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COVID-19

Chloroquine and hydroxychloroquine in the management of COVID-19: Much kerfuffle but little evidence

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Summary Chloroquine and hydroxychloroquine are drugs that have shown in vitro activity on the replication of certain coronaviruses. In the context of the SARS-Cov-2 epidemic, the virus responsible for the novel coronavirus disease (COVID-19), these two drugs have been proposed as possible treatments. The results of the first clinical studies evaluating the effect of hydroxychloroquine do not support any efficacy of this drug in patients with COVID-19, due to major methodological weaknesses. Yet, these preliminary studies have aroused considerable media interest, raising fears of massive and uncontrolled use. In the absence of evidence of clinical benefits, the main risk is of exposing patients unnecessarily to the well-known adverse

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effects of hydroxychloroquine, with a possibly increased risk in the specific setting of COVID-19. In addition, widespread use outside of any recommendation risks compromising the completion of good quality clinical trials. The chloroquine hype, fueled by low-quality studies and media announcements, has yielded to the implementation of more than 150 studies worldwide. This represents a waste of resources and a loss of opportunity for other drugs to be properly evaluated. In the context of emergency, rigorous trials are more than ever needed in order to have, as soon as possible, reliable data on drugs that are possibly effective against the disease. Meanwhile, serious adverse drug reactions have been reported in patients with COVID-19 receiving hydroxychloroquine, justifying to limit its prescription, and to perform suitable cardiac and therapeutic drug monitoring.

Abbreviations

ACE2	angiotensin converting enzyme 2
ADRs	adverse drug reactions
COVID-19	novel coronavirus disease 2019
CYP	cytochrome P450
ECG	electrocardiogram
HCSP	French High Council of Public Health
MERS-Cov	Middle East respiratory syndrome coronavirus
QTc	corrected QT interval
SARS-Cov	severe acute respiratory syndrome coronavirus
TDM	therapeutic drug monitoring
TNFα	tumor necrosis factor α

Introduction

Chloroquine (Nivaquine®) and its hydroxylated derivative hydroxychloroquine (Plaquenil®) are old drugs with antimalarial properties, the use of which has become progressively reduced with the appearance of chloroquine-resistant strains of *Plasmodium falciparum*. They also have anti-inflammatory and immunomodulatory activity by regulating the production of tumor necrosis factor (TNF)α, interferon and certain cytokines [1—3]. These properties justify the use of hydroxychloroquine in certain autoimmune diseases, such as lupus or rheumatoid arthritis.

In addition, since several decades, these drugs have been shown to have an inhibitory activity on the replication of many viruses [4]. Although the mechanisms of these antiviral properties are not fully understood, chloroquine and hydroxychloroquine are weak bases, which accumulate in lysosomes, modify their pH, and interfere with certain enzymes. They thus have the capacity to inhibit the pH-dependent entry of certain viruses into host cells, or even to block the replication of enveloped viruses by inhibiting the glycosylation of envelope proteins [5]. These in vitro antiviral effects have raised a lot of hope, opening-up consideration of the repositioning of these old and inexpensive drugs for the management of many viral infections, against which there are no or few effective treatments, or against which drugs exist but are not widely available, especially in countries with limited resources [6].

Chloroquine and hydroxychloroquine: are they effective antivirals?

The demonstration of antiviral activity in vitro is obviously not synonymous with efficacy on clinically relevant parameters. Several studies have demonstrated an effect of chloroquine and hydroxychloroquine on the replication of HIV in vitro [7]. However, a double-blind, randomized, controlled trial comparing hydroxychloroquine at 400 mg/day to placebo in 83 HIV positive patients not treated with antiretrovirals and having a CD4 level > 400/μL, showed after 48 weeks of treatment a greater decrease in CD4, and an increased viral load, in the group treated with hydroxychloroquine compared to controls [8]. Such paradoxical effect was also observed in a double-blind, randomized, controlled trial comparing the efficacy of 5 days of chloroquine to placebo in patients infected with chikungunya. Besides the lack of effect of chloroquine on the viremia, patients in the chloroquine group had significantly more arthralgia than those in the placebo group [9], despite an inhibitory effect of chloroquine on the replication of the virus *in vitro* [10]. In the case of chikungunya, this clinical versus laboratory discrepancy might be explained by the immunomodulatory effects of these drugs, which modify the cellular and humoral immune response to infection [10]. In other indications, such as the treatment of dengue fever or the prevention of influenza, chloroquine proved to be ineffective despite in vitro activity [3,11—13]. Finally, activity against the hepatitis C virus (HCV) has been described [14]. A pilot clinical trial conducted in a dozen patients with HCV (genotype 1) who did not respond to the combination of pegylated interferon alpha and ribavirin, showed a reduction in viral load and in ALT, but this effect ceased on stopping chloroquine (Table 1) [15]. Thus, to date, despite many promising in vitro leads, chloroquine or hydroxychloroquine have never been shown to have any real clinical efficacy in

Table 1 Examples of viral infections for which chloroquine or hydroxychloroquine have been tested in vitro and in clinical trials.

Infection	Drug tested	In vitro Activity	Efficacy in clinical trials
HIV	Hydroxychloroquine	Inhibits the replication of HIV	Decreased CD4 and increased viral load
Dengue	Chloroquine	Inhibits the replication of the virus	No clinical efficacy demonstrated
Chikungunya	Chloroquine	Inhibits the replication of the virus	No effect on viral load; increase in arthralgia
Influenza	Chloroquine (prophylactic)	Inhibits the replication of H1N1 and H3N2 viruses	No prevention of influenza; more adverse events in the chloroquine group
Hepatitis C	Chloroquine	Inhibits the replication of HCV	Decreased viral load; this effect ceased on stopping chloroquine

the treatment or prevention of viral infections [16]. Several reasons may explain the discrepancy between in vitro and clinical results, such as the validity of the experimental model, or pharmacokinetic issues, i.e. reaching sufficient inhibitory concentration at the site of infection.

Effects on coronaviruses

In 2002-2003, the coronavirus responsible for severe acute respiratory syndrome (SARS-Cov) quickly affected many countries. Since then, the hypothesis of chloroquine as a therapeutic option has emerged, based on its antiviral properties and also on its immunomodulatory effect [6]. This hypothesis was confirmed in vitro, chloroquine inhibiting the replication of the SARS-Cov virus at concentrations close to those used in patients treated for malaria [17]. Subsequent work, still in vitro, confirmed these results and also suggested a possible prophylactic effect [18]. In 2014, chloroquine was described as having in vitro activity on the Middle East respiratory syndrome coronavirus (MERS-Cov) [19]. However, these results have not been followed by clinical trials demonstrating any clinical efficacy.

In the context of the current SARS-CoV-2 pandemic, responsible for the novel coronavirus disease (COVID-19), the chloroquine question has resurfaced. In vitro studies have shown that chloroquine [20], and also hydroxychloroquine, exhibits antiviral activity against SARS-CoV-2, with a lower EC50 for hydroxychloroquine (0.72 μM vs 5.47 μM at 48 h) suggesting more potent activity [21]. In this same article, the authors developed a PB/PK model for these two drugs, in order to simulate their diffusion in pulmonary tissue with several dosage regimens while taking into account the safety profile. Based on simulations, they propose for hydroxychloroquine a loading dose of 400 mg twice on day 1, followed by a maintenance dose of 200 mg twice a day for the next four days. At these dosages, simulations show apparently low plasma concentrations, of the order of 0.1 μg/mL, but which could allow significant pulmonary exposure with very high tissue-free inhibitor quotients, of about 85 from day 5 after the start of treatment [21,22].

Yet, other reports have shown about 10-fold higher EC50, or a higher potency for chloroquine than hydroxychloroquine, highlighting substantial variability between in vitro models [23,24]. This stresses the difficulty to extrapolate that appropriate drug concentration at the site of infection will be reached with safe doses of chloroquine or hydroxychloroquine.

The first clinical data in COVID-19 and their limitations

Based on these experimental data, around twenty clinical trials evaluating the efficacy of chloroquine or hydroxychloroquine on COVID-19 had been started in China by mid-March 2020 [25], while there were almost 150 trials registered worldwide at the end of April. This frenetic activity originates from positive preliminary results obtained on one hundred patients, which were announced without there being a formal interim analysis or a detailed report of these results published [26]. They were followed by different studies of various methodological qualities from single-arm, non-controlled studies, to cohorts with propensity score-matched controls, and randomized controlled trials.

In France, a pilot study published a few weeks later evaluated the effect of hydroxychloroquine on patients with COVID-19. This non-randomized, open-label study had as primary endpoint the presence of nasopharyngeal virus 6 days after inclusion. The main result was a higher proportion of patients in whom the virus was no longer detected in the hydroxychloroquine group (600 mg/d for 10 days) than in the control group (70% vs 12.5%; $P = 0.001$). In addition, six patients who received a hydroxychloroquine-azithromycin combination had negative samples from day 5 (D5) [27]. While they open up interesting perspectives, the results of this work must be considered with the greatest caution due to major methodological weaknesses. First, as in any non-randomized study, imbalances between the groups expose it to a major bias. In this case, the use of geographic controls does not protect against a possible selection bias. In addition, of the 26 patients who received hydroxychloroquine, six left the trial prematurely; among these, three were transferred to intensive care, and a fourth died. An intention-to-treat analysis should not exclude these patients, but should consider these cases as failures of treatment with hydroxychloroquine. In the control group, the result of the primary endpoint (PCR on day 6, D6)

was not available for 5 of the 16 subjects, nearly 30%, whom the authors considered to be positive, i.e. carrying the virus. Another point concerns PCR results, which were not reported in the same way for 10 patients in the control group (compared to the 6 other controls and 20 patients in the group treated with hydroxychloroquine). The results of these PCRs also seem to be very variable: two patients in the control group, negative on D5 were again positive on D6, and counted as such for the main endpoint. However, in the arm treated with hydroxychloroquine, a patient found negative on D5 and for whom the data was missing on D6 was presumed negative. By re-analyzing the data actually available on D6, the difference between the groups is no longer significant, suggesting a possible risk of p-hacking, which consists of multiplying the comparisons until obtaining a p-value below the significance threshold. This obviously increases the risk of a false positive. The article also concludes that azithromycin is effective, but these results relate only to a series of 6 patients, without randomization. These results were supplemented by the pre-publication, by the same team, of a cohort of 1061 patients receiving a combination of hydroxychloroquine-azithromycin early after the first symptoms, and followed-up during nine days or longer. The results show a rate of poor clinical outcome (prolonged hospitalisation, transfer to intensive care, or death) of 4.3%, and 0.75% mortality [28], i.e. close to that observed among infected people in France [29]. However, in the absence of a control group, it is not possible to conclude to the efficacy of this association.

Several studies based on data collected from routine care compared outcomes of patients with confirmed SARS-CoV-2 infection treated with hydroxychloroquine to those untreated, and used propensity score-based methods to account for between-group differences inherent to non-randomized studies. The first study, conducted in four hospitals in France, compared 84 patients with pneumonia requiring oxygen, and who received hydroxychloroquine, to 89 controls. The results show no difference between the two groups regarding the risk of transfer to intensive care or death [30]. A second study reports data from the Department of Veterans Affairs, in the USA. Among 368 patients, 97 received hydroxychloroquine alone, 113 had hydroxychloroquine and azithromycin, and 158 were unexposed to hydroxychloroquine. The results suggest a higher risk of death in patients treated with hydroxychloroquine alone, without any difference between the three groups regarding the risk of ventilation [31]. These two studies were later completed by two larger cohorts of patients admitted to the hospital in New York, USA. The first one ($n = 1376$) did not show any significant association between hydroxychloroquine use and the primary end point of respiratory failure (HR = 1.04; 95% CI: 0.82 to 1.32) [32]. Of note, almost 60% of patients receiving hydroxychloroquine in that cohort also received azithromycin, while this proportion was 37.2% in propensity-matched patients not receiving hydroxychloroquine. In the second large cohort ($n = 1438$), hydroxychloroquine used was not associated with a decrease in in-hospital hospital mortality, whether associated with azithromycin or used alone [33]. However, the non-randomized nature of these studies exposes to bias and prevents from drawing clear conclusions.

Few randomized clinical trials have been conducted, and properly reported so far. The summary of a small, randomized trial conducted in China (NCT04261517) on 30 patients, was published at the end of March 2020. The results do not show any difference between the groups treated with hydroxychloroquine (5 days at 400 mg/d) and the control group, neither virologically nor based on clinical criteria [34]. Another Chinese study (ChiCTR2000029559) concluded to the superiority of hydroxychloroquine (5 days at 400 mg/day) over standard treatment in treating fever, cough, and associated pneumonia [35]. Yet, we cannot exclude significant reporting bias, since the outcomes, the number of patients, and the dose regimens reported in this study differed from those initially declared in the registry. The largest randomized trial was an open-label, multicenter study including 75 patients treated with hydroxychloroquine compared with 75 patients receiving standard of care. The dose was higher than on the previous trials (loading dose of 1200 mg/day for three days followed by 800 mg/day for 2 or 3 weeks, according to the severity of the disease). The results do not show any difference in viral clearance at day 28 (primary outcome). Symptoms were also comparable between the two groups [36], while some differences were observed in post-hoc subgroup analyses. Altogether, available evidence is limited and most studies are biased. To date, the results of a live meta-analysis do not suggest any efficacy of hydroxychloroquine in patients with COVID-19 [37]. Nonetheless, the media and societal excitement generated by very early announcements, based on in vitro data and uncontrolled case studies, are at the origin of a huge demand for and significant use of this drug.

The safety of hydroxychloroquine use in COVID-19 and its monitoring

In the absence of evidence of efficacy, the first risk is to unnecessarily expose patients to adverse effects [38]. While it is relatively safe at the doses used to prevent malaria (100 mg/day), chloroquine has well-known dose-dependent adverse drug reactions (ADRs). The most common are abdominal pain or diarrhea, described in almost 10% of users, as well as pruritus and rashes. Headache, ringing in the ears, dizziness and tinnitus are also possible (signs of ''cinchonism'' described with quinine). Psychiatric ADRs are also reported, ranging from anxiety disorders and insomnia to psychotic decompensations. Psychotic effects (hallucinations, delusions) seem more frequent than thymic disorders (depression). Regarding hydroxychloroquine, serious psychiatric effects are relatively rare within the framework of the usual prescription. However, in the current context, with the anxiety-provoking nature of the epidemic and the confinement of the population, these ADRs are potentially more likely to appear.

Chloroquine and hydroxychloroquine also have well-known cardiac ADRs linked to their inhibitory effect on hERG potassium channels, which repolarize the action potentials of cardiomyocytes by potassium efflux in phase 3 of the action potential (IKr potassium current). This property increases the risk of prolonging the corrected QT interval (QTc) of the electrocardiogram (ECG) [39]. Although this ADR

is dose-dependent and therefore more frequent in the event of an overdose, serious arrhythmias have been reported at therapeutic doses. Among the risk factors for QT prolongation that can facilitate or precipitate such arrhythmias are a slow heart rate (< 55 bpm), being female [40], and especially hypokalemia and combination with other QTc-prolonging drugs, such as macrolides [41,42]. Besides this effect on IKr current, hydroxychloroquine may reduce heart rate by inhibiting If-current in phase 4 of the action potential [43].

Among patients receiving hydroxychloroquine for COVID-19, about 10% had to discontinue due to ECG modifications [44]. In the largest randomized trial, adverse events were observed in about 30% of patients who received hydroxychlorquine, vs 9% of patients who did not [36]. QTc > 500 ms was observed in 11% to 20% of patients receiving a combination of hydroxychloroquine and azithromycin [45,46]. This proportion reached 25% with the highest dose of hydroxychloroquine (1200 mg/day for 10 days) in a double-blinded, randomized trial comparing two dose regimens, in combination with azithromycin [47]. Among 40 patients with severe infection (admitted to intensive care units) who received either hydroxychloroquine or hydroxychloroquine + azithromycin, there was an increase in QTc in 37 of 40 patients (93%) after drug administration. QTc prolongation, defined as. ΔQTc > 60 ms or QTc $\geq$ 500 ms, was observed in 14 patients (36%) [48]. The risk was significantly lower, yet significant, when hydroxychloroquine was used alone [46,48]. This is consistent with observational data collected from patients with rheumatoid arthritis, in whom the combination hydroxychloroquine + azithromycin was associated with an increased risk of 30-day cardiac mortality, while hydroxychloroquine alone was not [49]. Details about the cardiac effects of hydroxychloroquine and other drugs used in COVID-19 have been reviewed recently [50].

In the light of what was known about the cardiac ADRs of hydroxychloroquine before the pandemic, all these results suggest an increased cardiac risk in patients with COVID-19. This could be explained by frequent hypokalemia in these patients, possibly due to a particular tropism of SARS-CoV-2 towards the ACE2 angiotensin-converting enzyme [51]. Also, diarrhea and vomiting can be associated with the infection. It is therefore essential to monitor potassium level and to correct any hypokalemia before administering hydroxychloroquine, which itself can cause diarrhea. Similarly, the combination of azithromycin with hydroxychloroquine justifies reinforced monitoring, with an ECG if possible before the start of treatment, and within 3—4 h after the first administration. And then twice a week for the duration of treatment or in the event of symptoms suggestive of a heart rhythm disorder.

Another dose-limiting ADR of hydroxychloroquine is the risk of retinopathy, which could affect up to 8% of treated patients, especially when the dose is high (> 5 mg/kg) or when treatment is prolonged (> 5 years) [52].

Finally, since hydroxychloroquine is metabolized by several isoforms of cytochrome P450 (CYP), in particular 3A4/5, 2D6 and 2C8, there is an increased risk of ADRs with drugs that inhibit these CYP. In the context of COVID-19, special care must be taken with other anti-infectives, such as the combination lopinavir/ritonavir, the latter being a powerful inhibitor of CYP3A.

In addition to the clinical surveillance mentioned above, hydroxychloroquine use justifies therapeutic drug monitoring (TDM), which is also recommended in the context of autoimmune diseases [53]. In the framework of prescription for the management of a SARS-CoV-2 infection, high variability in concentrations tested is expected in view of the populations to be treated (the elderly, resuscitation or dialysis patients) [54], with the possibilities of therapeutic ineffectiveness or ADRs. In addition, given the proposed short duration of treatment, a state of equilibrium is unlikely to be reached, which increases the pharmacokinetic variability.

The Pharmacology group - AC43 of the National Agency for Research on AIDS and Viral Hepatitis and the Therapeutic Drug Monitoring Group of the French Society of Pharmacology and Therapeutics proposed at the end of March 2020 that a TDM evaluation should be performed 48 hours after the start of treatment, to check the early attainment of an adequate residual concentration. Based on the data currently available, the minimum threshold to be reached is estimated at 0.1 μg/mL for a plasma assay and 0.3 μg/mL for a whole blood assay. TDM assays could then be proposed throughout the course of treatment to ensure that the threshold concentration considered as toxic (1 μg/mL in plasma or 2 μg/mL in whole blood) is not exceeded (Recommendations for Therapeutic Drug Monitoring of Lopinavir/r and Hydroxychloroquine in Patients Treated for SARS-CoV-2 (COVID-19) Infection, available on the website) [55].

The impact of massive use of hydroxychloroquine on the conduct of high-quality clinical trials

The available efficacy and safety results mentioned above are at high risk of bias, which leaves a degree of uncertainty regarding the relevance of hydroxychloroquine use in COVID-19. In addition, the pandemic emergency has justified early publication of most of these results as pre-prints. Since these reports are not peer-reviewed, they cannot be considered as validated information, and thus should not guide clinical practice [56]. In parallel, we have recorded a growing request for reliable information on the efficacy and safety of hydroxychloroquine from the population [57].

On March 23, 2020, in this context of public pressure, the French High Council of Public Health (HCSP) recommended not to use hydroxychloroquine, except in severe hospitalized cases with the collegial decision of physicians and under strict medical supervision. The HCSP insists that the prescription of hydroxychloroquine in the general population, or for non-severe forms, should be excluded. This advice was followed a few days later by a decree restricting the use of hydroxychloroquine for COVID-19 to health facilities.

Nonetheless, a majority of the French population favored the large use of hydroxychloroquine, sometimes encouraged by healthcare professionals. This has likely impacted rigorous clinical research, penalizing the recruitment in randomized controlled trials. Indeed, taking hydroxychloroquine (or any other drug with antiviral properties) over the days or weeks preceding randomization is usually a criterion for non-inclusion in such trials.

In addition, some patients refused to participate in randomized trials fearing to receive placebo instead of hydroxychloroquine, which would have been perceived as not being given the best possible treatment [58]. Interestingly, alternative designs have been proposed, such as open-label randomized trials nested in a cohort, with randomization derived from Zelen's method [59]. Although this approach prevents from the above-mentioned issue, it raises several concerns [60].

Not being able to quickly conclude as to the efficacy of treatments is extremely detrimental in terms of public health. In the context of an epidemic it is indeed difficult to correctly carry out and complete randomized controlled trials. For example, the only therapeutic trial of this type conducted during the Ebola virus epidemic of 2014-2016 in West Africa could not be completed, leaving unanswered the question as to the potential efficacy of the humanized monoclonal antibodies ZMapp® [61].

Conclusion and perspectives

Chloroquine and hydroxychloroquine have been shown to have activity on the SARS-Cov-2 coronavirus in vitro, with possibly superior effect for hydroxychloroquine, which is currently at the center of attention. However, mid-May 2020, available clinical data do not support any efficacy of these drugs in patients with COVID-19. Good quality randomized, controlled trials are still underway. It is essential to be able to include patients in these trials rapidly, in order to have reliable data on whether these drugs are truly effective against COVID-19. Yet, the chloroquine hype, fueled by low-quality studies and media announcements, has yielded to the implementation of more than a hundred studies, with the risk of wasting resources and delaying rigorous trials. Pending their results, the lack of evidence of a possible benefit must be weighed against the known ADRs of hydroxychloroquine, which may be magnified in COVID-19 patients. Although relatively safe at a therapeutic dose and for a short period of time, this drug has a narrow therapeutic index, which requires regular cardiac and therapeutic drug monitoring. Serious adverse reactions of hydroxychloroquine have already been reported in patients with COVID-19, especially when it is combined with azithromycin.

Disclosure of interest

The authors declare that they have no competing interest.

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