Hydroxychloroquine: A relatively obscure antimalarial takes centre stage in COVID-19

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Hydroxychloroquine (HCQ) is an old antimalarial known for its use to treat rheumatoid arthritis. Recently, it has been shown to be useful in COVID-19 treatment, although its efficacy is being debated and the data from large-scale clinical trials are yet to be available. Overall, it appears that HCQ-Azithromycin-Remdesevir may be a viable treatment option on a case to case basis. There is also a case to evaluate curcumin as an adjunct food supplement to prevent and treat COVID-19.

Spanish Flu was the deadliest virus infection in the 20th century. It emerged in March 1918 during the First World War and came in three waves infecting 500 million people worldwide and claiming the lives of 20–50 million people. In 21st century, we are witnessing a similar pandemic affecting almost every country in the world. At the time of writing this article, COVID-19 virus (SARS-CoV-2) has infected close to 2.6 million people, killing more than 170,000 people. The figures keep changing by the hour. This virus emerged in December 2019 from Wuhan, China, although one does not really know whether it incubated in China much longer. It has taken the globe by storm, and one hopes that with advanced medical care and clampdowns, COVID-19 would be contained sooner than later. There have been constant updates through print media and TV channels (24×7). An unprecedented effort has also gone into the development of medical equipment, diagnostics, drugs and vaccines. At the last count, 115 vaccine trials are underway. In terms of drug development, we should think of testing every kind of chemical with therapeutic potential including antiretrovirals, anticancer drugs, natural molecules, interferon-α/β, combination therapies, etc. Plasma from recovered patients and monoclonal antibodies against the viral proteins are additional therapeutic options. We will confine to the hydroxychloroquine (HCQ) story of how a relatively obscure antimalarial has taken centre stage to treat COVID-19 and provide our views on the potential of nutraceuticals like curcumin as adjunct therapy for COVID-19, as it falls within our area of research interest.

History of chloroquine use

The story of HCQ (also known as Plaquenil) has come full circle. Chloroquine (CQ, Aralen) was introduced to treat the US army at home and combat areas with severe malaria problems during World War II in the 1940s. HCQ, considered less toxic than chloroquine, was introduced in the mid-1950s. These two drugs belong to the class of 4-aminoquinolines (Figure 1). The two main *Plasmodia* species causing malaria are *Plasmodium falciparum* and *Plasmodium vivax*. *P. falciparum* is more deadly and accounts for 90% of the deaths, especially in Africa. *P. vivax* causes morbidity and incapacitation for prolonged periods and is reported to become more virulent in recent years. In the 1970s, *P. falciparum* became resistant to chloroquine and artemisinin derivative-based combination therapies (ACTs) have become the choice to treat malaria patients, although CQ is still effective against *P. vivax* malaria[1].

HCQ and COVID-19

It was shown that CQ has strong antiviral effect against SARS-CoV-1 infected primate cells in *in vitro* cultures[2]. In a recent study, Chinese authors have shown that Remdesevir (a nucleoside analogue) and CQ could inhibit SARS-CoV-2 at EC_{90} concentrations of 1.76 and 6.90 μM respectively, in vero cell culture[3]. This is followed by another publication from Chinese authors, who claimed the following: 'Chloroquine phosphate, an old drug for treatment of malaria, is shown to have apparent efficacy and acceptable safety against COVID-19 associated pneumonia in multicenter clinical trials conducted in China. The drug is recommended to be included in the next version of the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19 issued by the National Health Commission of the People's Republic of China for treatment of COVID-19 infection in larger populations in the future'. The authors indicated that the study was performed in 10 hospitals in Wuhan with more than 100 patients[4]. In another study with French patients, it has been shown that HCQ was effective in clearing virus load in 50% of the patients (7/14 on day 5 post therapy), HCQ-Azithromycin was effective in 100% of the patients (6/6), with untreated controls showing clearance in 18.8% of the patients (3/16)[5]. Azithromycin is a macrolide antibiotic, known to inhibit bacterial protein synthesis.

Given this background, CQ/HCQ has been added to the treatment protocols in some countries including China, South Korea, Belgium and India with varying levels of conditionality, although large-scale clinical data from randomized trials are not yet available. Reports have indicated that UK has decided to wait for the outcome of the trials and Swedish hospitals have abandoned trials on CQ

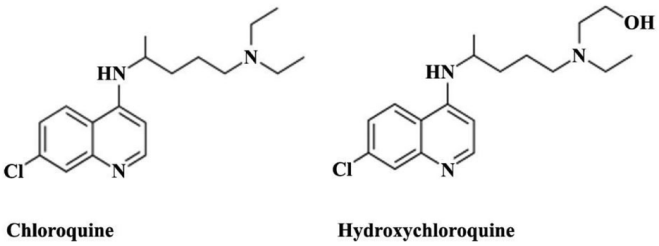

Figure 1. Structures of chloroquine and hydroxychloroquine.

because of side effects. In a manuscript submitted to the *New England Journal of Medicine* from a study carried out with 32 patients admitted to the hospital in Detroit, HCQ administration was found to increase the need for ventilator support. There were no benefits on mortality, lymphopenia orneurtophil–lumphocyte ratio improvement. However, no data was provided on clearance of viral load[6]. In a very recent study, the same French group mentioned earlier (led by Didier Raoult), has released data with the following findings: 'From 3 March to 9 April 2020, 59,655 specimens from 38,617 patients were tested for COVID-19 by PCR. Of the 3,165 positive patients placed in the care of our institute, 1061 previously unpublished patients met our inclusion criteria. Their mean age was 43.6 years old and 492 were male (46.4%). No cardiac toxicity was observed. A good clinical outcome and virological cure was obtained in 973 patients within 10 days (91.7%). Prolonged viral carriage at completion of treatment was observed in 47 patients (4.4%) and was associated to a higher viral load at diagnosis ($p < 10^{-2}$), but viral culture was negative at day 10 and all but one were PCR-cleared at day 15. A poor outcome was observed for 46 patients (4.3%); 10 were transferred to intensive care units, 5 patients died (0.47%) (74–95 years old) and 31 required 10 days of hospitalization or more. Among this group, 25 patients are now cured and 16 are still hospitalized (98% of patients cured so far). Poor clinical outcome was significantly associated to older age (OR 1.11), initial higher severity (OR 10.05) and low hydroxychloroquine serum concentration. In addition, both poor clinical and virological outcomes were associated to the use of selective beta-blocking agents and angiotensin II receptor blockers ($P < 0.05$). Mortality was significantly lower in patients who had received >3 days of HCQ-AZ than in patients treated with other regimens both at IHU and in all Marseille public hospitals ($p < 10^{-2}$). Interpretation: The HCQ-AZ combination, when started immediately after diagnosis, is a safe and efficient treatment for COVID-19, with a mortality rate of 0.5%, in elderly patients. It avoids worsening and clears virus persistence and contagiosity in most cases.' In another recent study, Shangai Jiao Tong University School of Medicine has shown in a randomized trial that HCQ limits the inflammatory response and hastens alleviation of symptoms. Over all, based on published and unpublished results, it looks HCQ-Azithromycin could be a potential treatment option in the clinical management of COVID-19 disease.

Unfortunately, a potential therapy to save lives has become a political hotbed with US President claiming it as a 'Game Changer' and the latest results by the French group being released to the French President! It is not clear whether the drumbeat is just to claim priority or there is something deeper in terms of Chinese versus US/Europe conspiracy theories.

Mechanism of action of CQ/HCQ

CQ and HCQ, although generally known as antimalarial and antiamoebic drugs, have been reported to have a wide range of clinically relevant effects. The non-protonated form of chloroquine can cross the endosomal membrane within a cell and can get protonated in the acidic compartment. The protonated CQ is held within the endosome, raising the pH. Some viruses, including flaviviruses, retroviruses and coronaviruses enter the target cells by endocytosis. The acidic pH in the endosomal (lysosomal) compartment leads to disruption of the viral particle with the release of the nucleic acid and enzymes necessary for viral replication (Figure 2). CQ prevents the pH-dependent entry of the virus and the viral replication. CQ was proposed as a therapy for SARS virus and some preliminary results were obtained using *in vitro* cultures[7]. However, CQ did not seem to have undergone any clinical trial for SARS-CoV-1. Perhaps, SARS did not spread like COVID-19 and reports indicate that SARS-CoV infected around 8000 people with almost 700 deaths. Further, these drugs have immunomodulatory effects and they can suppress the release of inflammatory cytokines (TNF-α and IL-6), with a potential to inhibit the inflammatory consequences of viral infections and other chronic diseases. Thus, HCQ and CQ have been used in the treatment of rheumatoid arthritis, systemic lupus erythematosis and other inflammatory diseases, although the mechanisms involved are still being analysed[8].

In terms of the anti-malarial action of CQ, the effects have been understood based on the toxicity of free heme to the parasite. The equivalent of the endosome/lysosome compartment is referred to as 'food vacuole' in the parasite, in which the haemoglobin taken up by the parasite from the host red cell is degraded to give rise to amino acids and heme. While the amino acids are utilized

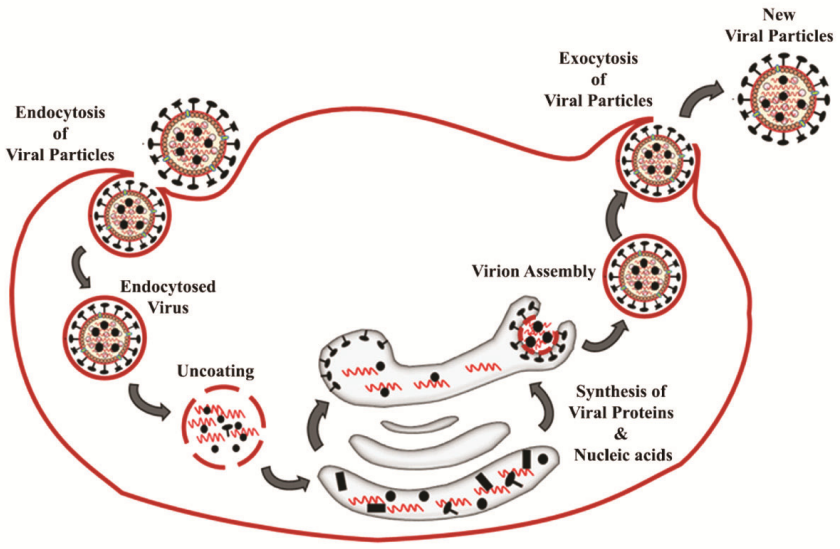

Figure 2. Steps involved in the entry and assembly of corona and related viruses. SARS-CoV-2 has a positive stranded RNA genome and is about 30 kb in size. It codes for many proteins needed for viral replication and viral assembly, including the characteristic spike protein that binds to the ACE-2 receptor of the host and facilitates the virus entry into the host cell through endocytosis. Figure based on ref. 7.

by the parasite for its own protein synthesis, free heme is highly toxic to the parasite. Therefore, the parasite converts free heme into a polymer called hemozoin – a brown pigment that accumulates in the parasite. CQ binds to free heme and prevents the conversion of free heme into hemozoin, leading to the death of the parasite[9]. As an extension of this mechanism, an interesting argument has been made for the treatment of COVID-19 with vitamin C in the perspective of acute respiratory distress syndrome (ARDS). A detailed review records the basis for this proposition[10]. ARDS patients have a 30–50% mortality rate. This is due to an uncontrolled cascading event starting with increased permeability of pulmonary capillary endothelial cell and leakage of fluid into the pulmonary parenchyma. This is followed by cytokine storms marked by acute inflammatory responses[11]. It has been proposed that the ARDS seen in critically ill COVID-19 patients is due to cell-free haemoglobin in the lung air space[12]. Hemolysis leads to free heme generation that contains iron in ferric (Fe^{3+}) state and this needs to be reduced and kept in the ferrous (Fe^{2+}) state. Ascorbic acid (not made by the human body) is involved in this process and is regenerated through a cyclic mechanism involving cytochrome b561. It has been suggested that oral ascorbic acid can save the lives of critically ill COVID-19 patients[10].

Safety of CQ/HCQ

Despite the use of CQ as an anti-malarial for decades and HCQ for rheumatoid arthritis, it can cause side effects and overdose can even be fatal. These drugs have common as well as independent side effects as described in the medical literature[13,14]. Therefore, these are prescription drugs and self-medication needs to be strictly avoided.

Potential of curcumin as an adjunct food supplement to prevent and treat COVID-19

Several studies in our laboratory have demonstrated the beneficial effects of curcumin from turmeric as an adjunct nutraceutical in preventing malaria parasite recrudescence and cerebral malaria in the animal model along with the antimalarial, artemisinin derivative[15–17]. Preliminary studies were initially carried out with chloroquine–curcumin combination, but detailed studies were performed later on with artemisinin derivative, as it is a more effective antimalarial in the mouse model. Curcumin has immunomodulatory effects and has anti-oxidative and anti-inflammatory properties. Formulations to increase the bioavailability of curcumin are already available. A clinical trial to test the efficacy of curcumin in malaria has been approved by DCGI as well. Here, we would like to propose the evaluation of curcumin as a simple food supplement to prevent as well as treat COVID-19 along with approved therapy such as HCQ, Azithromycin and Remdesevir. Finally, while vaccines and drugs would be very useful to prevent and treat the infection, a pandemic of this dimension can only be controlled through public health measures such as social distancing, quarantine and personal hygiene. This has been the experience in earlier corona virus infections as well.

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ACKNOWLEDGEMENT. Studies on curcumin in the malaria parasite were carried out in a Centre of Excellence project supported by the Department of Biotechnology, Government of India.

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