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PERSPECTIVES IN RHEUMATOLOGY

Hydroxychloroquine and chloroquine in COVID-19: should they be used as standard therapy?

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Abstract

The pandemic of the new coronavirus, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has urged the nations to an unprecedented world-wide reaction, including an accelerated exploration of therapeutic options. In the absence of a vaccine and specifically designed antivirals, the medical community has proposed the use of various previously available medications in order to reduce the number of patients requiring prolonged hospitalizations, oxygen therapy, and mechanical ventilation and to decrease mortality from coronavirus disease 2019 (COVID-19). Hydroxychloroquine and chloroquine are among the proposed drugs and are the most widely used so far, despite the lack of robust evidence on their usefulness. The objective of this article is to review and discuss the possible role of these drugs in the therapy of COVID-19.

Keywords Chloroquine · COVID-19 · Hydroxychloroquine · SARS-CoV-2

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has urged the nations to an unprecedented world-wide reaction, including drastic containment measures and the search of therapeutic options. The main focus has been to slow down the spread of this virus, but until now, this is an ongoing process. SARS-CoV-2 produces the coronavirus disease 2019 (COVID-19) characterized by lung infection and many other possible manifestations, in humans [1], with potential high mortality. Until a vaccine or specifically developed antiviral is available, the need to control the disease in those with a severe presentation, and to reduce mortality, has moved the medical community to evaluate empirically the use of previously available drugs. Hydroxychloroquine (HCQ) and chloroquine (CQ) are among the drugs proposed.

HCQ and CQ are weak bases that accumulate in acidic compartments, such as lysosomes and inflamed tissues, and have a large volume of distribution and long half-life, giving them a slow onset of action and effects that last after suspension. Their mechanisms of action include the interference of lysosomal activity and autophagy, the alteration of membrane stability, and the disruption of signaling pathways and transcriptional activity. These actions mean that at the cellular level, these drugs can inhibit immune activation, by decreasing Toll-like receptor (TLR) signaling and modulating other co-stimulatory molecules, and by reducing the production of cytokines [2].

The objective of this article is to review and discuss the possible role of these drugs in the therapy of COVID-19.

Mechanisms of action with possible role in COVID-19 therapy

CQ, which has been used to prevent and treat malaria and as an anti-inflammatory agent for the treatment of rheumatoid arthritis and lupus erythematosus, has shown a potential broad-spectrum antiviral activity [3].

It can inhibit a pre-entry step of the viral cycle by interfering with the binding of viral particles to their receptors on the cell surface. CQ inhibits quinone reductase 2 [4], which participates in the biosynthesis of sialic acids that are critical components of ligand recognition. Human coronavirus HCoV-O43 and the orthomyxoviruses use sialic acids as

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receptors [5]. If SARS-CoV-2 targets sialic acids, this could be affected by CQ [6, 7].

It can also be hypothesized that, in the presence of SARS-CoV-2, CQ could interfere with the glycosylation of the angiotensin-converting enzyme 2 (ACE2) receptor, preventing the virus from binding to its target cells. This hypothesis is based on in vitro evidence of reduced glycosylation of the SARS-CoV1 surface receptor, ACE2, on Vero cells [8].

For the SARS-CoV1, Dengue, and Chikungunya viruses, a pH-dependent mechanism of entry into target cells that can be interfered by CQ has also been reported [9–13]. Preliminary data indicates that CQ may interfere with SARS-CoV-2 acidification of lysosomes and inhibit cathepsins, which require low pH for cleavage of SARS-CoV-2 spike protein [14], necessary for the formation of the autophagosome [15].

Another possible mechanism of action is the inhibition of phosphorylation (activation) of the p38 mitogen-activated protein kinase (MAPK) in THP-1 cells by CQ [16]. This phosphorylation is required by various viruses to achieve their replication cycles [17]. In the model of HCoV-229 coronavirus, CQ inhibition of the virus appears to occur by this mechanism [18]. Regarding SARS-CoV-2, the inhibition of kinases such as MAPK could also be a mechanism of action for CQ.

Other proposed effect of CQ is that it can increase the soluble viral antigens in the cytosol of dendritic cells and enhance a cytotoxic CD8+ T cell response against them [19]. In the influenza virus model, CQ improved the cross-presentation of non-replicating virus antigen by dendritic cells to CD8+ T-cells, eliciting a protective immune response [20].

Also, CQ is capable of mediating an anti-inflammatory response [21]. It inhibits interleukin-1 beta (IL-1β) mRNA expression in THP-1 cells and reduces IL-1β release [16]. CQ-induced reduction of tumor necrosis factor-alpha (TNFα), IL-1, and IL-6 cytokines has also been reported [16, 22–26]. In the Dengue virus model, CQ was found to inhibit interferon-alpha (IFNα), IFNβ, IFNγ, TNFα, IL-6, and IL-12 gene expression in U937 cells infected with Dengue-2 virus [27].

HCQ, a less toxic aminoquinoline, has an *N*-hydroxyethyl side chain in place of the *N*-diethyl group that makes it more soluble than CQ. As CQ, HCQ also increases pH and confers antiviral effects and has a modulatory effect on activated immune cells. The antimalarial activity seems equivalent to CQ, but HCQ is preferred because of its lower toxicity [28]. HCQ binds strongly to melanin and can deposit in melanin-containing tissues such as the skin and the eyes, which might explain the retinopathy risk. Clinical observations suggest that HCQ confers a lower risk of retinopathy than CQ, and this could be explained by its lower volume of distribution and lower tissular accumulation [29]. HCQ was, in vitro, at least as effective as chloroquine in inhibiting SARS-CoV-2 infection, although it should be noted that studies on its mechanisms of action are not as extensive as with CQ [30].

Clinical studies of CQ and HCQ in COVID-19

The described preconceptions led quickly to studies in China. On February 15, 2020, the Chinese government recommended that antimalarials should be included in the guidelines for prevention, diagnosis, and treatment of COVID-19 pneumonia, issued by the National Health Commission of the People's Republic of China [3, 31], but it should be noted that all the positive reports available until March 31, 2020 were anecdotal reports and open-label studies without control groups. On that date, a report of a blinded, randomized, controlled trial of HCQ from Wuhan was published. It analyzed 31 patients in the treatment group (HCQ 400 mg per day for 5 days) and 31 in the control group [32]. The median age was 44.7 years, the male-female ratio was even, and all patients had pneumonia by computed tomography (CT) scan. Both groups received a not well-defined standard-of-care (oxygen therapy, antiviral drugs, antibiotics and even immunoglobulin, with or without corticosteroids). At presentation, more patients in the treatment group had fever and cough as compared to the control group. The treatment group showed significant improvements in comparison to the control group in fever, in cough, and in pneumonia by CT scan. Although this was the first controlled study to show any benefit from HCQ, it should be noted that the original registered trial informed a design for 100 control patients, 100 patients to receive a low dose of HCQ, and 100 more to receive a higher dose. The trial design also mentions as endpoints results for viral RNA, and for T cell recovery time [33]. This was not shown in the final publication. A previous Chinese controlled, pilot, study showed no benefit when 30 treatment-naïve patients were randomized 1:1 to HCQ 400 mg per day for 5 days or conventional treatment only [34]. Neither trial reported serious adverse events, but both excluded patients with cardiac arrhythmias, as high doses of hydroxychloroquine can induce QT interval prolongation.

In a French Clinical trial, 20 patients that received 600 mg of HCQ per day were compared with a group of untreated patients from another center. Viral load in nasopharyngeal swabs was tested daily. Azithromycin was added in 6 patients of the HCQ group. Results showed that the virus was not found after 6 days in all the patients treated with HCQ and azithromycin, in 57.1% of the patients treated with HCQ only, and in 12.5% of the control group patients ($p < 0.001$). No side effects or clinical evolution of patients were described. The authors said that azithromycin was added by clinical decision to treat a possible bacterial infection, but they also mention in their discussion that azithromycin may have an antiviral effect based on in vitro studies [35]. This study was questioned by a multinational team that reanalyzed its statistics performing a Bayesian A/B test and reported that for the original data, there was a strong statistical evidence for the positive effect of HCQ monotherapy on viral reduction, but that the level of evidence dropped to moderate when the deteriorated patients were

included in the analysis, and to anecdotal evidence when the patients that were not tested on the day of the primary outcome (day 6) were excluded [36].

The same group recently reported the results of a cohort of 80 patients that received HCQ 600 mg per day for 10 days, and azithromycin 500 mg the first day, and 250 mg per day for the next 4 days. Only 2 patients, according to their report, did not improve (an 86-year-old patient who died and a 74-year-old patient still in intensive care unit [ICU] at the time of the report). In 83% of the patients, the virus was not found at the nasopharyngeal sample tested by PCR at Day 7 (93% at Day 8). Virus cultures from patient respiratory samples were negative in 97.5% at day 5. The mean length of hospital stay was 5 days [37]. These results support the original report in that between 5 and 7 days of treatment, few patients had detectable virus by nasopharyngeal swab. The lack of comparison (hydroxychloroquine monotherapy, or standard of care) is problematic.

In contrast to these results, another French study evaluated 11 consecutive patients treated with the same combination (HCQ 600 mg per day and azithromycin, 500 mg the first day, and 250 mg per day thereafter). The mean age was 58.7 years, and 8 had significant comorbidities (2 obese, 5 with cancer, 1 with HIV). In this group, the combination was ineffective as 1 patient died, 2 had to be admitted to the ICU, and 8 (of 10) were still positive for the virus by nasal swab on day 5 or 6 after treatment. One patient had to discontinue therapy on day 4 because of prolongation of the QT interval [38].

Also, a multicenter, open-label, randomized controlled trial from China analyzed 75 patients receiving standard of care, and 75 receiving 1200 mg of HCQ per day for the first 3 days and then 800 mg per day for 2 weeks or 3 weeks (mild/moderate or severe disease, respectively). Specimens from the upper or lower respiratory tract were analyzed for viral RNA at screening, and then at days 4, 7, 10, 14, 21, and 28. The number of negative tests was similar between the two groups after 28 days (85.4% in the HCQ group, 81.3% in the standard of care group). Post hoc analysis did not identify any subgroups that showed a difference in these results. The alleviation of symptoms was also similar, but the adverse events were more frequent in the HCQ group (30% vs 9%), being diarrhea the most frequent [39].

Regarding the need to be admitted to ICU, a retrospective study from France analyzed 181 patients who were receiving oxygen therapy. Eighty-four received HCQ (600 mg per day) and the rest did not receive HCQ. The composite primary endpoint was transfer to an ICU within 7 days or death from any cause, and the secondary endpoint was the development of acute respiratory distress syndrome (ARDS). There were no statistical differences between the two groups. Eight of the patients in the HCQ group had electrocardiogram changes that required to stop the medication [40].

In relation to the cardiovascular risk, it is worth mentioning a study from the USA where 84 patients were treated with HCQ plus azithromycin combination. A notable QT interval prolongation was found in 30% of the patients, and in 11%, the interval increased to > 500 ms, with a high risk for arrhythmia. The mean age was 63, 74% of the patients were male, 65% had hypertension, and 20% were diabetic. The development of renal failure while on the drug combination was a strong predictor of QT interval prolongation [41]. Also, a multinational collaboration presented data from health care systems in Germany, Japan, Netherlands, Spain, UK, and the USA where the safety of HCQ and azithromycin combination versus HCQ and amoxicillin combination was compared. In users of the HCQ and azithromycin combination, a 15% increased risk of angina/chest pain, 22% increased risk of heart failure, and 2 times increased risk of cardiovascular mortality at 30 days of treatment was found in 323,122patients [42]. Finally, a study from Brazil analyzed 81 patients in two treatments arms, CQ 1200 mg per day for 10 days or low dose (900 mg on the first day, 450 mg for the next 4 days). All patients also received azithromycin and ceftriaxone. The high-dose arm showed more QT interval prolongation (> 500 ms) and a trend toward higher mortality (17%) than the lower dose group. The overall mortality rate was 13.5%, similar with their historical rate of patients not receiving CQ. The authors had to stop recruiting patients for the high-dose arm due to the cardiovascular events. The authors mention that they did not use a placebo control group as the use of placebo in Brazil in severe cases of COVID-19 infections was not considered ethically acceptable by national regulatory health agencies [43].

Discussion

Discovering therapeutic options is difficult, even more if most patients will recover with the current standard of care. It is important to observe the progression of the disease and standard outcomes, for example how many patients need mechanical ventilation, how many need supplemental oxygen and for how long, the length of stay in critical care units, and the length of hospital stay. These are patient-centered outcomes. Substitute endpoints, as viral load, will not necessarily relate to patient-centered outcomes; it has to be proven and their usefulness should not be assumed in advance.

The evidence for the use of hydroxychloroquine or chloroquine in COVID-19 is not good so far, not only because of the negative results of most of the studies but also because of their design, when publishing results of a very low number of patients, when reporting favorable results but without having a control group that allows comparison, when choosing results for which it will be very difficult to find significant differences, such as mortality, or for which their clinical relevance is uncertain.

Some countries and healthcare centers have adopted the use of hydroxychloroquine as a norm in patients hospitalized for COVID-19, due to political and social pressure given the publicity it has received. This makes the study of its possible beneficial effects even more difficult, and it has increased the reporting of its adverse effects.

Although due to the proposed mechanism of action, it could be postulated that the use of these antimalarials should be in the early stages of the disease; there is no clinical evidence to support this, and it could lead to serious problems in the availability of these drugs for patients with diseases in which the usefulness of these antimalarials is confirmed, not to mention the cardiovascular risks to which we would expose patients by indicating high doses of antimalarials without adequate monitoring.

It is hoped that the studies in progress can answer several of the questions that remain to be solved, such as what is the objective of treatment with antimalarials (decrease hospitalizations, decrease hospital stay, decrease the need for mechanical ventilation, etc.), what is the time suitable for its use, at what dose, for how long, what monitoring is necessary, and which patients are at the greatest risk of suffering adverse effects. Until then, we believe that the use of hydroxychloroquine or chloroquine should be in the context of strict studies or records that allow the detection of possible benefits and adverse effects.

Compliance with ethical standards

Disclosures None.

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