Hydroxychloroquine and COVID-19

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ABSTRACT
Hydroxychloroquine and chloroquine are medications that have been used for a long time. Their most common use is for the treatment and prophylaxis of malaria. However, these antimalarial drugs are known to also have anti-inflammatory and antiviral effects and are used for several chronic diseases such as systemic lupus erythematosus with low adverse effects. The antiviral action of hydroxychloroquine and chloroquine has been a point of interest to different researchers due to its mechanism of action. Several in vitro studies have proven their effectiveness on severe acute respiratory syndrome virus and currently both in vitro and in vivo studies have been conducted on 2019 novel coronavirus (COVID-19). The purpose of this article is to review the history and mechanism of actions of these drugs and the potential use they can have on the current COVID-19 pandemic.

INTRODUCTION
Antimalarial drugs such as chloroquine and hydroxychloroquine have been in the market for over a century. These drugs have been used not only for malaria but also for several rheumatic diseases given their anti-inflammatory properties, affordability and the fact that they have shown a good safety profile. Knowing that these drugs have proven effective for a wide range of diseases has made several researchers wonder about their use in other areas such as cancer and viral infections. This is why, in the midst of a global pandemic, the question of antimalarial use in treatment and prophylaxis of COVID-19 has been raised.

HISTORICAL USES AND SIDE EFFECTS
Uses of Cinchona bark for a 'febrile disease' have been reported since 1630 in Lima, Peru. In 1894, Dr J.F. Payne was the first one to administer it at St. Thomas Hospital in London for something other than malaria. He used it in the treatment of a rash that we now can infer was a lupus rash. Chloroquine was discovered in 1939 by the Germans. It substituted quinacrine by the end of World War II for the treatment of malaria proving to be safer and more effective. During the war, soldiers were given prophylactic antimalarial drugs which incidentally improved lupus erythematosus rashes and arthritis. Hydroxychloroquine was introduced to the market in 1955, differing from chloroquine by a hydroxyl group that makes it safer. These drugs have also proven to be effective in other inflammatory diseases such as rheumatoid arthritis, and in 1989, Geser et al described the decreased incidence of Burkitt lymphoma in patients treated for malaria prophylactically with chloroquine. These different uses are summarised by Ben Zvi et al.

Hydroxychloroquine and chloroquine are relatively safe drugs. Their most common side effects include gastrointestinal symptoms, pruritus and dermatological changes that can occur in up to 10% of the patients. The most severe side effects have low incidence. They include neuromyopathy of proximal muscles, cardiotoxicity and irreversible retinopathy. The latter is well documented in long-term users with high doses. This can be prevented or controlled with dose calculation based on body weight, reducing the dose after 5 years of use and routine ophthalmologic evaluation. On the other hand, the neuromuscular changes can slowly improve by discontinuing the medication. The cardiotoxicity may include QT prolongation syndrome, especially in patients with renal or hepatic dysfunction. Overdose toxicity has also been observed between 1 and 3 hours after a high-dose intake, especially with chloroquine. Toxic effects have occurred when dosing at 20 mg/kg and have been fatal at over 30 mg/kg. The overdose presents with visual changes, nausea, hypokalaemia, drowsiness, shock, convulsions and even death. Diazepam has been used as an antidote. Hydroxychloroquine overdose is rare and the dose has not been well established.

ANTI-INFLAMMATORY EFFECT
The effect of hydroxychloroquine and chloroquine on the immune system has been well established. Several of their anti-inflammatory mechanisms have been recognised, for example, interference with lysosomal acidification and antigen presentation, inhibition of phospholipase A2, absorption and blocking UV light cutaneous reactions, binding and stabilising DNA, inhibition of toll-like receptor signals, inhibition of T and B cell receptors, and especially, decreasing cytokine production by macrophages such as interleukin (IL)-1 and IL-6. Interaction with toll-like receptors and T cell receptors makes hydroxychloroquine effective in different sites of the signalling cascade in the inflammatory response. These interactions prevent autoimmunity without immunosuppressing the patient. Sperber et al also demonstrated the inhibition of IL-1 and IL-6 in T cells and monocytes and several other studies have demonstrated tumour necrosis factor (TNF)-alpha inhibition or attenuated inflammatory effect by hydroxychloroquine. The effective inhibition of inflammatory cytokines such as IL-6, IL-1 and TNF-alpha decreases tissue damage and endothelial inflammation thus preventing initiation and propagation of autoimmune inflammation. This cytokine inhibition is of great importance at this time, since it has been demonstrated that several viruses upregulate the expression of IL-1, IL-6 and TNF-
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<th>Name</th>
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| Randomized Controlled Clinical Trials of Lopinavir/Ritonavir or Hydroxychloroquine in Patients With Mild Coronavirus Disease (COVID-19) | NCT04307693    | Sung-Han Kim, Asan Medical Center, Seoul, South Korea          | 1. Multicentre, open-labelled, randomised clinical trial  
2. Viral load                                                            | Lopinavir/ritonavir 200 mg/100 mg two tablets by mouth, every 12 hours for 7–10 days | Hydroxychloroquine 200 mg two tablets by mouth, every 12 hours for 7–10 days     | No lopinavir/ritonavir and hydroxychloroquine                                    |
| Hydroxychloroquine Post Exposure Prophylaxis for Coronavirus Disease (COVID-19) | NCT04318444    | Elizabeth Oelsner, Columbia University, NY, USA                | Parallel assignment (randomised)                                                | Hydroxychloroquine two tablets (400 mg) twice daily on day 1; for days 2–5, they will be instructed to take one tablet (200 mg) twice daily | Two tablets (400 mg) twice daily on day 1; for days 2–5, they will be instructed to take one tablet (200 mg) twice daily | Two tablets (400 mg) twice daily on day 1; for days 2–5, they will be instructed to take one tablet (200 mg) twice daily |
| Hydroxychloroquine Chemoprophylaxis in Healthcare Personnel in Contact With COVID-19 Patients (PHYDRA Trial) (PHYDRA) | NCT04318015    | National Institute of Respiratory Diseases, Mexico City, Mexico | 1. Triple blinded, randomised controlled trial  
2. Symptomatic covid-19 infection rate                                     | 1. High risk: hydroxychloroquine 200 mg per day for 60 days  
2. Low risk: hydroxychloroquine 200 mg per day for 60 days | Chloroquine (400 mg 2 times per day, 12/12 hours)+azithromycin (500 mg once a day) | 1. High risk: placebo tablet per day for 60 days  
2. Low risk: placebo tablet per day for 60 days |
| Chloroquine Prevention of Coronavirus Disease (COVID-19) in the Healthcare Setting (COPCOV) | NCT04303507    | Oxford University, Oxford, UK                                  | 1. Parallel assignment  
2. Number of symptomatic covid-19 infections                                   | Chloroquine A loading dose of 10 mg base/kg followed by 150 mg daily (250 mg chloroquine phosphate salt) will be taken for 3 months | Placebo                                                                          | Chloroquine (400 mg 2 times per day, 12/12 hours)+azithromycin (500 mg once a day) |
| Safety and Efficacy of Hydroxychloroquine Associated With Azithromycin in SARS CoV-2 Virus (Alliance COVID-19 Brasil II) | NCT04321278    | Hospital Israelita Albert Einstein, Sao Paulo, Brazil          | 1. Parallel assignment  
2. Evaluation of clinical status                                                  | Hydroxychloroquine (400 mg 2 times per day, 12/12 hours)+azithromycin (500 mg once a day) | Hydroxychloroquine (400 mg 2 times per day, 12/12 hours)                         | Placebo                                                                          |
| Post-exposure Prophylaxis for SARS-Coronavirus-2                       | NCT04308668    | University of Minnesota, Minnesota, USA                        | 1. Parallel assignment  
2. Incidence of disease                                                                 | Hydroxychloroquine 200 mg tablet; 800 mg orally once, followed in 6–8 hours by 600 mg, then 600 mg once a day for four consecutive days | Placebo                                                                          | Post-exposure Prophylaxis for SARS-Coronavirus-2                                  |

Table 1: Ongoing clinical trials for Hydroxychloroquine on COVID-19.
alpha in vitro, suggesting the effectiveness of hydroxychloroquine in viral infections.

**INFLAMMATORY RESPONSE IN VIRAL INFECTIONS**

The role of inflammatory cytokines in viral disease is not well understood; nevertheless, numerous hypotheses have been proposed to describe the viral inflammatory response. It is believed that IL-6 can have both inflammatory and anti-inflammatory effect in the body depending on which signal is activated: IL-6 trans signalling is inflammatory while regular IL-6 signalling is anti-inflammatory. However, several studies point to negative implications of IL-6 involvement in the immune response to viral infections. Three mechanisms have been suggested: (1) in vitro secretion of IL-6 by toll-like receptor activation causes an inhibition of CD8 T cell response, which is confirmed by an in vivo blockage of IL-6 with monoclonal antibodies. This blockage causes reduction in viral loads and increases production of interferon (IFN)-gamma; (2) the synergistic interaction of IL-6 and IL-17 generates persistence of viral infection and worsens clinical outcomes; and (3) it is suggested that IL-6 upregulates PD-1 and PDL-1. Normally PD-1 and PDL-1 interaction prevents autoimmunity through programmed cell death. However, during a viral infection, it alters the immune response against the virus reducing CD8+ cytolytic function that is worsen by the upregulation from infection, it alters the immune response against the virus reducing CD8+ cytolytic function that is worsen by the upregulation from infection.

**HYDROXYCHLOROQUINE/CHLOROQUINE ANTIVIRAL EFFECT**

The effect of hydroxychloroquine and chloroquine on viral replication goes beyond cytokine inhibition. These medications are weak bases that can affect acid vesicles and inhibit several enzymes. This characteristic allows them to inhibit the viral entry to the cell when the endocytosis is pH dependent. It also inhibits glycosyl-transferases, viral post-translational modifications and replication of some viral families. The antiretroviral effect has been considered to be caused by the inhibition of viral glycosylation, a major antiviral mechanism of these drugs. It was also recently described that chloroquine might inhibit quinone reductase-2, an enzyme involved in sialic acid biosynthesis. If this were to be true, it would explain further its effect on HIV, SARS and orthomyxoviruses because sialic acid is present on HIV-1 glycoproteins, SARS angiotensin-converting enzyme 2 (ACE2 receptor and orthomyxovirus receptors).

Severe acute respiratory syndrome (SARS), better known as SARS-CoV, was a novel coronavirus that emerged in China in November 2002. It rapidly spread to more than 30 countries, causing 8096 cases and 774 deaths. The syndrome is described with fever, chills, malaise, cough and dyspnoea that can progress to respiratory failure. Savarino et al were the first ones to hypothesise that hydroxychloroquine and chloroquine might be helpful in treatment of SARS, since endocytosis might be involved in viral entry to the cell and there was an important immune response that was causing clinical worsening, probably due to inflammatory cytokines such as TNF-alpha and IL-6. This was later confirmed by two different in vitro studies. Kayeets et al demonstrated the inhibition of SARS-CoV by chloroquine in Vero E6 cells at different postinfection times. Vincent et al proved the effective dose-dependent inhibition of the virus in Vero E6 cells immediately after viral absorption and also 3–5 hours after absorption. They also showed that pretreated cells with chloroquine were refractory to the virus and revealed an impairment of terminal glycosylation of ACE2 receptor, decreasing viral-receptor affinity and therefore reducing the initiation of the infection. This experiment can illustrate the possibility of using hydroxychloroquine for coronavirus prophylaxis the same way as in malaria.

**THE CURRENT CORONAVIRUS OUTBREAK**

In December 2019, a coronavirus outbreak started in Wuhan, China. The viral infection presented with high fever, dry cough, headache, dyspnoea and diarrhoea. The first cases that showed these symptoms were all associated with a food market in the city of Wuhan. Since then, there have been several cases that progressed to respiratory failure, with an estimated mortality rate of 2%. According to the WHO, to this date, 23 March 2020, there are 332 935 confirmed cases, 14 510 deaths and 190 affected countries. In order to understand this outbreak, the genetic sequencing of the virus has been extracted and is now known to share 79% of its genome with SARS-CoV from the Coronavirus family. However, it is sufficiently different from it to be considered a novel virus. The WHO renamed it covid-19 (SARS-CoV-2) in February 2020 and declared it a Pandemic. It has also been demonstrated that this novel virus not only shares sequencing with SARS-CoV but also uses the same cell entry ACE2 receptor. Even though the novel virus shares genomic sequencing with SARS-CoV, data show that their shedding patterns in infected patients differ substantially, making covid-19’s more similar to the pattern of influenza virus. Furthermore, the viral load of covid-19 has been similar in asymptomatic patients and symptomatic patients, thus, making the virus transmissible in early stages of the infection. On the contrary, transmission of SARS-CoV occurred within days of initial symptoms, making the epidemiological strategies different for the two infections.

**USES OF HYDROXYCHLOROQUINE AND CHLOROQUINE IN COVID-19 (SARS-CoV-2)**

The worldwide effects of the SARS-CoV-2 pandemic has been unparalleled and it has prompted the scientific community to consider all possible solutions. Due to covid-19 similarities to SARS-CoV, several researchers have proposed the use of hydroxychloroquine and chloroquine on the novel virus. Wang et al tested the effect of several Food and Drug Administration-approved antiviral drugs on the virus in vitro. Remdesivir showed post entry blockage of viral infection with an effective concentration at 50% of EC50=0.77 µM and a cytotoxic concentration of 50% of CC50>100 µM. Chloroquine was found to have an effective concentration at 50% of EC50=1.13 µM, a cytotoxic concentration at 50% of CC50>100 µM and an effective concentration at 90% EC90 of 6.90 µM. Chloroquine showed effectiveness at an entry and post entry level, while remdesivir was only effective at a post entry level. This further suggests the possible prophylactic use of chloroquine on SARS-CoV-2. Yao et al also tested the effect of hydroxychloroquine and chloroquine in vitro. They divided the experiment into two phases: treatment study and prophylaxis study. In the treatment study, the EC50 values for chloroquine were 23.90 and 5.47 µM at 24 and 48 hours, respectively, and the EC50 values for hydroxychloroquine were 6.14 and 0.72 µM at 24 and 48 hours, respectively. In the prophylaxis study, the EC50 values for chloroquine were >100 and 18.01 µM at 24 and 48 hours, respectively, and the EC50 values for hydroxychloroquine were 6.25 and 5.85 µM at 24 and 48 hours, respectively. They concluded that hydroxychloroquine is more effective in vitro than chloroquine for both prophylaxis and treatment.
The promising results of the in vitro experiments have led to the creation of multiple clinical trials to further investigate the chloroquine effect on covid-19. Currently, there are two trials with available data. Gao et al demonstrated the superiority of chloroquine over the control treatment in more than 100 patients regarding inhibition of pneumonia exacerbation, improvement in lung imaging findings, promoting a virus negative conversion and shortening the disease course in more than 10 hospitals in China. Gautret et al treated 20 patients with hydroxychloroquine and compared the results with 16 controls in France. They used PCR to measure the viral load on day 3, 4, 5 and 6 of postinclusion. The treatment group had a higher age mean, but no difference in gender was made between the two groups. Asymptomatic patients and patients with both lower and upper respiratory tract infections were treated. They concluded that hydroxychloroquine was effective in viral load reduction. The results on day 3 indicated that 50% of the hydroxychloroquine-treated patients had a viral load reduction with a p=0.005; on day 4, it showed a 60% reduction with a p=0.04; on day 5, a 65% reduction with a p=0.006; and on day 6, 70% of the patients showed viral load reduction with a p=0.001. Furthermore, they described the synergistic effect of azithromycin when using it alongside hydroxychloroquine in decreasing the viral load. The dual treatment showed 100% decrease on the viral load with a p<0.001 by day 6, while hydroxychloroquine alone showed a 70% decrease.

ONGOING STUDIES AND GUIDELINES
Several randomised clinical trials regarding antimalarial drug use in covid-19 have been proposed. On the US National Institute of Health’s (NIH) medical library, there are already 143 trials on covid-19 registered from all over the world and 14 of them are specifically about the use of antimalarial drugs on the virus. These trials aim to demonstrate the effectiveness of hydroxychloroquine and chloroquine in pre-exposure and postexposure prophylaxis and treatment. Table 1 enlists six of these studies, but further information can be found at the NIH web page. Moreover, on the Chinese clinical trial entry there are over 500 studies enrolled and 16 of them are about hydroxychloroquine and chloroquine use on covid-19. This information can be found on their web page. Multiple institutions worldwide are already using hydroxychloroquine in their treatment guidelines, including the Centers for Disease Control and Prevention (CDC), pointing to the relevance of this drug in the current pandemic.

CONCLUSION
In the midst of a pandemic with a high mortality rate, the collapsing of health systems and the devastating effects on the economy worldwide, a fast solution is crucial. Preventive medicine is key in any pandemic. Preventing cases rather than treating them would decrease world morbimortality. Vaccinations have been the centre of prophylactic measures on infectious diseases. However, because of the novelty of the covid-19 virus, the undetermined immune reaction and its rapid spread, the creation of a successful vaccine for this virus in the short term is uncertain. Current measures such as self-quarantine and social distancing are recommended by institutions like the WHO and the CDC of Atlanta.

Covid-19 pandemic presents the classic conflict between the clinical bedside medicine and academic medicine. While clinical bedside medicine incorporates evidence-based medicine, it also allows for prescription of the therapies which may not be based on the most rigorous clinical evidence, but based on biological plausibility, in vitro preclinical data or limited biological plausibility and a high degree of biologic plausibility of an antiviral mechanism. In vitro data showed a synergistic effect of azithromycin and hydroxychloroquine when using it alongside hydroxychloroquine in decreasing the viral load. The dual treatment showed 100% decrease on the viral load with a p<0.001 by day 6, while hydroxychloroquine alone showed a 70% decrease.

Main messages
- Hydroxychloroquine has shown several antiviral mechanisms, including the inhibition of inflammatory cytokines such as IL-1, IL-6 and TNF-alpha.
- The effect of hydroxychloroquine on SARS-CoV-2 (covid-19) has been studied in vitro, demonstrating its pre-entry result, probably due to the inhibition of the virus ACE2 receptor and the viral inhibition post-entry. Also, in vivo studies have demonstrated clinical improvement and decrease in the viral load.
- Several double-blinded, randomised, placebo-controlled clinical trials are being conducted to further investigate the effects of hydroxychloroquine on covid-19.

Current research questions
- Should hydroxychloroquine be used as primary prophylaxis on covid-19?
- Can hydroxychloroquine be used as post-exposure prophylaxis on covid-19?
- Is hydroxychloroquine better than placebo in the treatment of covid-19?
- Is hydroxychloroquine better than other antiviral drugs such as ritonavir or lopinavir in the treatment of covid-19?

Key references
clinical evidence. In a setting like this, many hospitals have already adopted hydroxychloroquine-based therapies for covid-19 under the auspices of clinical protocols, the key ingredient of the clinical protocols being a mechanism to collect data prospectively so that we can learn as much as possible about the safety and efficacy of hydroxychloroquine therapy. In order to obtain the highest grade of evidence, double-blinded, randomised, placebo-controlled trials have also been started by a few other groups to determine the effectiveness of hydroxychloroquine on covid-19. The authors of this review article do not make any strong recommendations about which one of the above pathways to take. Well-evaluated safety profile, encouraging but limited in vitro and in vivo data supporting benefit, low cost and easy availability, makes a clinical protocol pathway an understandable one for this novel disease with high mortality. We encourage the ongoing investigation on this area to have a clearer guideline to prevent and treat this disease.

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REFERENCES


For more details, please refer to the full text of the article.


Answers

1. A (False), B (True), C (True), D (False)

2. A (False), B (True), C (True), D (False), E (True), F (False), H (True)

3. A (True), B (False), C (True), D (False), E (False)

4. A (True), B (True), C (False), D (True), E (False)

5. A (True), B (False), C (True)