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Pharmacological effects of hydroxychloroquine in patients with COVID-19: Systematic literature review

Efeitos farmacológicos da hidroxicloroquina em pacientes com COVID-19: Revisão sistemática da literatura

Efectos farmacológicos de la hidroxicloroquina en pacientes con COVID-19: Revisión sistemática de la literatura

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Abstract
An outbreak of the new coronavirus (COVID-19) started in December 2019, and spread throughout China, spreading to the world in a short time, which requires immediate searches for its treatment. Thus, the use of chloroquine (CQ) and hydroxychloroquine (HCQ) has aroused interest in COVID-19. This systematic review follows the reporting items indicated for systematic reviews and Meta-Analysis Guidelines (PRISMA). The descriptors were selected: "*SARS-CoV-2*", "*COVID-19*", "*Hydroxychloroquine*" and "*Chloroquine*" and included in the databases Science Direct, United States National Library of Medicine (PubMed), Latin American bibliographic information on health sciences (LILACS), Scientific Electronic Library Online (SciELO) and Web of Science to search for studies. After screening, a total of 6.339 studies were found. After reading and applying the eligibility criteria, a total of 8 articles were selected to prepare the outcomes of this review. The results of the studies showed that the use of HCQ has outcomes with no significant improvement in the clinical picture, including association with adverse events (including serious) in patients with COVID-19. Thus, randomized clinical trials did not provide evidence of the efficacy of HCQ in patients with COVID-19, showing that an HCQ is not significantly effective, and has negative results regarding its safety for patients with COVID-19.
Keywords: Hydroxychloroquine; COVID-19; Coronavirus; Treatment.

Resumo

Um surto do novo coronavírus (COVID-19) teve início em dezembro de 2019 e se espalhou pela China, alastrando-se pelo mundo em pouco tempo, o que requer pesquisas imediatas para seu tratamento. Assim, o uso de cloroquina (CQ) e hidroxicloroquina (HCQ) tem despertado interesse no COVID-19. Esta revisão sistemática segue os itens de relatório indicados para revisões sistemáticas e Diretrizes de Meta-Análise (PRISMA). Os descritores selecionados foram: "*SARS-CoV-2*", "*COVID-19*", "*Hydroxychloroquine*" e "*Chloroquine*" e incluídos nas bases de dados Science Direct, United States National Library of Medicine (PubMed), informações bibliográficas latino-americanas em saúde ciências (LILACS), Scientific Electronic Library Online (SciELO) e Web of Science para busca de estudos. Após a triagem, um total de 6.339 estudos foram encontrados. Após a leitura e aplicação dos critérios de elegibilidade, foram selecionados 8 artigos para elaboração dos resultados desta revisão. Os resultados dos estudos mostraram que o uso de HCQ apresenta desfechos sem melhora significativa do quadro clínico, incluindo associação com eventos adversos (inclusive graves) em pacientes com COVID-19. Assim, os ensaios clínicos randomizados não forneceram evidências de eficácia da HCQ em pacientes com COVID-19, mostrando que a HCQ não é significativamente eficaz e apresenta resultados negativos em relação à sua segurança para pacientes com COVID-19.

Palavras-chave: Hidroxicloroquina; COVID-19; Coronavírus; Tratamento.

Resumen

Un brote del nuevo coronavirus (COVID-19) se inició en diciembre de 2019, y se extendió por toda China, extendiéndose al mundo en poco tiempo, lo que requiere búsquedas inmediatas para su tratamiento. Por lo tanto, el uso de cloroquina (CQ) e hidroxicloroquina (HCQ) ha despertado interés en COVID-19. Esta revisión sistemática sigue los elementos de informe indicados para las revisiones sistemáticas y las Pautas de metaanálisis (PRISMA). Los descriptores fueron seleccionados: "*SARS-CoV-2*", "*COVID-19*", "*Hidroxicloroquina*" y "*Cloroquina*" e incluidos en las bases de datos Science Direct, Biblioteca Nacional de Medicina de los Estados Unidos (PubMed), Información bibliográfica latinoamericana en salud. ciencias (LILACS), Scientific Electronic Library Online (SciELO) y Web of Science para la búsqueda de estudios. Después del cribado, se encontraron un total de 6.339 estudios. Después de leer y aplicar los criterios de elegibilidad, se seleccionaron un total de 8 artículos para preparar los resultados de esta revisión. Los resultados de los estudios mostraron que el uso de HCQ tiene resultados sin una mejora significativa en el cuadro clínico, incluida la asociación con eventos adversos (incluidos los graves) en pacientes con COVID-19. Por lo tanto, los ensayos clínicos aleatorizados no proporcionaron evidencia de la eficacia de HCQ en pacientes con COVID-19, lo que demuestra que un HCQ no es significativamente efectivo y tiene resultados negativos con respecto a su seguridad para pacientes con COVID-19.

Palabras clave: Hidroxicloroquina; COVID-19; Coronavirus; Tratamiento.

1. Introduction

An outbreak of a new coronavirus (COVID-19) began in December 2019, where it spread in a market in the city of Wuhan in China, being the point of transmission to cities and provinces in that country. The coronavirus has the capacity to infect mammals, logo, transmission of diseases from animals to humans is known as zoonotic overflow, which is most likely originated or a new outbreak of coronavirus, being an alteration of vectors or bats (Rodriguez-Morales et al., 2020). Thus, the first coronavirus capable of infecting humans was identified in 1960, where COVID-19 is the best known as coronavirus known to act under humans (Cui et al., 2019). Also evoking a severe acute respiratory syndrome (SARS) that emerged in 2002 as a threat to global health. From that period on, or investment in drugs that could combat SARS began to be more studied in the scientific field (Donnelly et al., 2003).

The new coronavirus had its first case registered in December 2019, and in March 2020 a pandemic was declared by the World Health Organization (WHO), thus demonstrating the high growth of infected individuals. In the updated platform made available by the WHO, 163,869,893 cases of coronavirus were found in the world and a total of 3,398,302 confirmed deaths, showing progression of new infections (World Health Organization, 2021).

The virus incubation period is between 1 to 14 days, and can extend to 24 days (Hui et al., 2020). Clinical manifestations include hyperthermia above 37.3 °, cough, hypoxemia, diarrhea, nasal secretions, sore throat, tachypnea and fatigue, and pneumonia may develop in severe cases (Xia et al., 2020). In addition, something around 20% of those infected evolve to SARS and may result in death (Van Der Brand et al., 2014). In view of this, the appearance of gastrointestinal signs

such as pain, diarrhea and vomiting can also be configured in the disease (Channappanavar, & Perlman, 2017). In analyzed lungs of patients who suffered from SARS, pulmonary edema with pleural effusions, hemorrhage and mucopurulent material was found, thus causing prominent alveolar damage to the lungs (Gu et al., 2005). The main form of transmission is through respiratory droplets and the contact of particles with mucous membranes (Zhu et al., 2020). In this way, the market for the sale of food and wild animals in the city of Wuhan in China may have facilitated transmission by crowding people, as the virus may also be transmitted by asymptomatic individuals (Riou, & Althaus, 2020). Droplet deposition occurs in the epithelium of the airways, serving as a site for SARS-CoV replication. Therefore, the angiotensin-converting enzyme 2 (ACE2) serves as a receptor for the coronavirus, which is very present in the upper respiratory system, causing infection preference in well-differentiated hair epithelial cells to which they express ACE2 (Jia et al., 2005).

Coronaviruses infect host cells through protein-mediated fusion on their surface called the spike in the virion, being activated by cellular proteases. The spike protein can be activated by furins (proteases with high expression power), this activation being considered unusual, but possible to occur (Millet, & Whittaker, 2014). The genomes of coronaviruses evolve through gains or losses of genes, these in turn, have high power of plasticity, therefore, the long genome brings greater probabilities of adaptive mutation, thus generating high diversity for the spike protein to mutate in order to adapt itself. to other cell receptors in order to perform interactions with cells for new infections (Forni et al., 2017).

Faced with this global reality of pandemic originating from COVID-19, there is an intense search for treatment in the scientific community (Kupferschmidt, & Cohen, 2020). In view of this, there is evidence present in the scientific literature, such as the research carried out by Keyaerts et al. (2004), where the potential protective effect of chloroquine (QC) on coronavirus *in vitro* was evaluated, showing inhibition in the replication of coronavirus in Vero E6 cells. In addition, there are some advantageous events when referring to QC such as its affordable price, good handling and its easy formulation, which are stimulating factors in the search for more evidence on the use of this drug for use in the disease (Keyaerts et al., 2004).

Thus, QC is widely used for the treatment of malaria in several countries with high incidence of the disease. Therefore, the drug is approved by the FDA for malaria, it still exists for indications of other pathologies such as rheumatoid arthritis and systemic lupus erythematosus (Goel, & Gerriets, 2019). In view of this, it is known that this class of drugs blocks infection by the virus, increasing the endosomal pH necessary for the fusion between virus and cell, in addition to interfering in the glycosylation of coronavirus cell receptors (Vincent et al., 2005). However, the drug can present possible adverse effects such as retinopathies, neuropathies, convulsions and hallucinations (Goel, & Gerriets, 2019). In addition, there is also hydroxychloroquine (HCQ) as a derivative of QC, presenting less toxic effects (Liu et al., 2020). Its use is generally indicated for individuals with autoimmune pathologies and malaria, with direct effects on the lysosomal and autophagic activity of cells. Based on this, its effects are dependent on other factors of the individual's metabolic environment such as the level of systemic inflammation (Schrezenmeier, & Dorner, 2020). However, they can also have adverse effects due to their use as retinopathies due to loss of the internal and external segment of the retina (Proano et al., 2019).

Currently, there is no specific treatment against the new virus, and the pandemic is still ongoing (Yazdany, & Kim, 2020). Based on this, the systematic literature review aimed to elucidate the outcomes on the pharmacological effects of HCQ and QC, given the great need to disseminate this scientific information in the face of the unrestrained use of drugs during the course of the COVID-19 pandemic.

2. Methodology

This systematic review follows the reporting items indicated for systematic reviews and Meta-Analysis Guidelines (PRISMA) (Page et al., 2021). Original studies on the effects of HCQ administered to patients with COVID-19 published only

in the English language between 2020 and 2021 were considered. Review articles, society consensus documents, guidelines and preprints were excluded.

2.1 Eligibility criteria

The critical discussion of the systematic review was: "What are the pharmacological effects of HCQ in patients with COVID-19?". The population, intervention, comparison, and outcome (PICO) format was used to define the eligibility criteria:

Population: Patients tested for SARS-CoV-2 infection;

Intervention: Administration of HCQ or QC;

Comparison: no treatment;

Result: Status of the patients' clinical condition;

2.2 Research Strategy

We searched Science Direct, National Library of Medicine of the United States (PubMed), Latin American bibliographic information on health sciences (LILACS), Scientific Electronic Library Online (SciELO) and Web of Science until June 15, 2021. The search strategy was composed by the combination of terminology duly registered in the Medical Subject Headings (MeSH) and "*Entry Terms*": "*SARS-CoV-2*, " "*Hydroxychloroquine*, " "*COVID-19*" and "*Chloroquine*". Then, the Bollean operators "*and*" or "*or*" were used as a search strategy among the terminologies for filtering the studies in the databases (the search strategy can be verified in complementary material). In addition, reference lists of selected relevant papers were hand-selected for potentially relevant new manuscripts. The language of this review was restricted to English.

2.3 Selection of studies

Using Rayyan QCRI software (Rayyan QCRI, Qatar Computing Research Institute, HBKU, Doha, Qatar), study selection was performed on the basis of title and abstract. Two investigators (FIP and FPG) independently selected all abstracts for inclusion criteria. Differences were resolved by a third investigator (GMD).

The studies included were those studying the pharmacological effects of HCQ or CQ in patients with COVID-19, comparing the studies, and thus elucidating clinical status outcomes. Studies were excluded if they met one of the following criteria: 1) Review studies; 2) It is not an original review (such as guidelines, prepress, conference.); 3) Studies of predatory journals; 4) Double publication: if the article occurred more than once in one of the databases, only the original manuscript was included; 5) Studies that did not include patients tested positive for Sars-cov-2; 6) *In vitro* or animal studies; 7) Studies that associated the treatment of HCQ or QC with another therapy.

The selected articles were read in full to verify the adequacy of the eligibility criteria by two authors (FIP and FPG) independently. Disagreements were resolved by consensus.

2.4 Study characteristics and data extraction

The following data were extracted from the included studies: authorship and year of publication, number of patients, mean age of participants, pharmacological intervention, and the main findings of the included and analyzed studies described in table 1.

2.5 Summary of results

The qualitative synthesis report followed the nine-item checklist of the Synthesis Report Without Meta-analysis

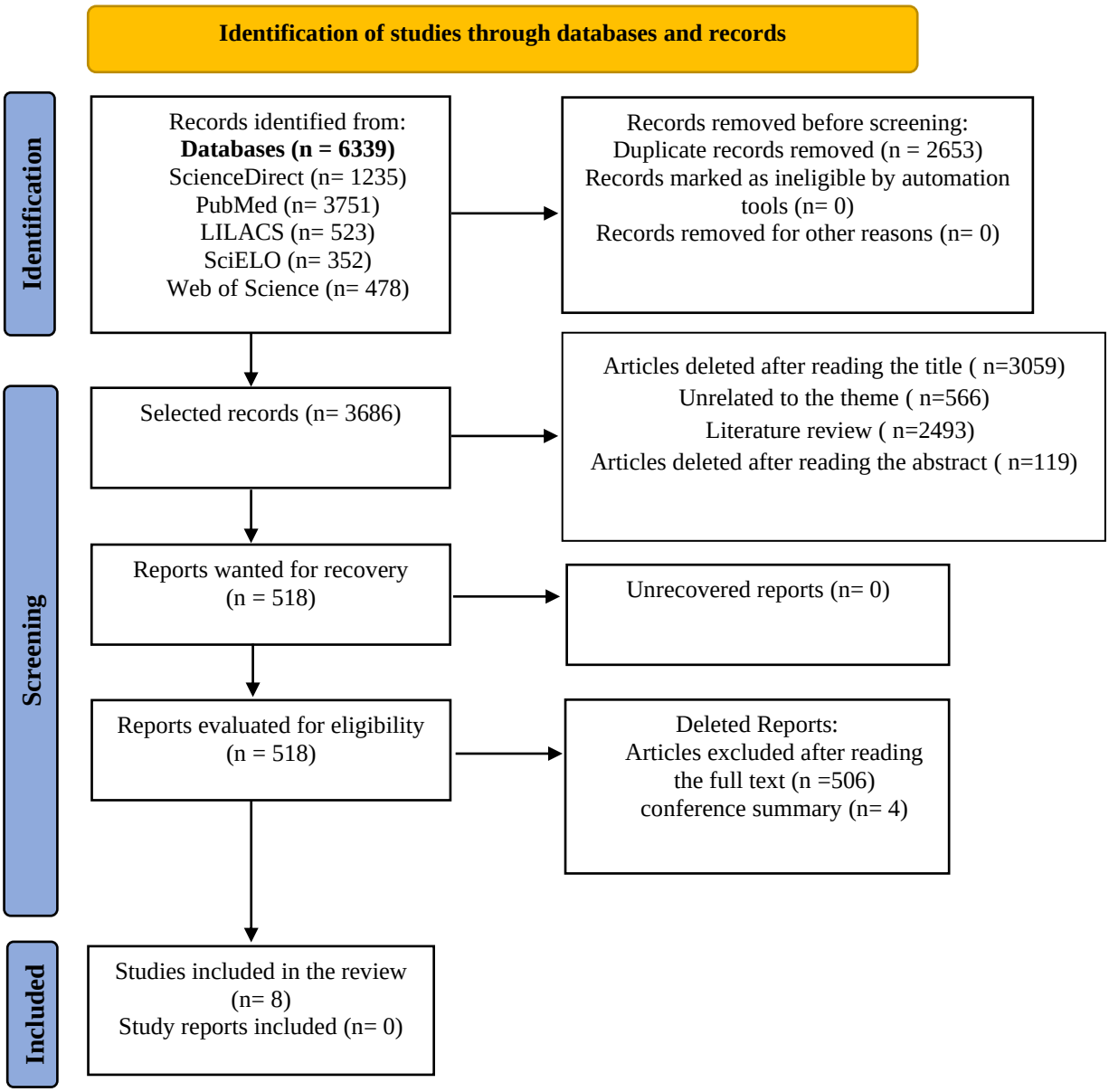
(SWiM) Guideline (Campbell et al., 2020). In addition, exploratory analyzes were performed to verify possible causes of heterogeneity between studies that contributed to the identification of different results. However, due to the significant heterogeneity of the included studies, it was impossible to perform a meta-analysis.

3. Results and Discussion

3.1 Description of Included Studies

The search strategy retrieved potentially eligible articles, and 2653 duplicates were excluded. Among the 3686 manuscripts retrieved in the initial search, 2940 were excluded after reading the title and abstract, and 518 articles seemed to meet our selection criteria. However, after reading the articles in full, 8 original studies remained and were included in the qualitative synthesis (Figure 1). The characteristics of the included studies are shown in Figure 1.

Figure 1. PRISMA flowchart of systematic review.



Source: Authors.

The studies included in this systematic review showed high heterogeneity, which prevented quantitative synthesis through a meta-analysis. The studies used HCQ and CQ and the pharmacological effects associated with their use in patients with COVID-19 were evaluated.

In relation to the doses, the lower the concentration used, the studies included at some point were 200mg, and the higher it was 800mg. In some two studies, the dose did not follow a dose pattern, and it was used a single attack dose to target the therapeutic janelle, and followed by subsequent doses for its maintenance.

Regarding the main findings and general characteristics of these 8 studies, the publication was selected from the period 2020 to 2021, when the pandemic started in December 2019 in China, and all 8 articles were published in international journals. Different clinical designs were used in these articles, where the selected studies identified the clinical condition of the patients included, as well as their respective clinical outcomes according to the treatment used.

Table 1. Description of study findings.

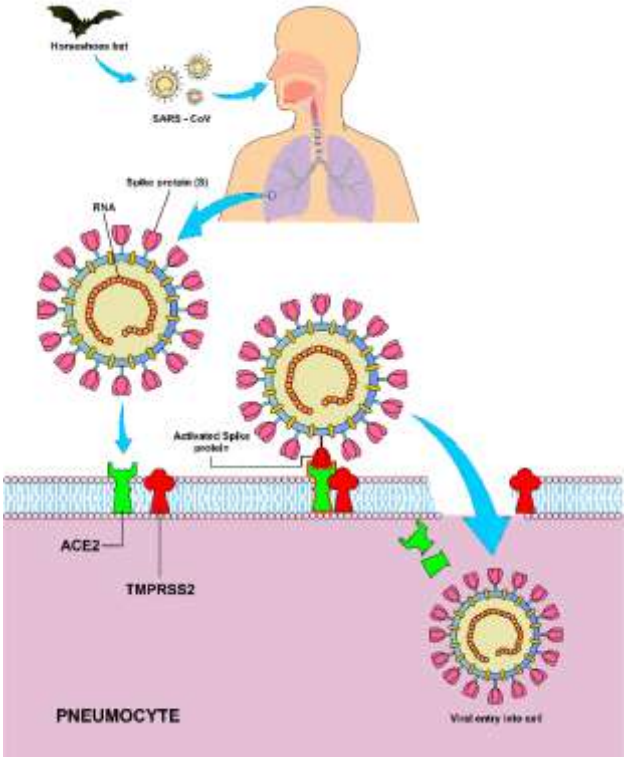
AUTORES	NUMBER OF PATIENTS	MIDDLE AGES	PHARMACEUTICAL - INTERVENTION	CLINICAL OUTCOMES
Skipper et al. (2020)	491 patients randomly in a group (423 contributed with primary outcome data). However, 341 (81%) positive for infection or exposure to a confirmed patient.	Average of 41 years	Oral HCQ (1x 800mg, followed by 600mg for 6 to 8 hours, then 600mg per day for another 4 days).	In 14 days, 24% (49 of 201) with HCQ showed continuous symptoms compared to 30% (59 of 194) with placebo. Adverse effects occurred in 43% (92 of 212) with HCQ versus 22% (46 of 211) who received placebo. With placebo, there were 10 hospitalizations (2 unrelated to COVID-19), including 1 hospitalized death. With hydroxychloroquine, 4 hospitalizations occurred plus 1 non-hospitalized death.
Self et al. (2020)	Among the 479 randomized patients, 242 (50.5%) were randomized to the HCQ group and 237 (49.5%) were randomized to the placebo group.	Average of 57 years	Patients randomized to receive hydroxychloroquine (400 mg, 2x daily, for 2 doses, then 200 mg 2x daily for 8 doses) (n = 242) or placebo (n = 237) in 14 days.	There was no significant improvement in clinical status at 14 days associated with HCQ treatment compared to placebo. 25 of 241 patients (10.4%) in the HCQ group and 25 of 236 (10.6%) in the placebo group died. At 5 days after randomization, 13 patients (5.9%) were observed in the HCQ group and 7 patients (3.3%) in the placebo group with assessed QTc, registering a QTc interval greater than 500 ms. A total of 30 serious adverse events were reported, including 18 serious adverse events in 14 patients (5.8%) for the HCQ group and 12 serious adverse events in 11 patients (4.6%) for the placebo group.
Chen et al. (2020)	30 patients	Average of 50 years	400mg de HCQ/ day for 5 days	On the 7th day after enrollment, 13 cases (86.7%) in the test group and 14 cases (93.3%) in the control group had tests with negative results. 1 patient in the experimental group had severe illness, and the medication was discontinued on the fourth day. During the 2-week visitation period, the tests of all individuals were negative. In addition, the occurrence of adverse events in patients with moderate to severe disease was not related to medication. Thus, the study suggests that current patients with COVID-19 in general have better overall treatment effects.
Huang et al. (2020)	22 patients	Average of 44 years	Wo groups: 10 patients (3 severe and 7 moderate) 500mg of QC 2x daily for 10 days and 12 patients (5 severe and 7 moderate) Liponavir / ritonavir 400 / 100mg orally 2x daily for 10 days.	1 patient in the QC group tested negative after treatment for 2 days, and the other negative also during treatment. 5 patients suffered 9 adverse events, such as vomiting, pain, diarrhea, nausea, rash or itching, shortness of breath and cough.
Barbosa et al. (2020)	63 individuals (32 in the HCQ group and 31 in the standard support care group)	Average of 62 years	400mg HCQ orally twice a day	The HCQ group had a significantly higher respiratory rate of need for support in 5 days compared to the supportonly group. In addition, there was greater mortality in the hydroxychloroquine group 4/32 (12.90%) compared to the supportive care group 1/31 (3.13%).
Ip et al. (2021)	Among 1274 outpatients tested with SARS-CoV-2 infection in polymerase chain reaction (PCR), 97 received HCQ or note of an	Average of 55 years	86 patients received 400 mg of HCQ twice a day, on day 1, and 400 mg / day on days 2 to 5. The other participants received 200 mg prescribed 3x a day (n = 6) or another (n = 5). The median	In a propensity-matched cohort of 1,067 patients it was observed that 21.6% of outpatient HCQ exposure were hospitalized and 31.4% without exposure were hospitalized. However, QTc prolongation events have been reported in 2% of patients using HCQ. Arrhythmia events were not reported among patients.

	outpatient exposure to HCQ.		pretension duration of pharmacotherapy was 5 days.	
Sogut et al. (2021)	There were 78 male patients (51.3%) and 74 female patients (48.7%) confirmed for SARS-CoV-2 in real time polymerase chain reaction (RT-PCR) who received outpatient treatment.	Average of 47 years	Suggested pharmacotherapy of HCQ in a loading dose was performed with 400 mg 2x on day 1, followed by 400 mg per day (200 mg twice a day) for an additional 4 days.	An increase in the median QTc1 interval from 425.0 to 430.0 milliseconds (ms) was observed. However, an increased risk of ventricular tachycardia was not associated. None of the patients had ΔQTc of> 60 ms or a QTc2 of> 500 ms. Significant clinical improvement was observed in 91.4% of patients (except for 13 patients in whom non-serious adverse drug reactions were reported or who had severe COVID-19 and progressed to hospitalization. Based on this, the adverse effects associated with HCQ did not were considered severe and occurred in approximately 52.8% (n = 80) of patients.
Fteiha et al. (2021)	Among 297 patients confirmed for COVID-19, 149 were treated with HCQ.	Average of 65 years	In the treatment with HCQ the dose was 400 mg twice a day on day 1, and 200 mg twice a day for 5 to 10 days.	A total of 39 patients (43%) of the total were in a serious or critical illness. In addition, 23 (26%) of the patients had clinical conditions for hypertension and obesity. There was an evolution of QTc prolongation in 14 of the patients (16%). In patients over 65 years of age, clinical conditions associated with QTc prolongation were present. In patients who received HCQ treatment, QTc prolongation associated with the presence of traditional risk factors, such as hypokalaemia and treatment with furosemide.

Source: Authors.

Horseshoe bats serve as natural hosts and are reservoirs for SARS-CoV. The first step in the viral replication cycle is mediated by the viral protein spike (S) and offers several potential therapeutic targets. Protein S uses the angiotensin-2-converting enzyme receptor (ACE2) for entry into the cell, but also sialic acids linked to cell surface gangliosides (Fantini et al., 2020). In addition, coronavirus S protein is a facilitator of viral entry into target cells, as described in Figure 2. Therefore, entry requires the activation of protein S by cellular proteases, which affects the cleavage of protein S at the S1 / S2 and S2 'sites, thus allowing the fusion of viral and cellular membranes, where this process is driven by the S2 subunit. In this way, the SARS-S receptor gets involved with the angiotensin-converting enzyme 2 (ACE2 / ECA2) as the input receptor (Li et al., 2003) and releases the cellular serine protease TMPRSS2 to influence and activate the protein S (Matsuyama et al., 2010; Shulla et al., 2010).

Figure 2. Bats are hosts of SARS-cov. In human contamination, viral replication is mediated by the spike protein that uses the angiotensin-converting enzyme receptor-2 (ACE2) for entry into the cell.



Source: Authors.

SARS-S and SARS-2-S share 76% amino acid identity, but studies have yet to show how SARS-2-S or SARS-S employs ACE2 and TMPRESS2 to adhere to target cells (Hoffmann et al., 2020). In this context, the transmembrane serine protease TMPRSS2 activates the coronavirus peak protein (Iwata-Yoshikawa et al., 2019). In addition, in this study, less expression of cytokines and chemokines observed in mice deficient in TMPRSS2 became evident than in mice that had TMPRSS2 activity after coronavirus infection. Viral replication is probably one of the main causes of the high levels of inflammatory chemokine observed in WT mice, however, the possibility of TMPRSS2, a serine protease, also being able to contribute to inflammatory reactions was also evaluated. Thus, activation of coronavirus S proteins by target cell proteases is essential for viral entry into cells and encompasses protein S cleavage at S1 / S2 and S2 'sites. The S1 / S2 cleavage site of SARS-2-S houses several arginine residues (multibasic), which indicates high cleavage activity (Menachery et al., 2020; Yang

et al., 2014).

TMPRSS2 is then dispensable for development and homeostasis and, therefore, constitutes an attractive target for drugs (8L), for this reason, it constitutes a more attractive target for drugs (Kim et al., 2006). Thus, CQ, an existing drug, has been gaining north, which has been studied for some time and used clinically since 1944. In response to SARS, work began in mid-2004, when this research was QC phosphate was used in VeroE6 cells. In conclusion, the inhibition of SARS-CoV entry into the functioning cell type was noticeable (Keyarerts et al., 2004).

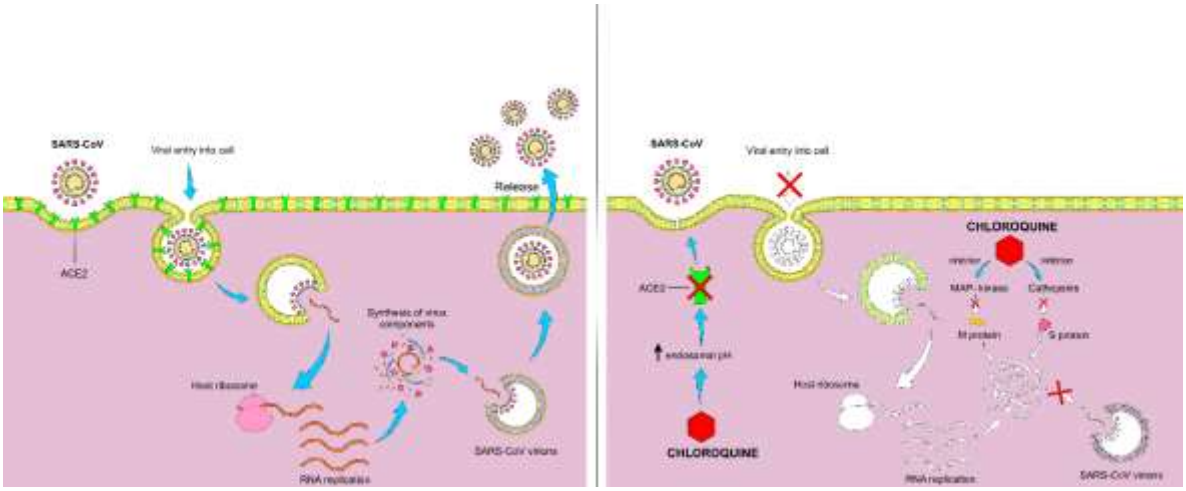
The existence of a probable prophylactic effect of QC or HCQ against COVID-19 generates the use of higher doses, but a better understanding of how they act in SARS-Cov-2 would be of great value for optimization in the development of preventive and therapeutic strategies, so that the ideal dosage is evaluated (Hu et al., 2020). According to Colson et al. (2020) HCQ and CQ have similar mechanisms of action in antiviral activity, where the search for more assertive doses also depends on the form of use, assuming that it may still be necessary to administer a load dose followed by a dose of maintenance.

QC increases endosomal pH and interferes with the glycosylation of the COVID-19 cell receptor and therefore has the potential to block viral infection (Wang et al., 2020).

> "QC also inhibits quinone reductase-2, which is involved in the biosynthesis of sialic acid (an acid monosaccharide of cellular transmembrane proteins necessary for the recognition of ligands) that makes this agent a broad antiviral agent. It is important to note that both the human coronavirus HCoV-O43 and the orthomyxoviruses use portions of sialic acid as a receptor. In addition, QC alters the pH of lysosomes and probably inhibits cathepsins, which play an important role in generating peptides by cleavage. QC altering the ph of lysosomes inhibits cathepsins. intracellular and pathogens and end up degrading the S protein (Spike) main binding protein of SARS-CoV-2. In addition, CQ, through the inhibition of MAP kinase, this protein participates in the process of gene expression, proliferation potency interfering in the molecular crosstalk (physical or chemical process that a signal is transmitted by the organism to catalyze enzymes or proteins), in processing of protein M that will directly interfere in the assembly of the virus (Wang et al., 2020). "

This drug also works by altering the pH of lysosomes with the possibility of inhibiting cathepsins leading to the formation of the autophagosome that denatures the spike protein of COVID-19 as well as the inhibition of MAP kinase will interfere in the molecular crosstalk, in addition to altering the assembly of the virion interfering in the processing of M. protein, as described in figure 3 (Colson et al., 2020).

Figure 3. Proposed hydroxychloroquine mechanism of action for SARS-COV-2 mediated by lysosomal pH change.



Source: Authors.

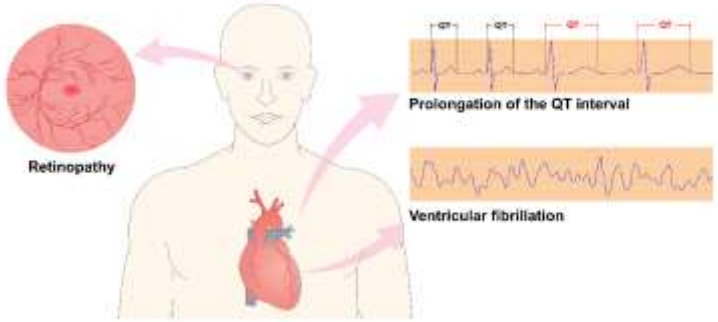
Previous studies have shown that QC has antiviral activity against SARS-COV-1, which can be attributed to a deficit in glycosylation receptors present on the surface of the virion so that it does not allow binding to ACE2 produced mainly by the lung, but also present in the lung, kidney, heart and intestine (Vincent et al., 2005). The new virus studied in 2020 had characteristics very similar to SARS-CoV-1, in addition to the name also having the same or similar glycoproteins, so it is believed that the new virus has the same ACE2 receptor on its surface. Based on this, it has been theorized that QC can also interfere in the binding of COVID-19 to target cells (Colson et al., 2020; Wang et al., 2020; Lu, 2020).

In analyzed *in vitro* studies, the use of both QC and its derivative to HCQ proved to be a great alternative. Liu et al. (2020) tested *in vitro* the cytotoxicity of CQ and HCQ in VeroE6 cells of the African green monkey kidney (ATCC-1586) determined by CCK-8 assay, the result showed the cytotoxic dose (CC50) of QC and HCQ were 273.20 and 249.50μM, respectively. These cells were added to 04 different doses of 0.01, 0.02, 0.2 and 0.8 mg / kg in MOIs (multiplicities of infection) of the two drugs. The control cells were treated with PBS for 1h, thus, they were able to observe with these tests the result comparing HCQ and QC, which confirmed that HCQ effectively inhibited the entry stage, as well as the post-entry stages of SARS-CoV -2, which were also found slightly softer in the treatment with QC. However, *in vitro* studies limit pharmacodynamic and pharmacokinetic parameters, which must be analyzed in an organism by *in vivo* studies.

According to Jie et al. (2020), the use of QC for the purpose of involution of COVID-19 replication in humans can be 500mg, being administered twice daily, for 10 days. In cases of severe gastrointestinal symptoms, the use is reduced to 500mg once a day or discontinued. In the study by Marmor (2020), administration is up to 10 days in dosages of 500mg of QC, twice a day or 400mg of HCQ four times a day, as the dosages proposed for COVID-19 are 4 to 5 times higher.

In study by Gautert et al. (2020) the synergistic effect of the combination of HCQ and azithromycin (AZT) was introduced, in which previous studies of azithromycin proved to be effective *in vitro* against the Zika and Ebola viruses, in addition to preventing serious infections of the respiratory tract when administered to patients with viral infection (Retallack et al., 2016; Madrid et al., 2015; Bosseboeuf et al., 2018). In a case report prepared by Gabriels et al. (2020), a 72-year-old woman was medicated with 400mg of HQC twice daily and 500mg of intravenous AZT for 5 days. Throughout the treatment, changes in the cardiac profile were observed with an electrocardiogram to observe the prolongation of the QT interval, since they are already known adverse effects, for this it is up to the risk assessment. The adverse effects of the drug are already established in relation to the treatments to which it was destined as malaria, mainly in populations at risk, as described in Figure 4.

Figure 4. Retinopathy, QT interval prolongation, and ventricular fibrillation are adverse events described in the literature associated with the use of chloroquine and hydroxychloroquine.



Source: Authors.

Given the range of studies analyzed, some specific studies that demonstrate contradictory effects are noticeable, such as a study by Barbosa et al. (2020) which statistically measured the therapeutic effect of HCQ compared to a standard treatment, 63 patients with a mean age of 62 years. In conclusion, a greater need for respiratory support was found in 5 days of treatment in the HCQ group when compared to the group with standard treatment. At the end of the treatment days, the mortality rate in the group with standard treatment was lower with 3.13% when compared to the group with HCQ, in which the mortality rate reached 12.90%. In the study presented by Gabriels et al. (2020) 400 mg of HCQ with AZT was administered to a 72-year-old patient who, during treatment, showed ventricular fibrillation, increased beats per minute to 150 and cardiac arrhythmia. But, in a case report presented in a 66-year-old patient also with HCQ and AZT, there was complete treatment without arrhythmic events (Mitra et al., 2020). However, in case of case reports, there are limitations and variables that should be better analyzed in larger and more rigorous studies.

In a study by Borba et al (2020), reported the administration of CQ in high doses of 600mg and CQ in low doses of 450mg for 10 days in 81 patients, who were presented throughout the study 11/81 deaths among the study patients. However, the study showed 54/81 (67.5%) of patients with some type of comorbidity. Thus, another preliminary randomized clinical study by Barbosa et al. (2020) administered 400mg of HCQ 2x daily in 63 patients, and the study outcome showed higher mortality in the HCQ 4/32 group (12.90%) in relation to the supportive care group 1/31 (3.13%). In a study of systematic review and meta-analysis it was observed that the effects of the treatment of HCQ alone was not associated with reduced mortality in patients hospitalized with COVID-19. However, it has been reported that the combined treatment of HCQ + AZT significantly increased mortality in patients (Fiolet et al., 2021).

In observed observational studies, the outcomes below have been reported. In these studies, it was reported that a percentage of participants received from HCQ had prolonged QTc intervals (Ip et al., 2021; Sogut et al., 2021; Fteiha et al., 2020). In the study by Sogut et al. (2021), 91.4% of the participants showed significant improvement, however 13 had adverse events (Sogut et al., 2021). However, the results of these studies were very limited given the small number of participants. It was reported that in comparison, the HCQ group had a 21.6% hospitalization rate, versus 31.4% hospitalized who did not receive HCQ. The result was not very significant, also considering the presence of 2% of QTc prolongation events (Ip et al., 2021). However, the greatest limitations of these studies are their observational methodology, in which there is no possibility to analyze rigorously and safely the comparison of pharmacological effects.

In contrast, randomized controlled clinical trial studies provide us with this rigorous comparative analysis methodology for accurate assessment of pharmacological effects in patients with COVID-19. It was reported in the study by Skipper et al. (2020), that the HCQ group had 4 hospitalizations and the placebo group with 10 hospitalizations. Continuous symptoms were 24% for the HCQ group and 30% for the placebo group. However, adverse events for the HCQ group were 43%, versus 22% for the placebo group, seen more than twice as many participants with HCQ-associated adverse events compared to placebo. In the study by Self et al. (2020), there were no significant clinical improvements compared to placebo. In addition, 5.9% of participants in the HCQ group had prolonged QTc intervals, versus 3.3% in the placebo group. Regarding the reported serious adverse events, 5.8% were observed in the HCQ group and 4.6% in the placebo group. In studies of randomized clinical trials to evaluate the effectiveness of HCQ with a prophylactic measure of post-exposure to COVID-19 with 87.6% of the participants (719 out of 821) who reported high risk exposure to a confirmed contact with COVID-19, it was reported that HCQ or CQ did not prevent the disease or confirmed infection used within 4 days of exposure (Boulware et al., 2020). Therefore, randomized clinical trial studies have not proven the efficacy of HCQ for patients with COVID-19.

4. Conclusion

Although *in vitro* studies have observed viral replication inhibition outcomes, caution should be exercised, since, when conducting the study for humans, there is every metabolic alteration by the organism, in which the pharmacodynamic and pharmacokinetic factors are involved. In observational, pilot studies or case reports, minor or non-significant difference outcomes have been reported with respect to improving the patients' clinical status, and even in these, adverse events have been observed, mainly prolongation of the QTc interval. In randomized controlled clinical trials, no significant difference was observed between admissions and, and in relation to the improvement of clinical status, there was no significant difference, and it was not associated with improvement in clinical symptoms. In addition, these outcomes were associated with adverse events, mainly prolongation of the QTc interval. The results of the studies showed that the use of HCQ has outcomes with no significant improvement in the clinical picture, including association with adverse events (including serious) in patients with COVID-19. Thus, randomized clinical trials did not provide evidence of the efficacy of HCQ in patients with COVID-19, showing that an HCQ is not significantly effective, and has negative results regarding its safety for patients with COVID-19.

HCQ proves to be ineffective and unsafe for patients with COVID-19. Therefore, the studies already elucidate enough information about this clinical approach, therefore, the discussion about the search for the resolution of the COVID-19 pandemic should be guided by effective and safe proposals, as the presence of vaccines are also available to the population.

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