasma. A proof-of-concept single arm multicenter trial. Haematologica. 2020 Jul 23:haematol.2020.261784. doi: 10.3324/haematol.2020.261784. Epub ahead of print.
https://haematologica.org/article/view/9826

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Convalescent plasma for coronavirus disease 2019 in pregnancy: a case report and review. Am J Obstet Gynecol MFM. 2020;2(3):100174. doi:10.1016/j.ajogmf.2020.100174
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A potentially effective treatment for COVID-19: A systematic review and meta-analysis of convalescent plasma therapy in treating severe infectious disease.
Int J Infect Dis. 2020;98:334-346. doi:10.1016/j.ijid.2020.06.107
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7334933/

Salazar E, Christensen PA, Graviss EA, et al.
Treatment of Coronavirus Disease 2019 Patients with Convalescent Plasma Reveals a Signal of Significantly Decreased Mortality.
Am J Pathol. 2020;190(11):2290-2303. doi:10.1016/j.ajpath.2020.08.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7417901/

Salazar E, Christensen PA, Graviss EA, et al.

In particular, mention should be made of the findings of the recent (12.10.2020) Cochrane review* on the efficacy of plasma convalescent[158] :

> * The Cochrane Collaboration is an international nonprofit initiative established for the purpose of collecting, critically evaluating, and disseminating information regarding the effectiveness and safety of health care interventions.

" *We are not sure whether convalescent plasma is useful for people hospitalized with COVID-19. Information on grade 3 and 4 adverse events was limited to determine the effect of convalescent plasma therapy on clinically relevant adverse events. In the absence of a control group, we are unable to assess the relative safety of convalescent plasma therapy. Although significant efforts have been made to conduct research on COVID-19, recruiting the expected number of participants for these studies is problematic. The early completion of the first two RCTs studying convalescent plasma and the lack of data from 20 studies that have completed or were due to be completed at the time of this update illustrate these critical issues. Priority should be given to well-designed studies. In addition, studies should report results in the same way and should consider the importance of maintaining comparability in terms of co-interventions administered in all study arms. There are 138 ongoing studies evaluating convalescent plasma and hyperimmune immunoglobulins, of which 73 are RCTs (three already completed). This is the second ongoing update and we will continue to update this review periodically. Future updates may show different results from those reported here.*"

Mechanism of action

To date, despite the effectiveness of CP and IVIG treatment in various autoimmune and inflammatory diseases, the mechanism by which this occurs remains poorly defined. [159]

For a more detailed discussion, please refer to the review "*Convalescent plasma in Covid-19: Possible mechanisms of action*" by Manuel Rojas et al in which the possible mechanisms of CP action and their repercussion in COVID- 19 pathogenesis are discussed in detail, including direct virus neutralization, control of a hyperactive immune system (cytokine storm, Th1/Th17 ratio, complement activation) and immunomodulation of a hypercoagulable state .[160]

Significantly Decreased Mortality in a Large Cohort of Coronavirus Disease 2019 (COVID-19) Patients Transfused Early with Convalescent Plasma Containing High-Titer Anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Spike Protein IgG
[published online ahead of print, 2020 Nov 4]. Am J Pathol. 2020;S0002-9440(20)30489-2. doi:10.1016/j.ajpath.2020.10.008
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7609241/

Khulood D, Adil MS, Sultana R, Nimra .
Convalescent plasma appears efficacious and safe in COVID-19.
Ther Adv Infect Dis. 2020;7:2049936120957931. Published 2020 Sep 28. doi:10.1177/2049936120957931
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7534072/

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Convalescent Plasma Therapy for Coronavirus Disease 2019.
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Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review.
Cochrane Database Syst Rev. 2020 Oct 12;10:CD013600. doi: 10.1002/14651858.CD013600.pub3. PMID: 33044747.
https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013600.pub3/full
https://www.cochrane.org/CD013600/HAEMATOL_plasma-people-who-have-recovered-covid-19-effective-treatment-people-covid-19

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High-Dose Intravenous Immunoglobulin in Skin Autoimmune Disease
Front Immunol. 2019 Jun 11;10:1090. doi: 10.3389/fimmu.2019.01090.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6579842/

Pandey A, Nikam AN, Shreya AB, et al.
Potential therapeutic targets for combating SARS-CoV-2: Drug repurposing, clinical trials and recent advances. Life Sci.
2020;256:117883. doi:10.1016/j.lfs.2020.117883
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7263255/

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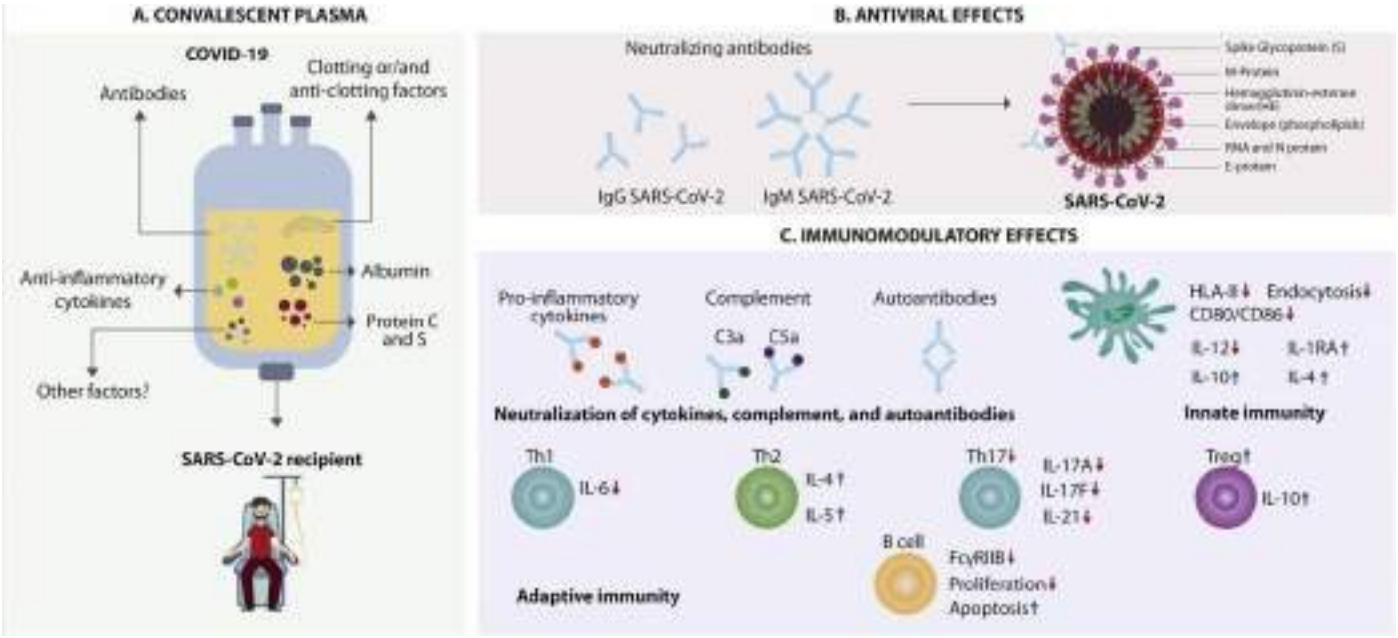
Convalescent plasma in Covid-19: Possible mechanisms of action.
Autoimmun Rev. 2020;19(7):102554. doi:10.1016/j.autrev.2020.102554
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198427/

The composition of CP is complex and includes a wide range of components derived from blood, including a mixture of inorganic salts, organic compounds, water, and more than 1,000 proteins. The other factors identified are albumin, immunoglobulins, complements, coagulation factors, and antithrombotic factors.

The main components of convalescent plasma are illustrated in the following Fig. A[161] . In addition to neutralizing antibodies (NAbs), additional protective antibodies that might show antiviral effect are found in plasma, including immunoglobulin G (IgG) and immunoglobulin M (IgM). Immunoglobulin A (IgA) may also be important, especially for viral mucosal infections. Non-NAbs that bind to the virus but do not affect its ability to replicate could contribute to prophylaxis and/or improved recovery. The humoral immune response mainly affects the spike protein (S). (Fig. B)[162] , while the anti-inflammatory effects of CP include the autoantibody network and regulation of an overactive immune system.
In addition, some antibodies prevent the complement cascade (i.e., C3a and C5a) and limit the formation of immune complexes (Fig. C)[163] .



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198427/

Schematic representation of the components of convalescent plasma and its mechanisms of action.

A. Main components of convalescent plasma.

B. Antiviral effects of NAbs. IgG and IgM are the main isotypes, although IgA may also be important, particularly in viral mucosal infections. Other non-NAbs may exert a protective effect. The humoral immune response is mainly directed toward the spike protein (S).

C. The anti-inflammatory effects of CP include the autoantibody network and control of a hyperactive immune system (e.g., cytokine storm, Th1/Th17 ratio, complement activation, and regulation of a hypercoagulative state) (see text for details). N: Nucleoprotein; M: membrane; E: envelope

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[161] Benjamin RJ, McLaughlin LS.
Plasma components: properties, differences, and uses.
Transfusion. 2012 May;52 Suppl 1:9S-19S. doi: 10.1111/j.1537-2995.2012.03622.x. PMID: 22578375.
https://onlinelibrary.wiley.com/doi/full/10.1111/j.1537-2995.2012.03622.x

[162] Bloch EM et al
Deployment of convalescent plasma for the prevention and treatment of COVID-19. J Clin Invest. 2020 Jun 1;130(6):2757-2765. doi: 10.1172/JCI138745.
https://www.jci.org/articles/view/138745

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Anti-inflammatory effect of intravenous immunoglobulin mediated through modulation of complement activation.
Clin Rev Allergy Immunol. 2005 Dec;29(3):207-12. doi: 10.1385/CRIAI:29:3:207. PMID: 16391395.
https://link.springer.com/article/10.1385/CRIAI:29:3:207

Basta M, Van Goor F, Luccioli S, Billings EM, Vortmeyer AO, Baranyi L, Szebeni J, Alving CR, Carroll MC, Berkower I, Stojilkovic SS, Metcalfe DD. F(ab)'2-mediated neutralization of C3a and C5a anaphylatoxins: a novel effector function of immunoglobulins.
Nat Med. 2003 Apr;9(4):431-8. doi: 10.1038/nm836. Epub 2003 Mar 3. PMID: 12612546.
https://www.nature.com/articles/nm836

The possible mechanisms underlying the efficacy of IVIGs revolve mainly around their ability to modify the immune response.
In particular, mechanisms related to the Fab'2 and Fc regions of antibodies in plasma should be noted:

- **F(ab´)2** (antigen-binding antibody region): preparation of IVIg includes anti-idiotypic antibodies that block autoreactive recipient antibodies[164] . This reaction is essential to control autoantibodies in patients with autoimmune diseases.
In this regard, a recent report in patients with COVID-19 showed that critically ill patients showed positivity for anti-cardiolipin IgA antibodies as well as anti-β2-glycoprotein I IgA and IgG antibodies[165] .

This evidence may suggest that CP-COVID-19 may neutralize this type of autoantibody by reducing the likelihood of thrombotic events (i.e., antiphospholipid syndrome-like disease), especially in critically ill patients.
Along the same lines, a recent report on a patient with Sjögren's syndrome and COVID-19 successfully treated with CP may suggest that this strategy is safe and effective in autoimmune conditions[166] .

In addition, some antibodies inhibit the complement cascade (i.e., C3a and C5a) and limit the formation of immune complexes[153] . Complement-deficient mice with induced SARS-CoV infection showed a marked decrease in viral titers, inflammatory cytokine and chemokine secretion, and immune cell infiltration within the lung compared with controls.
These findings suggest that complement activation largely contributes to systemic inflammation and neutrophil migration to the lungs, perpetuating tissue damage[167] . Further studies have shown that plasma-transferred IgG neutralizes cytokines such as IL-1β and TNFα[168] .
In this sense, passive immunity by infusion of CP-COVID-19 can limit the inflammatory cascade driven by pathogenic antibodies as well as cell damage induced by activation of the complement cascade in excessive inflammatory environments.

- **Fc** (effector region): The FcRn receptor is a critical regulator of IgG half-life and acts by preventing the degradation and elimination of IgG, through a pinocytosis mechanism that allows the uptake of antibodies within the cell and their subsequent excretion[169] .

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[164] Rossi F, Dietrich G, Kazatchkine MD.
Anti-idiotypes against autoantibodies in normal immunoglobulins: evidence for network regulation of human autoimmune responses. Immunol Rev. 1989 Aug;110:135-49. doi: 10.1111/j.1600-065x.1989.tb00031.x. PMID: 2676846.
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Spalter S.H., Kaveri S., Kazatchkine M.D. Anti-idiotypes to autoantibodies in therapeutic preparations of normal polyspecific human IgG (intravenous immunoglobulin, IVIg) In: Shoenfeld Y., Kennedy R.C., Ferrone Infection and Cancer SBT-I in MA, editors. Idiotypes Med. Autoimmun. Infect. Cancer. Elsevier; Amsterdam: 1997. pp. 217-225. Editors

Luc Mouthon, Michel D. Kazatchkine, Autoantibodies in Therapeutic Preparations of Human IgG (IVIg), Editor(s): James B. Peter, Yehuda Shoenfeld, Autoantibodies, Elsevier Science B.V.,1996, Pages 91-95, ISBN 9780444823830, https://doi.org/10.1016/B978-044482383-0/50014-5.
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Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19.
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Treatment with convalescent plasma for COVID-19 patients in Wuhan, China
[published online ahead of print, 2020 Apr 15]. J Med Virol. 2020;10.1002/jmv.25882.
doi:10.1002/jmv.25882 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262027/pdf/JMV-9999-na.pdf

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Complement Activation Contributes to Severe Acute Respiratory Syndrome Coronavirus Pathogenesis.
mBio. 2018;9(5):e01753-18. Published 2018 Oct 9. doi:10.1128/mBio.01753-18
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6178621/

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Anti-cytokine nature of natural human immunoglobulin: one possible mechanism of the clinical effect of intravenous immunoglobulin therapy. Immunol Rev. 1994 Jun;139:5-19. doi: 10.1111/j.1600-065x.1994.tb00854.x.
https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-065X.1994.tb00854.x?sid=nlm%3Apubmed

[169] Sand KM, Bern M, Nilsen J, Noordzij HT, Sandlie I, Andersen JT.
Unraveling the Interaction between FcRn and Albumin: Opportunities for Design of Albumin-Based Therapeutics.

It has been shown that saturation of this receptor by IVIg may be the most likely mechanism to eliminate autoantibodies under autoimmune conditions by shortening their duration[170] and thus saturation of FcRn may provide an additional immunomodulatory pathway in COVID-19 patients receiving CP.

Fcγ receptors are found in almost all immune cells and are critical factors in modulating or inhibiting the activity of immune cells, including lymphocytes. Activation of the Fcγ receptor by IgG induces upregulation of FCγRIIB associated with inhibitory effects.[171]

This has been demonstrated in B cells, where upregulation of FCγRIIB is a major determinant of response to IVIg in patients with Kawasaki disease[172] .
One possible mechanism is that high-dose IVIG infusion prevents FcγR activation through saturation of the binding of these receptors with infused IgG, as well as through increased expression of inhibitory FcγRIIb.[173]

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Chaigne B, Mouthon L.
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The MHC class I-like Fc receptor promotes humorally mediated autoimmune disease. J Clin Invest. 2004;113(9):1328-1333. doi:10.1172/JCI18838
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC398424/

[171] Nimmerjahn F, Ravetch JV.
Fcgamma receptors: old friends and new family members.
Immunity. 2006 Jan;24(1):19-28. doi: 10.1016/j.immuni.2005.11.010. PMID: 16413920.
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Role of activating FcγR gene polymorphisms in Kawasaki disease susceptibility and intravenous immunoglobulin response.
Circ Cardiovasc Genet. 2012;5(3):309-316. doi:10.1161/CIRCGENETICS.111.962464
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3444514/

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Focosi D, Anderson AO, Tang JW, Tuccori M.
Convalescent Plasma Therapy for COVID-19: State of the Art.
Clin Microbiol Rev. 2020;33(4):e00072-20. Published 2020 Aug 12. doi:10.1128/CMR.00072-20
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7430293/

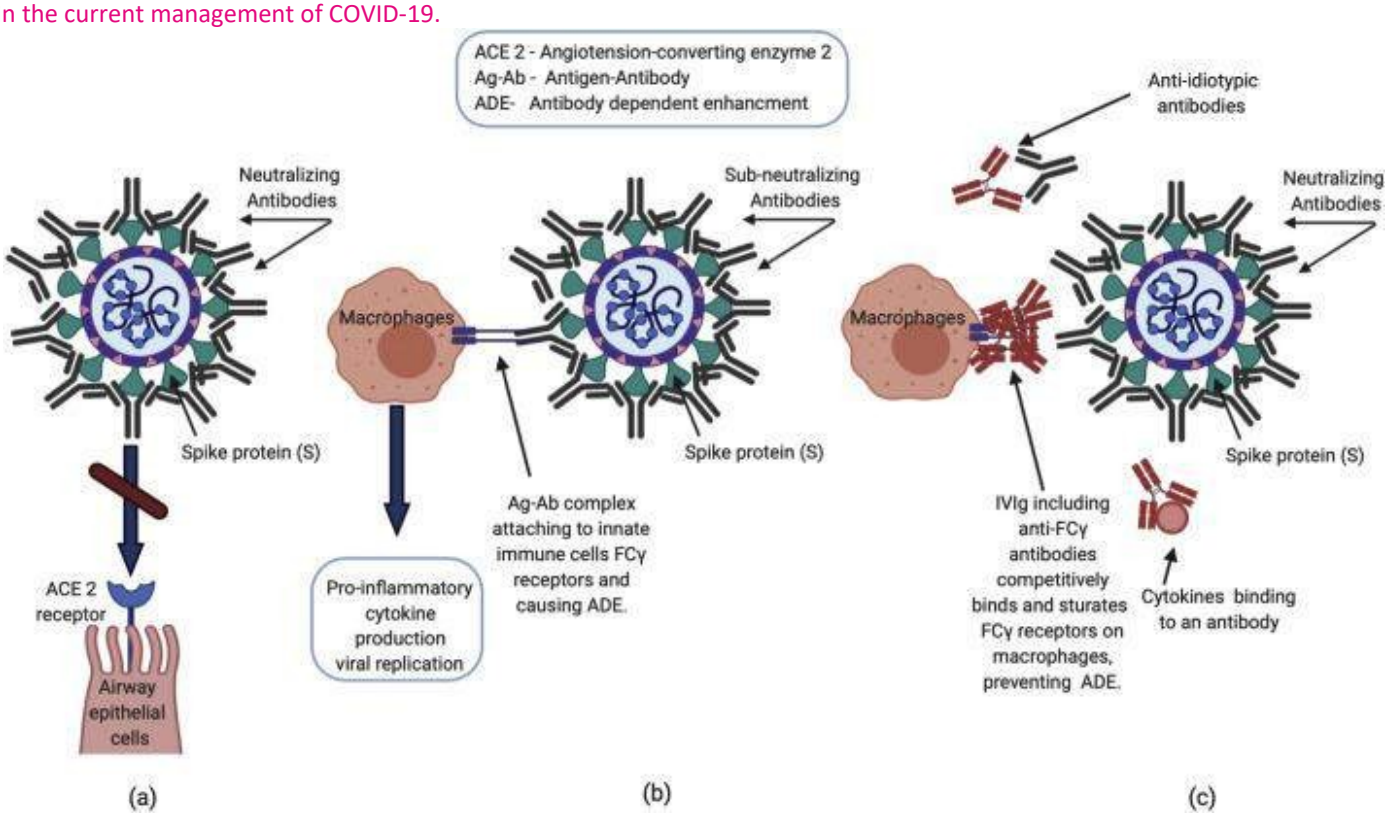
Piyush R, Rajarshi K, Khan R, Ray S.
Convalescent plasma therapy: a promising coronavirus disease 2019 treatment strategy.
Open Biol. 2020;10(9):200174. doi:10.1098/rsob.200174
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7536086/

Rojas M, et al.
Convalescent plasma in Covid-19: Possible mechanisms of action
Autoimmun Rev. 2020;102554. doi:10.1016/j.autrev.2020.102554
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198427/

Fischer JC, et al.
The role of passive immunization in the age of SARS-CoV-2: an update.
Eur J Med Res. 2020;25(1):16. doi:10.1186/s40001-020-00414-5
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7220618/

Brown BL, McCullough J.
Treatment for emerging viruses: Convalescent plasma and COVID-19.
Transfus Apher Sci. 2020;102790. doi:10.1016/j.transci.2020.102790
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194745/

It follows that CP infusion can help modulate the immune response via Fcγ receptors and deserves attention
In the current management of COVID-19.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211658/
Proposed mechanisms of neutralizing antibodies and IVIG in COVID-19 infection.
(a) Neutralizing antibodies prevent the SARS-CoV2 spike protein from binding to the ACE2 receptor, inhibiting viral entry into the cell**.**
(b) Immune complexes consisting of viral antigens and sub-neutralizing antiviral antibodies can activate Fcγ receptors on innate immune cells (e.g., macrophages) in the lung, triggering an exaggerated inflammatory response leading to acute lung injury through antibody-dependent enhancement (ADE) . In addition, antibody-associated virus can be internalized through Fcγ receptors, enhancing viral replication.
(c) Proposed mechanisms by which IVIG exerts anti-inflammatory action include saturation of Fcγ receptor binding, anti-idiotypic binding to anti-viral antibodies, and binding of proinflammatory cytokines.

Bloch EM, et al.
Deployment of convalescent plasma for the prevention and treatment of COVID-19.
J Clin Invest. 2020;130(6):2757-2765. doi:10.1172/JCI138745
https://www.jci.org/articles/view/138745

Sullivan HC, Roback JD.
Convalescent Plasma: Therapeutic Hope or Hopeless Strategy in the SARS-CoV-2 Pandemic
Transfus Med Rev. 2020;S0887-7963(20)30025-0. doi:10.1016/j.tmrv.2020.04.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7179481/

Knudson CM, Jackson JB.
COVID-19 convalescent plasma: phase 2.
Transfusion. 2020;10.1111/trf.15842. doi:10.1111/trf.15842
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7267265/

de Alwis R, Chen S, Gan ES, Ooi EE.
Impact of immune enhancement on Covid-19 polyclonal hyperimmune globulin therapy and vaccine development.
EBioMedicine. 2020;55:102768. doi:10.1016/j.ebiom.2020.102768
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7161485/

Morabito CJ, Gangadharan B.
Active Therapy with Passive Immunotherapy May Be Effective in the Fight against COVID-19.
Clin Transl Sci. 2020;13(5):835-837. doi:10.1111/cts.12816
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7485949/

Regarding the **limitations of using IVIG and hyperimmune plasma**, a recent study of secondary DENV infection in an immunocompetent mouse animal model showed that IVIG-treated mice had **increased mortality, exacerbation of disease severity, and elevated virus replication in a dose-dependent manner**. Further examination found sub-neutralizing titers of anti-DENV antibodies in the serum of IVIG-treated mice as a contributing factor to the exacerbation[174] .

It follows that the administration of IVIG should be preceded by detection and purification from nonneutralizing antibodies in serum In case of infections in which ADE is possible as disease potentiation. [175]

ANTISTAMINES

Coronaviruses develop special mechanisms to invade the body and immune cells, including mast cells.
Host response to RNA virus invasion activates TLR3 present on mast cells with production of antiviral IFN and chemokines (positive effect).
However, often the virus only causes sensitization of mast cells with the synthesis of IgE that binds to the FcεRI receptor and triggers a violent inflammatory reaction, or it stimulates mucosal mast cells to release pro-inflammatory cytokines such as TNF, IL-1, IL-6 and proteases, which aggravate the inflammatory state and autoimmune responses (negative effect).[176]

Virus-activated mast cells produce histamine, prostaglandin D2 (PGD2) and leukotriene C_4 (LTC_4), which induce acute bronchoconstriction and lung inflammation. (mast cell activation syndrome) [177]

[174] Med Microbiol Immunol 2014 Aug;203(4):231-50. doi: 10.1007/s00430-014-0334-5.
Subversion of Early Innate Antiviral Responses During Antibody-Dependent Enhancement of Dengue Virus Infection Induces Severe Disease in Immunocompetent Mice
Vivian V Costa et al
https://link.springer.com/article/10.1007/s00430-014-0334-5

Fleming AB, Raabe V.
Current studies of convalescent plasma therapy for COVID-19 may underestimate risk of antibody-dependent enhancement.
J Clin Virol. 2020;127:104388. doi:10.1016/j.jcv.2020.104388
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7187833/

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Corona (COVID-19) time musings: Our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning.
Autoimmun Rev. 2020;19(6):102538. doi:10.1016/j.autrev.2020.102538
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7131471/

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What the physicians should know about mast cells, dendritic cells, urticaria, and omalizumab during COVID-19 or asymptomatic infections due to SARS-CoV-2?
Dermatol Ther. 2020 Jul 25:e14068. doi: 10.1111/dth.14068. Epub ahead of print. PMID: 32713127.
https://onlinelibrary.wiley.com/doi/10.1111/dth.14068

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Self-reactive IgE exacerbates interferon responses associated with autoimmunity.
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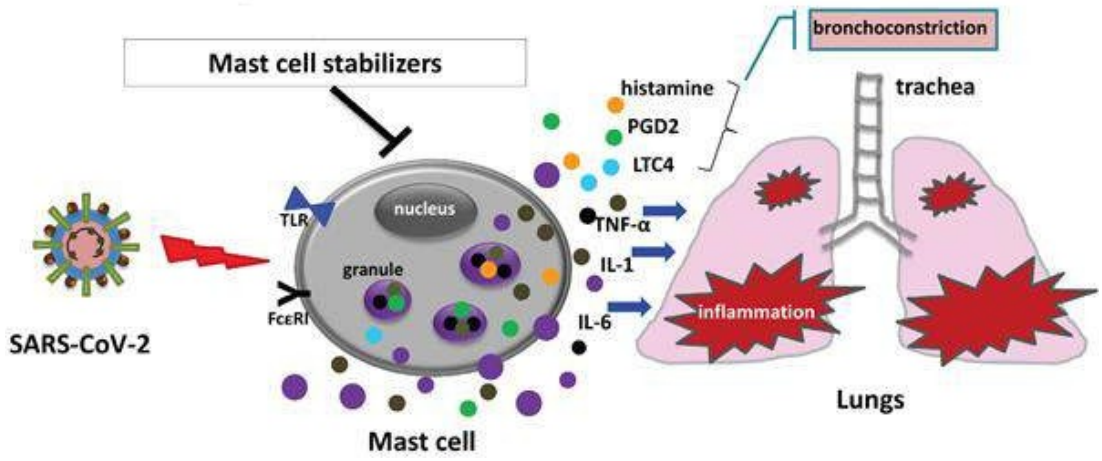
Covid-19 hyperinflammation and post-Covid-19 illness may be rooted in mast cell activation syndrome.
Int J Infect Dis. 2020;100:327-332. doi:10.1016/j.ijid.2020.09.016
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7529115/

Conti P, Caraffa A, Tetè G, Gallenga CE, Ross R, Kritas SK, Frydas I, Younes A, Di Emidio P, Ronconi G. Mast cells activated by SARS-CoV-2 release histamine which increases IL-1 levels causing cytokine storm and inflammatory reaction in COVID-19. J Biol Regul Homeost Agents. 2020 Sep-Oct,;34(5):1629-1632. doi: 10.23812/20-2EDIT. PMID: 32945158.
https://www.biolifesas.org/biolife/wp-content/uploads/2020/09/Conti_cytokine_storm.pdf



https://www.cebm.net/covid-19/mast-cell-stabilisers-leukotriene-antagonists-and-antihistamines-a-rapid-review-of-effectiveness-in-covid-19/

For an in-depth discussion of the mechanism of mast cell activation syndrome in COVID-19, please refer to the chapter
PULMONARY COMPLICATIONS-IMMUNOPATHOLOGY

Allergy-targeted drugs (antihistamines) and natural compounds that inhibit mast cell activation can then be used (e.g., polyphenols) to alleviate the symptoms of COVID-19.
It is known that vitamin D is necessary to maintain mast cell stability and its deficiency causes mast cell activation,[178] vitamin C has an antihistamine effect, hydroxychloroquine improves IgE-mediated asthma, and azithromycin has an anti-inflammatory effect on histamine-induced inflammation. [179]

Histamine is an endogenous biogenic amine ubiquitously distributed in cells and is present in high concentrations in the lungs, skin, and gastrointestinal tract where it acts as a local mediator in the immune system.

Histamine causes complex physiological changes, including chemotaxis, cytokine production, and gastric acid secretion. [180] These biological changes occur through four subtypes of G-protein-coupled receptors (GPCRs): H1 receptor (H1R), H2 receptor (H2R), H3 receptor (H3R) and H4 receptor (H4R).
H1R is expressed in various cell types, such as neurons, endothelial cells, adrenal medulla, muscle cells, hepatocytes, chondrocytes, monocytes, neutrophils, eosinophils, dendritic cells (DCs), T cells, and B cells. Activation of H1R leads to activation of Th1 lymphocytes and decreased humoral immunity.
H2R is expressed by gastric mucosal parietal cells, muscle, epithelial, endothelial, neuronal, hepatocyte, and immune cells. H2R antagonizes some of the H1R-mediated effects and leads to smooth muscle cell relaxation, causing vasodilation. In a mouse model of lung inflammation, loss of H2R has an effect on invariant natural killer T (iNKT) cells, exacerbating local inflammation. [181]

[178] Liu ZQ, Li XX, Qiu SQ, Yu Y, Li MG, Yang LT, Li LJ, Wang S, Zheng PY, Liu ZG, Yang PC.
Vitamin D contributes to mast cell stabilization.
Allergy. 2017 Aug;72(8):1184-1192. doi: 10.1111/all.13110. Epub 2017 Jan 17. PMID: 27998003.
https://pubmed.ncbi.nlm.nih.gov/27998003/

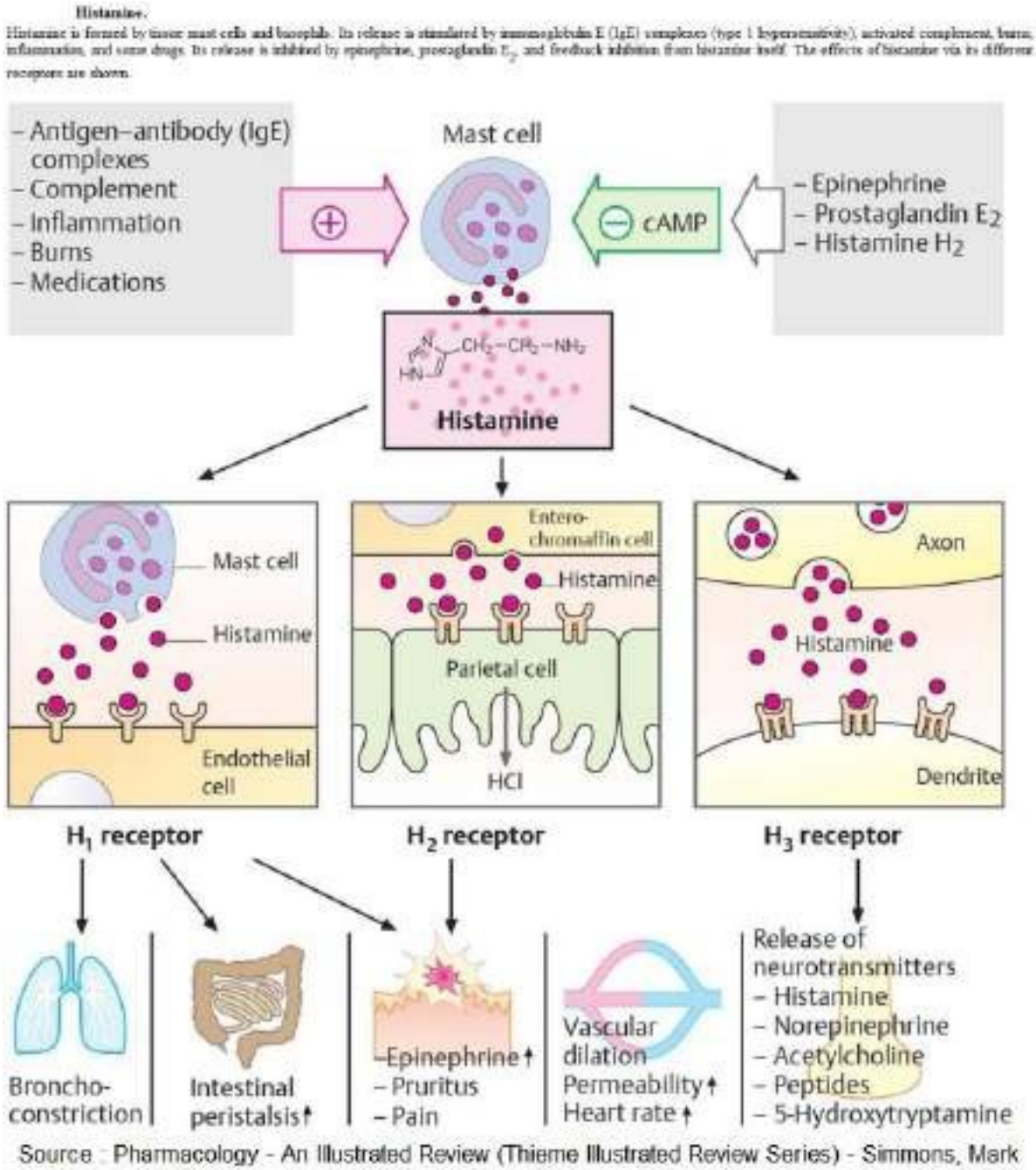
[179] Immunological mechanisms explaining the role of IgE, mast cells, histamine, elevating ferritin, IL-6, D-dimer, VEGF levels in COVID-19 and dengue, potential treatments such as mast cell stabilizers, antihistamines, Vitamin C, hydroxychloroquine, ivermectin and azithromycin
Arumugham, Vinu
https://zenodo.org/record/3748304?fbclid=IwAR3dnb-C_h5cVSieLUw6XCR1-JfMyxROlYNhgHD_LUH8JWnWEwxPpsn6U#.X3z-ZS1abjC

[180] Eldanasory OA, Eljaaly K, Memish ZA, Al-Tawfiq JA.
Histamine release theory and roles of antihistamine in the treatment of cytokines storm of COVID-19.
ravel Med Infect Dis. 2020;37:101874. doi:10.1016/j.tmaid.2020.101874
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7470786/

[181] Ferstl R., Frei R., Barcik W., Schiavi E., Wanke K., Ziegler M.
Histamine receptor 2 modifies iNKT cell activity within the inflamed lung.
Eur J Allergy Clin Immunol. 2017;72 doi: 10.1111/all.13227.1925–35
https://onlinelibrary.wiley.com/doi/abs/10.1111/all.13227

The functions of **H3R** have been identified in the central nervous system and peripheral and presynaptic receptors to control the release of histamine and other neurotransmitters.
H4R is preferentially expressed in the gut, spleen, thymus, bone marrow, peripheral hematopoietic cells, and cells of the innate and adaptive immune systems. Expression of H4R is regulated by stimulation with TNF-α, IL-6, IL-10, and IL-13, which leads to inhibition of cAMP accumulation and activation of mitogen-activated protein kinases (MAPKs) by H4R.

Thus, histamine is a potent inflammatory mediator, commonly associated with allergic reactions, which promotes vascular and tissue changes and possesses high chemotactic activity.
The use of selective H4R ligands and/or modulation of H1 and H4 receptor synergism may be effective in treating inflammatory conditions of the lung.
Histamine also modulates the inflammatory response by acting on other cell populations, e.g., in human lung macrophages.
Binding of histamine to H1R induces the production of proinflammatory cytokines IL-6 and β-glucuronidase.
Blockade of H4R in a model of pulmonary fibrosis alleviates the inflammatory response by reducing cyclooxygenase 2 (COX 2) expression and activity, leukocyte infiltration, transforming growth factor beta (TGF-β) (profibrotic cytokine) production, and collagen deposition.



At present, there are few studies examining the use of antihistamine products in patients with COVID-19, however, the results so far are particularly significant[182] .

In a study of ten consecutive patients with COVID-19 who self-administered high-dose oral **famotidine** (80 mg three times daily (n = 6) for a median of 11 days (range: 5-21 days)) all 10 patients had marked improvement in COVID-19 symptoms.
he treatment was well tolerated and the combined symptom score improved significantly within 24 hours of starting famotidine, and peripheral oxygen saturation (n = 2) and device-recorded activity (n = 1) increased[183] .

Interestingly, analysis of the pharmacokinetic parameters of famotidine might indicate that it must be administered intravenously to be effective in COVID-19 treatment given its low gastrointestinal absorption and volume of distribution, and there is currently an ongoing double-blind randomized controlled trial in New York evaluating the efficacy of high-dose (360 mg/day) intravenous famotidine with standard of care for up to 14 days in hospitalized COVID-19 patients[184] .

In a retrospective propensity score-matched cohort study comparing the famotidine cohort (84 patients) with the non-famotidine cohort (1536 patients), a crude analysis showed that famotidine use was significantly associated with a reduced risk of death or intubation (adjusted hazard ratio (aHR) 0.42, 95% CI 0.21-0.85). The famotidine group received between 10 and 40 mg/day for a median of 5.8 days and 72% received it orally[185] .
Although famotidine is an H2R antagonist and is mainly used for peptic ulcer and gastroesophageal reflux disease, its potential benefit has been attributed to the binding and inhibition of the protease-like 3-chemotrypsin[186] .

[182] Efficacy of Famotidine for COVID-19: A Systematic Review and Meta-analysis
Rahul Sethia, Manya Prasad, Soumya Jagannath, Neeraj Nischal, Manish Soneja, Pramod Garg, Shalimar
medRxiv 2020.09.28.20203463; doi: https://doi.org/10.1101/2020.09.28.20203463
https://www.medrxiv.org/content/10.1101/2020.09.28.20203463v1.full.pdf

Malone RW, Tisdall P, Fremont-Smith P, et al.
COVID-19: Famotidine, Histamine, Mast Cells, and Mechanisms.
Preprint. Res Sq. 2020;rs.3.rs-30934. Published 2020 Jun 22. doi:10.21203/rs.3.rs-30934/v2
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7336703/

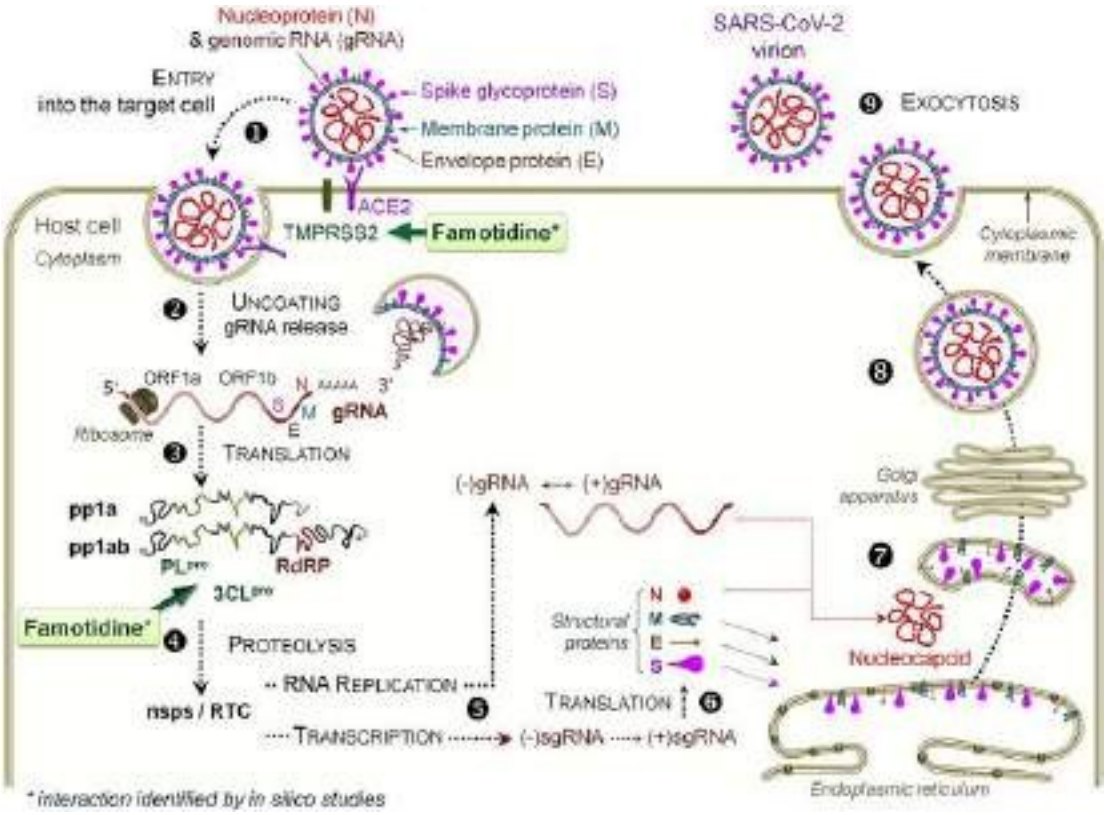
Hogan Ii RB, Hogan Iii RB, Cannon T, et al. Dual-histamine receptor blockade with cetirizine-famotidine reduces pulmonary symptoms in COVID-19 patients. Pulm Pharmacol Ther. 2020;63:101942. doi:10.1016/j.pupt.2020.101942
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7455799/

[183] Janowitz T, Gablenz E, Pattinson D, et al.
Famotidine use and quantitative symptom tracking for COVID-19 in non-hospitalized patients: a case series.
Gut. 2020;69(9):1592-1597. doi:10.1136/gutjnl-2020-321852
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7299656/

[184] Conigliaro J. Multi-site adaptive trials for COVID-19.
2020. https://clinicaltrials.gov/ct2/show/NCT04370262?type=Intr&cond=COVID&intr=famotidine+OR+cimetidine&draw=2&rank=1

[185] Freedberg DE, Conigliaro J, Wang TC, et al. Famotidine Use Is Associated With Improved Clinical Outcomes in Hospitalized COVID-19 Patients: A Propensity Score Matched Retrospective Cohort Study. Gastroenterology. 2020;159(3):1129-1131.e3. doi:10.1053/j.gastro.2020.05.053
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7242191/

[186] Ortega JT, Serrano ML, Jastrzebska B.
Class A G Protein-Coupled Receptor Antagonist Famotidine as a Therapeutic Alternative Against SARS-CoV2: An In Silico Analysis.
Biomolecules. 2020 Jun 24;10(6):954. doi: 10.3390/biom10060954.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7355875/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7673069/

PAINKILLERS AND ANTIPYRETICS

Although SARS-CoV-2 infection can be asymptomatic, the most common clinical symptoms in COVID-19 usually are fever, dry cough, fatigue, muscle pain, dyspnea, anosmia and ageusia a few days after contracting the virus.

Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, celecoxib, and indomethacin, are a group of medicines widely used for the acute (e.g., fever and pain) and chronic (e.g., rheumatoid arthritis) management of inflammatory conditions. [187]

NSAIDs are commonly used by COVID-19 patients to reduce fever and relieve muscle pain, and are more likely to be routinely used by older people (>60 years) with pre-existing conditions such as a high body mass index, increased waist circumference, or heart disease, which collectively represent risk factors for COVID-19.

Although the World Health Organization have stated that there is no evidence of an increased risk of developing COVID-19 or making the disease more severe by using NSAIDs, suggestions to avoid NSAIDs for COVID-19 patients have previously been provided based on numerous clinical trials and observations on non-COVID-19 infectious lung disease.

For example, apart from the commonly adverse effects of NSAIDs such as gastrointestinal, renal, and cardiovascular complications, NSAIDs have been found to cause more prolonged illness or complications when taken during respiratory tract infections.
It was reported that the use of NSAIDs for fever or nonrheumatologic pain during the early stages of infection increased the risk of severe bacterial superinfection. Some NSAIDs could also increase hypercoagulation and the incidence of thrombosis due to decreased thrombomodulin, a particular concern since COVID-19 patients often have coagulation abnormalities and increased vascular coagulation.
It is plausible that NSAIDs may inhibit host protective immune responses against coronavirus replication and potentiate the proinflammatory cytokine storm observed in the lungs of COVID-19 patients, for example, through

[187] Robb CT, Goepp M, Rossi AG, Yao C.
Non-steroidal anti-inflammatory drugs, prostaglandins, and COVID-19.
Br J Pharmacol. 2020;177(21):4899-4920. doi:10.1111/bph.15206

activation of inflammatory macrophages. In addition, because SARS-CoV-2 can infect human intestinal enterocytes, it is important to know whether NSAIDs synergize with SARS-CoV-2 infection to potentiate severe intestinal damage.

However, clinical studies conducted to evaluate the benefit/risk ratio of using ibuprofen or other NSAIDs found that both acute and chronic drug exposure showed no significant association with COVID-19-related mortality, and no significant difference in time to clinical improvement or length of stay was found compared with those who did not use NSAIDs. [188]

There is no evidence to date that the occasional use of an over-the-counter oral NSAID for a few days by a person with suspected or diagnosed mild COVID-19 infection will exacerbate the infection. Second, there is no reason to think that patients taking NSAIDs prescribed for a chronic painful condition should stop taking this drug for fear that it may increase their risk of contracting COVID-19 or exacerbate it if they take it.[189]

Paracetamol

Regarding the use of acetaminophen (acetaminophen, Tylenol ®, Tachipirin®), WHO initially recommended its use instead of ibuprofen, but now states that it is possible to use both. Paracetamol is recommended at a total dose of no more than 3 grams per day, unless contraindicated (e.g., liver disease).

It should be kept in mind, however, that acetaminophen (PAC) and its metabolites reduce GSH levels, even when administered at relatively low doses in healthy volunteers, and in elderly people 3 g of PAC for 14 days resulted in a significant reduction in sulfur amino acids.

It is worth mentioning that plasma levels of PAC can increase beyond expected concentrations, exacerbating thiol consumption, under conditions of gut dysbiosis, another common state in the COVID-19 risk population.
In addition, clinically achievable concentrations of PAC have been shown to reduce intracellular GSH in human lung macrophages, type II pneumocytes, and lymphocytes in vitro, and oxidized metabolites of PAC-quinon-imine form conjugates with GSH that inhibit glutathione reductase (GR), hindering the detoxification and antioxidant capacity of the GSH- GSSG cycle, and further aggravating the pro-oxidative state in the cell.

From a different toxicological perspective, PAC in the absence of adequate physiological levels of GSH may give rise to genotoxic quinon-imine metabolites, consequently in the case of severely reduced GSH levels, PAC should be administered with caution, especially in subjects with severe GSH depletion who, , again, are those at highest risk of developing a severe form of COVID-19. The quinon-imine metabolite is also the major contributor to the hepatic and renal toxicity of PAC, and 97% of acute drug-induced liver failure has been attributed to PAC.

Finally, it is important to consider that PAC has the ability to reduce fever and pain as well as NSAIDs and can mask symptoms by delaying objective classification of disease grade, but it lacks the anti-inflammatory and antiplatelet activities of NSAIDs that could be critical in containing the exacerbation of COVID-19.

Although purely anecdotal, there is extensive and cross-national evidence of patients left at home with mild symptoms for more than a week who received only PAC until their worsening condition required hospitalization and, not

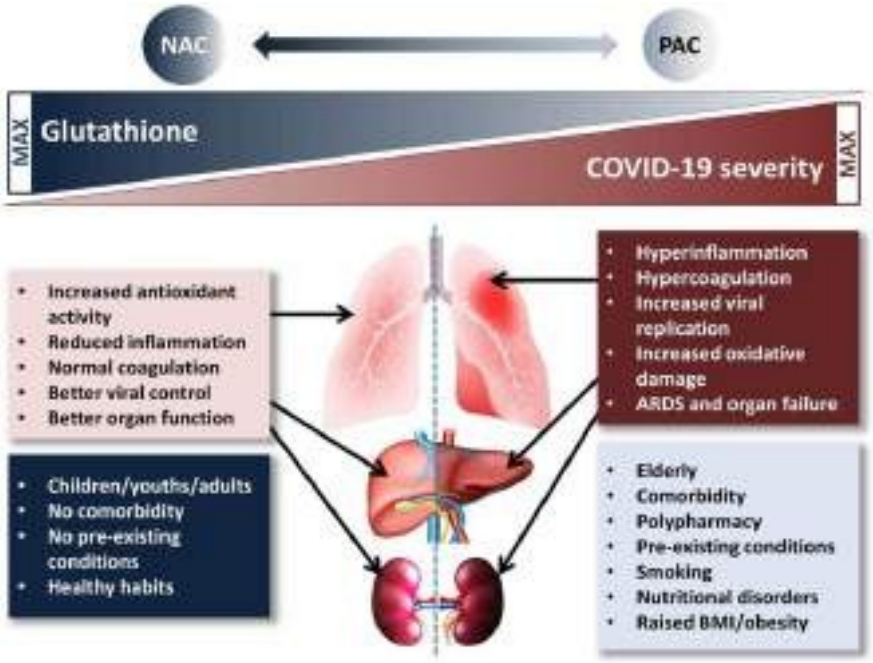
[188] Abu Esba, L.C., Alqahtani, R.A., Thomas, A. et al.
Ibuprofen and NSAID Use in COVID-19 Infected Patients Is Not Associated with Worse Outcomes: A Prospective Cohort Study.
Infect Dis Ther (2020). https://doi.org/10.1007/s40121-020-00363-w
https://link.springer.com/article/10.1007/s40121-020-00363-w

[189] Pergolizzi JV Jr, Varrassi G, Magnusson P, et al.
COVID-19 and NSAIDS: A Narrative Review of Knowns and Unknowns.
Pain Ther. 2020;9(2):353-358. doi:10.1007/s40122-020-00173-5
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7245573/

Vosu J, Britton P, Howard-Jones A, Isaacs D, Kesson A, Khatami A, Marais B, Nayda C, Outhred A.
Is the risk of ibuprofen or other non-steroidal anti-inflammatory drugs increased in COVID-19?
J Paediatr Child Health. 2020 Oct;56(10):1645-1646. doi: 10.1111/jpc.15159. Epub 2020 Aug 30.
https://onlinelibrary.wiley.com/doi/10.1111/jpc.15159

Smart L, Fawkes N, Goggin P, et al.
A narrative review of the potential pharmacological influence and safety of ibuprofen on coronavirus disease 19 (COVID-19), ACE2, and the immune system: a dichotomy of expectation and reality.
Inflammopharmacology. 2020;28(5):1141-1152. doi:10.1007/s10787-020-00745-z
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7427497/

rarely, admission to intensive care units. The routine use of PAC in at-risk groups, together with their inherently fragile conditions, may have further exacerbated the shortage of GSH, especially in Western countries where PAC consumption is particularly high. Such a situation may have made this population group even more susceptible to SARS-CoV2 at the time of its spread. To this end, a purely speculative but possible hypothesis is that the adoption of PAC may have contributed to the high virulence of COVID-19 observed in many EU countries and the United States where PAC is sold freely as an over-the-counter drug, increasing the risk of unintentional abuse and adverse effects. [190]

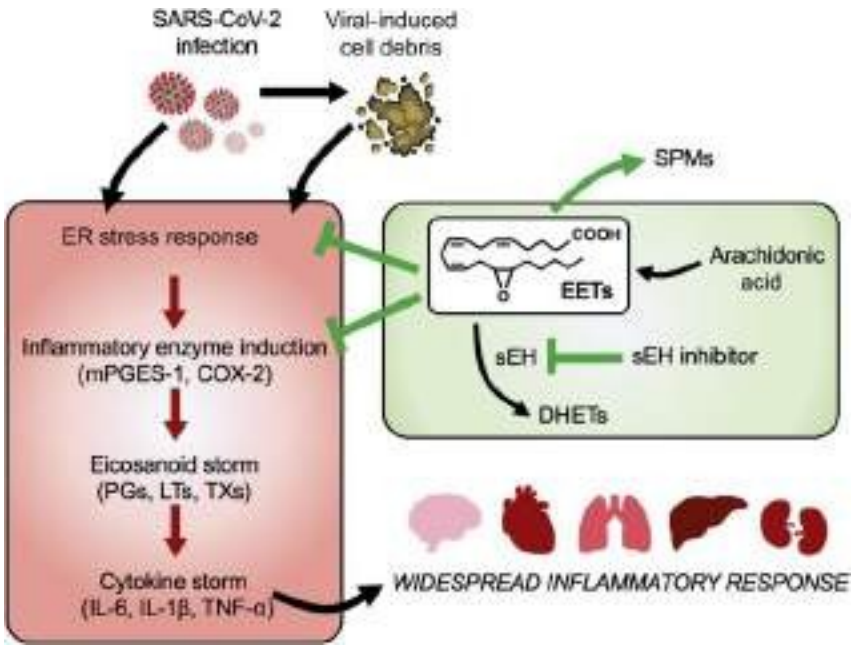


https://www.frontiersin.org/articles/10.3389/fphar.2020.579944/full
Proposed mechanism of glutathione-paracetamol-COVID-19 interactions. Low-risk population groups (blue box) have normal/high GSH levels that contribute to the beneficial effects of COVID-19 (pink box). In contrast, high-risk population groups (blue box) have low GSH levels that cannot help but modulate the deleterious events that cause (red brick box) severe COVID-19. N-acetylcysteine supplementation increases, while acetaminophen may reduce GSH availability and negatively impact lung, liver, and kidney function, especially in inherently low GSH subjects. ARDS, acute respiratory distress syndrome; BMI, body mass index; NAC, N-acetylcysteine; PAC, paracetamol.

Mechanism of action of NSAIDs

A hallmark of uncontrolled activation of the inflammatory response, such as that occurring in the cytokine storm present in critically ill patients with SARS-CoV-2 infection, is excessive production of pro-inflammatory cytokines and eicosanoids mediators of inflammation derived from lipids released from dead cell debris (eicosanoid storm), accompanied by exacerbated oxidative stress . [191]

[190] Sestili P, Fimognari C.
Paracetamol-Induced Glutathione Consumption: Is There a Link With Severe COVID-19 Illness?
Front Pharmacol. 2020;11:579944. Published 2020 Oct 7. doi:10.3389/fphar.2020.579944
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7577213/
[191] Hammock BD, Wang W, Gilligan MM, Panigrahy D.
Eicosanoids: The Overlooked Storm in Coronavirus Disease 2019 (COVID-19)?
Am J Pathol. 2020;190(9):1782-1788. doi:10.1016/j.ajpath.2020.06.010
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7340586/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7340586/
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection leads to severe tissue damage, which releases cellular debris. Both the primary infection and the accumulation of cellular debris initiate the endoplasmic reticulum (ER) stress response and up-regulate inflammatory enzymes, including microsomal prostaglandin E synthase-1 (mPGES-1) and prostaglandin-endoperoxide synthase 2 [cyclooxygenase 2 (COX-2)], which subsequently produce eicosanoids, including prostaglandins (PGs), leukotrienes (LTs) and thromboxanes (TXs). These proinflammatory lipid autacoids induce cytokine storms that mediate widespread inflammatory responses and organ damage in patients with severe coronavirus 2019 (COVID-19). In contrast, epoxycosatrienoic acids (EETs), which are stabilized by inhibition of their metabolizing enzyme, soluble epoxide hydrolase (sEH), are anti-inflammatory and prorisolvent mediators that promote cessation (resolution) of inflammation by suppressing ER stress response, inflammatory enzyme induction, and proinflammatory cytokine production. EETs also shift arachidonic acid metabolism to promote the production of specialized prorisolvent mediators (SPMs), which initiate downstream anti-inflammatory and prorisolvent programs. EETs and sEH inhibitors can counterregulate the unabated systemic inflammatory response and organ failure associated with COVID-19 infection. DHET, dihydroxyicosatrienoic acid; TNF-α, tumor necrosis factor-α.

NSAIDs act by reducing inflammation through blocking cyclooxygenases (COX, i.e., COX-1 and COX-2) and by inhibiting the biosynthesis of prostaglandins (PGs), a group of important lipid mediators that are formed when arachidonic acid (AA) is released from cell membrane phospholipids by the action of cytosolic PLA2 (cPLA2) and converted to PGH2 by COXs. [192]

Inhibition of prostaglandin expression induces vasoconstriction, altered blood flow, and activation of the "renin-angiotensin" system, which can lead to increased expression of ACE2 in the lungs, kidneys, and intestines.

It was hypothesized that this overregulation of ACE2 by **ibuprofen** could increase individual susceptibility to SARS-CoV-2 infections.
However, as seen above, this hypothesis has not been confirmed to date, and further studies are needed to better evaluate the adverse effects of ibuprofen and other anti-inflammatory drugs in the same therapeutic class.
Aspirin (ASA, acetylsalicylic acid) has a different mechanism of action than ibuprofen and other nonselective nonsteroidal anti-inflammatory drugs and is 170 times more potent in inhibiting COX-1 than COX-2. In addition to COX-1 inactivation, ASA promotes the conversion of ARA to 15-HETE [15-hydroxyicosatetraenoic acid] via the COX-2 pathway, decreasing prostaglandin concentrations, increasing leukotrienes and lipoxins, and promoting the resolution of inflammation.[193]

[192] Micallef J, Soeiro T, Jonville-Béra AP; French Society of Pharmacology, Therapeutics (SFPT).
Non-steroidal anti-inflammatory drugs, pharmacology, and COVID-19 infection.
Therapie. 2020;75(4):355-362. doi:10.1016/j.therap.2020.05.003
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7204680/

[193] Reference for bibliography given in superscript in red brackets Rogero
MM, Leão MC, Santana TM, et al.
Potential benefits and risks of omega-3 fatty acids supplementation to patients with COVID-19.
Free Radic Biol Med. 2020;156:190-199. doi:10.1016/j.freeradbiomed.2020.07.005
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7350587/

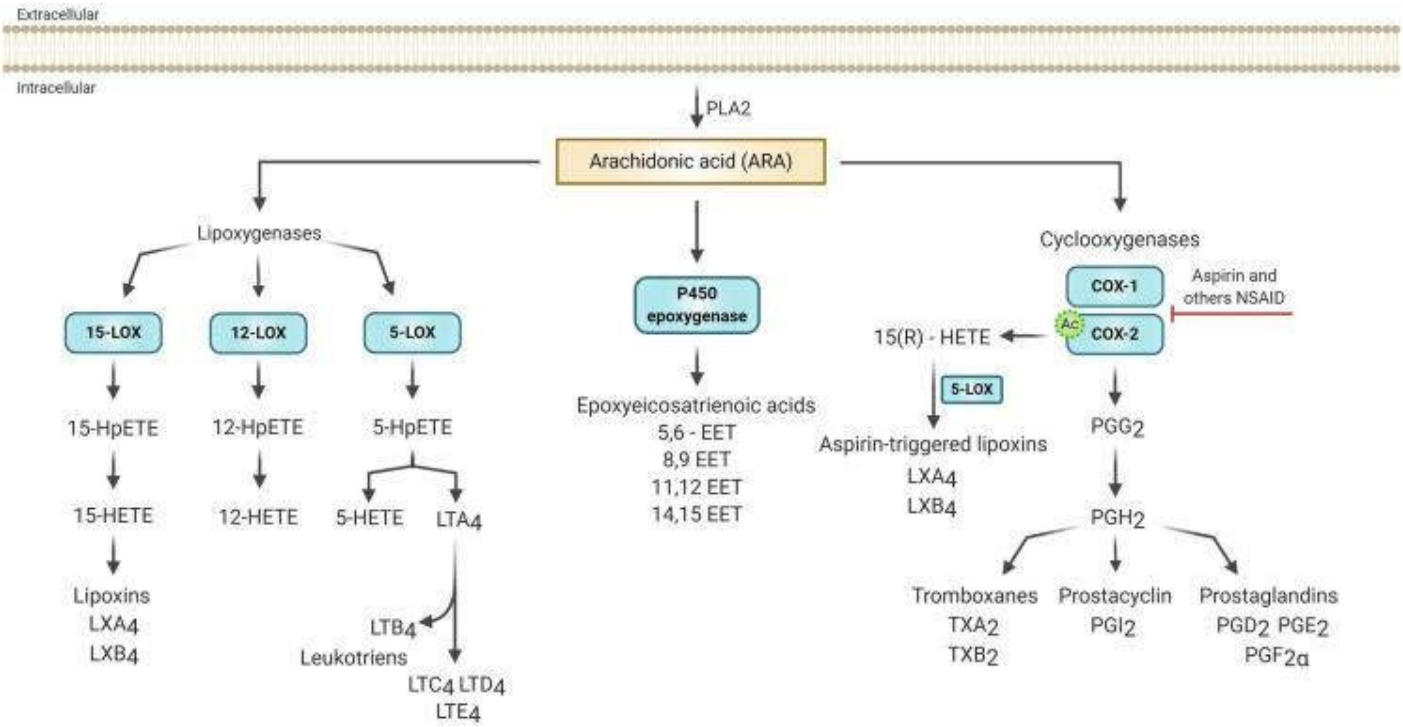
Panigrahy D, Gilligan MM, Huang S, et al.
Inflammation resolution: a dual-pronged approach to averting cytokine storms in COVID-19?
Cancer Metastasis Rev. 2020;39(2):337-340. doi:10.1007/s10555-020-09889-4
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7207990/

Long-chain unsaturated fatty acids

Human and animal studies have investigated the effect of EPA (eicosapentanoic acid) and DHA (acidodocohexanoic acid) in ALI (acute lung injury) and ARDS (acute respiratory distress syndrome), which is characteristic in patients with severe SARS-CoV-2.
In general, these studies have shown favorable results on multiple inflammatory, respiratory, and clinical outcomes.

A meta-analysis conducted by Pontes-Arruda et al.[86] reported a significant reduction in ventilator-free days, organ failure, length of stay in the intensive care unit, and mortality in individuals with ARDS. Equally, a recent Cochrane review showed that patients with ARDS receiving EPA and DHA supplementation had significantly improved blood oxygenation and reduced ventilator requirements, organ failures, ICU length of stay, and 28-day mortality[87] .

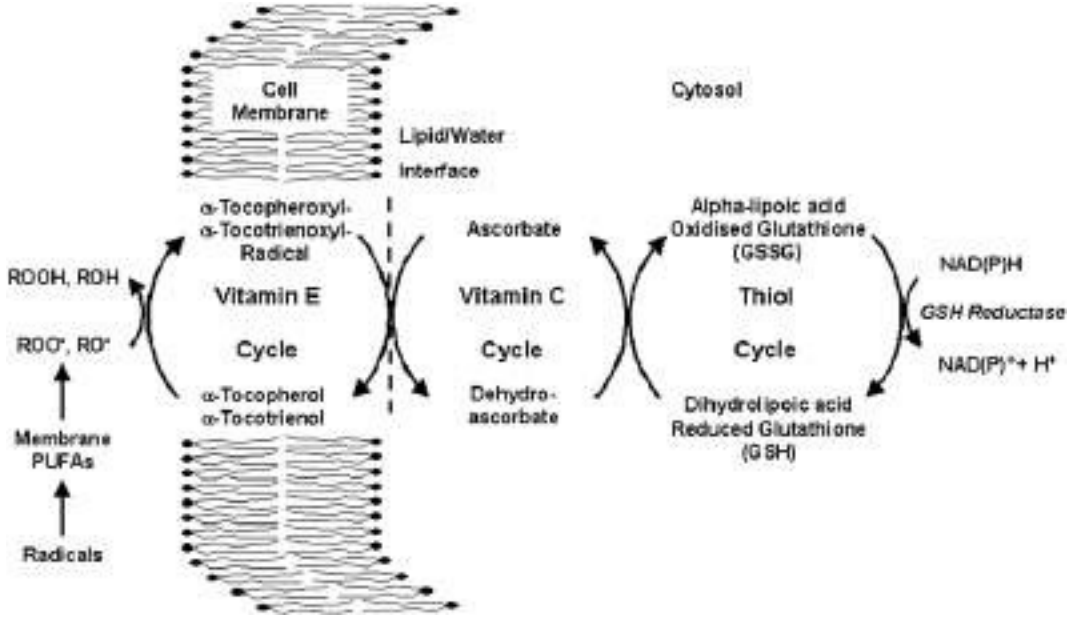
Therefore, supplementation with EPA and DHA could have important implications in critically ill patients with COVID-19.
Recently, several reviews have corroborated this idea [58,88], [89], [90], [91], [92]] .
These studies mainly focused on the anti-inflammatory properties of EPA and DHA, suggesting that the less inflammatory lipid mediators produced by these compounds together with SPMs (specialized pro-responsive mediators: resolvins, protectins and marensins) derived from EPA and DHA could help in managing the cytokine storm by ameliorating inflammation and lung damage[58],[87],[88] . In addition, both SPMs and EETs have also been shown to attenuate pathological thrombosis and promote clot clearance, which is now recognized as a key pathology of COVID-19 infection. 193



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7350587/
Biosynthesis of eicosanoids from arachidonic acid [ARA]. In response to various stimuli, ARA is released from cell membranes by phospholipase A2 [PLA2]. Free ARA can be metabolized to eicosanoids via the P-450 epoxygenase, cyclooxygenase [COX 1 and 2] or lipoxygenase [5-LOX, 12-LOX and 15- LOX] pathways. In epoxygenase P-450, ARA is metabolized to epoxycosatrienoic acids [EET]. COX enzymes catalyze the conversion of ARA to prostaglandin intermediate G2 [PGG2] and then to prostaglandin H2 [PGH2]. PGH2 serves as a substrate for the generation of biologically active products such as prostaglandins [PGD2, PGE2, PGF2α], thromboxanes [TXA2 and TXB2] and prostacyclin [PGI2], together these metabolites are called prostanoids. In the presence of aspirin, COX-2 is acetylated [indicated by 'Ac'], which increases COX-2-catalyzed formation of 15-R-hydroxyicosatetraenoic acid [15R -HETE] that can be converted by 5- LOX into the so-called aspirin-activated lipoxins [LXA4 and LXB4]. LOX first converts ARA into the respective hydroperoxy-eicosatetraenoic acids [5, 12 and 15 -HpETEs] to produce the corresponding hydroxy-eicosatetraenoic acid [5, 12 and 15 -HETEs]. 5-HpETEs is also further metabolized to form leukotriene [LT] A4 from 5-LOX. LTA4 is subsequently converted to LTB4, LTC4, LTD4 and LTE4. 15-HETE leads to the formation of lipoxins by 15-LOX.

However, it should be considered that hypoxemia caused by pneumonia reduces the energy supply of cellular metabolism, increases anaerobic fermentation, intracellular acidosis, and reactive oxygen species (ROS), and leads to the destruction of the phospholipid layer of the cell membrane.
Higher ROS levels followed by depletion of antioxidant defenses lead to the development of oxidative stress, chronic activation of immune responses, and inflammation implicated in tissue damage.

Therefore, considering the increased release of ROS during the cytokine storm due to SARS-CoV-2 infection, the increased unsaturated fatty acid esters of phospholipids, glycerol, and cholesterol should be investigated for increased susceptibility to nonenzymatic oxidation.
For this reason, it is recommended that supplementation with EPA and DHA be accompanied by antioxidants, such as vitamin C and E.[194]



https://www.nature.com/articles/1602255

OZONOTHERAPY: COVID-19 patient treatment proposal.

Dr. Vincenzo Simonetti
Executive Member
SIOOT
Email: simonetti.vinc@gmail.com

Importantly, the SIOOT (INTERNATIONAL SOCIETY OF OXYGEN OZONOTHERAPY, www.ossigenoozono.it), has published results of the use of ozone therapy on COVID-19 patients treated in 15 Italian hospitals, with a significantly better clinical course than the untreated.
Patients treated simultaneously with drugs and ozone had an even more favorable course and a clear reduction in side effects seen in patients treated with drugs alone.[195]

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[194] van Meeteren, M., Teunissen, C., Dijkstra, C. et al.
Antioxidants and polyunsaturated fatty acids in multiple sclerosis.
Eur J Clin Nutr 59, 1347-1361 (2005). https://doi.org/10.1038/sj.ejcn.1602255

[195] Valdenassi L, Franzini M, Ricevuti G, Rinaldi L, Galoforo AC, Tirelli U.
Potential mechanisms by which the oxygen-ozone (O2-O3) therapy could contribute to the treatment against the coronavirus COVID-19.
Eur Rev Med Pharmacol Sci. 2020 Apr;24(8):4059-4061. doi: 10.26355/eurrev_202004_20976. PMID: 32374009.
https://www.europeanreview.org/article/20976.

Franzini M, Valdenassi L, Ricevuti G, et al.
Oxygen-ozone (O2-O3) immunoceutical therapy for patients with COVID-19. Preliminary evidence reported. Int Immunopharmacol. 2020;88:106879.
doi:10.1016/j.intimp.2020.106879
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7414302/

Menendez-Cepero S, Marques-Magallanes-Regojo JA, Hernandez-Martinez A, Hidalgo Tallón FJ, Baeza-Noci J
Therapeutic Effects of Ozone Therapy that Justifies Its Use for the Treatment of COVID-19.
J Neurol Neurocrit Care Volume 3(1): 1-6. (2020)
https://clinalgia.com/JNNC-3-304_AC.pdf

Gavazza A, Marchegiani A, Rossi G, et al.

Ozone simultaneously performs the following functions:

- **Reduces tissue acidity, hypoxia and inflammation;**
- **Improves microcirculation and oxidative phosphorylation;**
- **Increases the production of ATP;**
- **is a powerful Antibiotic and Virustatic (so far no resistant virus or bacteria have been found).**
- Ozone, by oxidizing Spike liporotheins, inhibits ACE2 receptor contact and endocellular penetration of the virus.
- It also activates the NrF2 transcription factor, which regulates the antioxidant response and increases, through mitochondrial oxidative phosphorylation, ATP production.
- It also inhibits NF-KB factor p65 and reduces proinflammatory cytokines, including Th17 and the metalloproteases MMP2 and MMP9, thus reducing endothelial inflammation.
- It increases the production of erythrocyte Di-phosphoglycerate and the dissociation curve between Hb and oxygen; it has a hemorheological effect and increases the negative charge of red blood cells, not forgetting the important antiplatelet effect that reduces microthrombi.
- It increases oxidative phosphorylation, activation of the mitochondrial antioxidant system, and ATP production.
- Reduces mitochondrial outer membrane glycolysis and extracellular acidity.

Ozone therapy, therefore, can have favorable effects on all the intended goals; but functional recovery will be inversely proportional to the anatomical damage sustained.
As already pointed out, the reducing action performed by ozone therapy with regard to inflammation, thrombi, hypoxia, asthenia, and pain is decisive.

Ozone therapy can be practiced in the form of:

1) GAEI: large ozonated auto hemoinfusion. 200 ml of blood is taken in special San $_{O3}$ bag and mixed with ozone at the concentration of 45-50µgr/ml. Then follows the phase of slow reinfusion into the same vein. Reinfuse very slowly. If the patient should experience sensation of heat, cold, tachycardia or bradycardia: just slow the reinfusion rate further and these complaints will disappear. If the patient is in the acute phase we recommend: 1 daily session for 2-4 days and then 2-3 sessions per week for 2-3 weeks. Cautions for: hyperthyroidism, favism and hypoglycemia.

2) Rectal insufflation: 180 ml of ozone at a concentration of 35µgr/ml through a Nelaton #10 catheter introduced up to the rectal ampulla. Insufflate slowly; should the patient experience urge to evacuate or pain, simply pause a few minutes, without removing the catheter, and give a small abdominal massage to eliminate any discomfort. Rectal insufflation, almost as effective as GAEI, is particularly useful in cases of difficult venous access.

3) Patients with chronic or degenerative diseases will be able to reduce their risk of infection with periodic ozone therapies. All of our patients who periodically undergo ozone therapy even for years report to us that they have become more resistant to flu epidemics.

"Home ozone therapy, practiced by USCA (Special Continuing Care Units) or other appropriately trained and equipped medical teams, could significantly reduce the recovery time and need for hospitalization of symptomatic patients."

4) We consider the selection of the most suitable materials to produce the required amounts and concentrations of ozone for medical therapy to be important; they should meet the following unavoidable physical characteristics:

- Length of ozonation tubes. The longer the tubes are, if the electrical power they receive is adequate, the more suitable they are for generate low and high ozone concentrations, which depend on the oxygen flow velocity.
- For example, a 10-cm tube, even if it were hit by high electrical powers, could not produce high concentrations of ozone because of the short oxygen path; one would have to lower the oxygen flow so much that it would be unsuitable to be drawn for practicing medical therapy.
- The production of high voltages (6,000 to 18,000 volts) involves the use of transformers with a minimum weight of 2.5 kg (optimal for therapy, as it can generate high concentrations even at medium-high flows)

Ozone Therapy as a Possible Option in COVID-19 Management.
Front Public Health. 2020;8:417. Published 2020 Aug 25. doi:10.3389/fpubh.2020.00417
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7477102/

Regarding the application for both home and hospital therapeutic purposes of ozone therapy for critical COVID-19 patients we Refer to Dr. Simonetti's in-depth discussion:

OZONE: ANTIBIOTIC-VIRUSTATIC THAT DOES NOT INDUCE RESISTANCE OR ALLERGY AND LEAVES NO TRACE IN THE ENVIRONMENT

The document was approved by the working group of physicians collaborating with FRI

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