Using Chloroquine and Hydroxychloroquine in the Treatment of COVID-19: Does It Make Sense?

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ABSTRACT
The severe acute respiratory syndrome caused by the new coronavirus SARS-Cov-2 (COVID-19) has quickly turned into a pandemic, infecting more than 10 million people and causing more than 500,000 deaths worldwide. The absence of an effective treatment against this disease has led several researchers to investigate the possibility of redirecting drugs already known to be effective against other diseases in the treatment of COVID-19, among them the antimalarial drugs chloroquine and hydroxychloroquine. This review aims at showing how chloroquine and hydroxychloroquine came to be considered as possible drugs in the treatment of COVID-19 and how the recent in vivo experiments described so far shed light on the adequacy of this use.

Key words: COVID-19, SARS-Cov-2, Coronavirus, Chloroquine, Hydroxychloroquine, antiviral activity.

INTRODUCTION
“Using chloroquine and hydroxychloroquine in the treatment of COVID-19: does it make sense?” This question has arisen in recent months in view of the vast discussion witnessed in the media about the possible use of these drugs in the treatment of COVID-19. Really, at first, it seems quite uncommon because both chloroquine (CQ) and hydroxychloroquine (HCQ) are classically antimalarial drugs that have also been repositioned in the pharmaceutical market for the treatment of some autoimmune diseases, such as lupus and rheumatoid arthritis, among others. But, after all, why would CQ or HCQ, both antimalarial drugs, make sense in the treatment of an infectious disease caused by the new coronavirus? This issue was the driving force behind the gathering of data on this subject and the writing of this article. A combined systematic and narrative review on articles published until June 2020 reporting antiviral properties of both CQ and HCQ was carried out to bring more relevant and accessible technical information to a larger portion of society, thus contributing to enrich their understanding and better base their opinion on this subject.

This paper is organized in three main parts. The first one will address basic information about SARS-Cov-2, highlighting the onset of the disease, the viral structure and the stages of infection of this new coronavirus. A second step will take a brief historical approach to the emergence of CQ and HCQ and will comment on their antiviral and anti-inflammatory properties. Finally, the third part will comment on the results of the latest in vivo experiments with these substances against COVID-19 and discuss whether there is, in fact, any safe relationship between these molecules and their possible use in the treatment of this disease.

SEVERE ACUTE RESPIRATORY SYNDROME (COVID-19)

The term “COVID-19” represents the abbreviation of the English expression “Corona Virus Disease” and the number “19” of that term indicates the year in which the first report of this disease was made in...
the world (2019). On December 31, 2019, the World
Health Organization's office in China received a statement
that the first reports of an unknown pneumonia had
arisen in Wuhan city, Hubei province, China. A week
later, Chinese authorities reported the discovery of the
causative agent for this new type of pneumonia: a new
coronavirus (Figure 1), which was named SARS-CoV-2,
an English expression meaning Severe Acute Respiratory
Syndrome Coronavirus-2. This epidemic quickly turned
into a pandemic, being recorded in just a few months in
practically all 216 existing countries and surpassing the
mark of 10 million infected and 500 thousand deaths
worldwide.

This new coronavirus belongs to the Coronaviridae
family, which is subdivided into four genera based on
their genetic properties: Alphacoronavirus, Betacoronavirus,
Gammacoronavirus and Deltacoronavirus. Coronaviruses
can infect not only humans but also many species
of animals, such as dogs, cats, rodents, cattle, pigs,
horses, camels, birds, bats, rabbits and other wild animals.
SARS-CoV-2, which causes the current pandemic,
together with the species SARS-CoV and MERS-CoV
(coronavirus that causes respiratory syndrome in the
Middle East) which were responsible for recent epidemics
that occurred in 2002-2003 and 2012, respectively, all
belong to the Betacoronavirus genus and are zoonotic
pathogens that can cause more severe respiratory
diseases in humans. Besides them, the species HKU1,
NL63, OC43 and 229E also infect humans, but cause
milder symptoms.

Figure 1-A shows a photo of the SARS-CoV-2
coronavirus by electron microscope. This group of
viruses is characterized by a crown appearance, hence
the term “corona” in the name. Figure 1-B shows,
schematically, the main parts that constitute the
viral structure of the coronavirus, namely: the
“S” glycoprotein, also known as “Spike protein”,
represented in yellow; the membrane protein (M), the
main component of the envelope (E), both represented
by the brown structure; and finally the RNA genome,
single and positive strand, wrapped in the “N” protein,
thus forming a nucleocapsid that is represented by the
structure in green.

The process of infection and replication of the
coronavirus occurs in the epithelial cells of the human
respiratory tract and is schematically represented in
Figure 2. The lungs are the organs most compromised
by COVID-19 because the coronavirus readily recognizes
the cells of these organs due to the high affinity that the
Spike protein has by the receptor of the angiotensin-
converting enzyme 2 (ACE-2), more abundant in
the membrane of the alveolar cells of the lungs. The
adsorption step, therefore, represents the binding of the
virion to the membrane surface of the host cell
(Figure 2, Step 1). Through endocytosis, the endosomes
are then formed (Figure 2, Step 2) and then become
lysosomes (Figure 2, Step 3). The acidic pH of the
lysosome allows the envelope of the virus to join the
membrane of this organelle, launching the genomic
material out of the lysosome. Next, since it is released
into the cytoplasm (Figure 2, Step 4), the genomic RNA
will follow both the path of transcription - formation
of new RNA genetic materials and translation along
the ribosome to form the enzymes and proteins that
will make part of the future virus structure which will
be formed (Figure 2, Step 5). Finally, in the assembly
of Peru between 1628 and 1629, the viceroy’s wife, the Countess of Cinchón, was stricken with malaria in Lima. Because she was a very popular person, news of her illness quickly spread to all the people in the country side and finally reached the Lloxa region, where a Spaniard was in the government. He promptly sent the sick Countess a medicine obtained from the bark of a tree found in that region. Days later, the therapeutic result obtained was extraordinary: The Countess of Cinchón improved quickly and, therefore, because she was so delighted with the result, she distributed this remedy to the poor people of Lima who also suffered from tertiary fevers. It is believed that, based on this tale, the Swedish botanist Carl Linnaeus (1707-1778) would have named the tree as “Cinchona” in honour of the Countess of Cinchón. The discovery of Quina-Quina was a remarkable scientific fact in medicine at the time, because many people began to be cured of malaria using the powder from Quina-Quina bark, which in the subsequent years led to a rapid export of seeds from that plant to other parts of the world, mainly Europe and Asia.9

However, another memorable scientific breakthrough took on an even greater role in history almost 200 years later. In 1820, the French chemists Pierre Pelletier and Joseph Caventou described the isolation of quinine from the bark of Cinchona sp. and classified this substance as an alkaloid. Scientifically, this fact would definitely mark the history of Science because it started to have a pure substance (quinine) that would be used for the first time as an antimalarial drug. This allowed that adequate and standardized doses of this antimalarial could be administered in relatively smaller amounts compared to the amount of bark's powder used to achieve the same therapeutic effect. Subsequent studies on quinine showed that it, in fact, it inhibited the growth of Plasmodium falciparum, the causative agent of malaria, only in the acute phase of the disease. However, quinine was not as efficient in the chronic phase as it was unable to stop the sporulation of schizonts and gametocyte development.10

Later, during the First World War (1914-1918), it was observed that many armies were immobilized on the Balkan peninsula because of malaria, resulting in the loss of the lives of many soldiers. At that time, it was said that malaria killed more soldiers than the enemy artillery itself. Even so, many lives were saved in the British and French armies thanks to the use of quinine.11

In addition to their valuable medicinal use, antimalarial drugs also began to assume military and strategic importance, which stimulated many researches that followed in the post-war period in the search for new
antimalarial drugs inspired by the quinine’s structure (Figure 3, QN). Pamaquine (Figure 3, PAMQ), discovered in 1926, also known as plasmoquine, was the first synthetic antimalarial with an 8-aminoquinoline nucleus that later inspired primaquine synthesis in 1946 (Figure 3, PRIMQ). Mepacrine (Figure 3, MEPC), an acridine, was synthesized in 1932 and only then, in 1934, the preparation of 4-aminoquinoline chloroquine (Figure 3, CQ) was announced by Hans Andersag in Bayer laboratories. Initially, Bayer rejected CQ since this compound appeared to be toxic to human use. In May 1945, however, after being tested in many naturally infected patients, the CQ showed an efficient antiparasitic action with a rapid decrease in the symptoms of malaria, accompanied by a relatively low toxicity as evaluated by the American researchers who conducted the study. Thus, from the beginning of the 1950s, CQ started to be recommended as the medicine of choice for the treatment of all cases of malaria in the world. The purpose of replacing quinine with new antimalarial substances, such as CQ, in order to increase the number of drugs available on the market for the treatment of this parasitic disease, also led to the first synthesis of hydroxychloroquine (Figure 3, HCQ) described by the researchers Surrey and Hammer, in 1946, at Sterling-Winthrop Research Institute in New York, United States. They expected that the introduction of a hydroxyl in the primary carbon of one of the N-ethyl groups would bring greater water solubility and less toxicity to the molecule. In fact, HCQ was proved to be less toxic to animals than CQ itself. Only in 1955, HCQ was made available as a medicine after receiving approval from the Food and Drug Administration (FDA). In 1953, the physician J. Charles Shee reported an accidental discovery: the daily administration of CQ sulfate to a 62-year-old patient resulted in the cure of her advanced chronic lupus erythematosus. This achievement opened the door for the use of CQ and HCQ in the treatment of autoimmune diseases such as lupus and rheumatoid arthritis, among others.

WHAT IS THE RELATIONSHIP OF BOTH CQ AND HCQ WITH ANTIVIRAL ACTION?

In the early 1960s, several studies in different in vitro systems showed that, after the adhesion of viruses to the surface of the cytoplasmic membrane of animal cells, viruses entered the cytoplasm through structures called “pinocytic vesicles” or “phagosomes”. They observed that, inside these phagosomes which then turned into lysosomes, when exposed to the acidic pH and lysosomal enzymes of the host cell, the viruses use this hostile environment to lose their envelope next to the lysosomal membrane and release their genomic material into the cell cytoplasm. Therefore, substances that influence the

Figure 3: Molecular formulas of quinine and the first synthesized quinolinic antimalarials.
stability of the lysosome membrane could also affect the virus’s multiplication process within the host cell. Based on this hypothesis and in view of the observation made by Weissmann that CQ stabilizes lysosomal membranes under certain study conditions, Mallucci (1966) investigated the in vitro effect of CQ on the growth of rat hepatitis coronavirus (MHV-3) in macrophage culture. His results showed that the exposure of these macrophages to increasing concentrations of CQ caused an increasing inhibition in the growth of the virus, until the complete disappearance of the virus at the highest concentration of CQ tested (60 µg/mL). These results impelled the investigation of the antiviral action of these quinolinic antimalarials in other known viruses. It didn’t take long for new results from in vitro experiments to show that CQ and/or HCQ also inhibited the growth of several other viruses that are pathogenic to humans and animals. Table 1 displays twenty virus species belonging to eleven families as being susceptible to the antiviral action of CQ and/or HCQ in in vitro assays by means of different cell systems. Against SARS-Cov-2, it is curious to note that CQ was slightly more potent (EC\textsubscript{50} = 2.7–7.4 µM) than HCQ (EC\textsubscript{50} = 4.5–13 µM), although HCQ figures as less toxic than CQ.

In most of the studies listed in Table 1, the main mechanism of antiviral action of CQ and HCQ is attributed to the stabilization that these substances bring to lysosomal membranes due to the increase of pH within these organelles, thus causing interference in the loss of the virus envelope, which prevents the release of viral genomic material towards the cytoplasm of the host cell and thus interrupts its multiplication. Both CQ and HCQ are weak bases and, therefore, they are able to affect acidic vesicles and cause the dysfunction of several enzymes with the neutralization of this acidic pH. Outside the cell, CQ and HCQ are largely in protonated form and become unable to cross the plasma membrane due to their positive charge. However, non-protonated molecules can enter the intracellular compartment and become immediately protonated by the very acidic pH. Thus, these acidic organelles (endosomes, Golgi vesicles and lysosomes) accumulate more and more protonated CQ or HCQ, increasing their internal pH. In this way, both CQ and HCQ can inhibit virus replication by interfering with its endosome and lysosome-mediated entry (Figure 2, steps 2 and 3), or in the final stages of virus replication (Figure 2, steps 5–7) that happen with the participation of Golgi vesicles.8,20

Recently, structural and molecular modelling studies have revealed a new possible mechanism of action for CQ and HCQ against SARS-Cov-2 infection. Considering that the first stage of the viral replication cycle is the adhesion of the coronavirus to the surface of the respiratory cells through the affinity of the viral protein Spike (Protein S) to the angiotensin-2-converting enzyme (ACE-2) receptor, in silico studies have shown that both CQ and HCQ bind to sialic acids and gangliosides with high affinity, making the viral S protein no longer able to bind to the cell surface gangliosides. Thus, according to these results, CQ and HCQ would act against the infection of this new coronavirus by disrupting the adhesion of this virus to the cell surface, preventing its subsequent entry into the cell’s cytoplasm.8

Finally, it is worth remembering that CQ and HCQ also act on the human immune system. The accumulation of CQ and HCQ in lymphocytes and macrophages is responsible for the anti-inflammatory properties of these drugs, a fact that justifies them in the treatment of some autoimmune diseases such as rheumatoid arthritis, lupus erythematos and sarcoidosis, among others. All of these diseases show some relationship with the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF\textalpha). Both CQ and HCQ reduce the secretion of such cytokines, especially TNF\textalpha, which causes a decrease in the body’s inflammatory response. The mechanisms of TNF\textalpha inhibition by CQ and HCQ, together with other more detailed information on the anti-inflammatory action of these molecules, can be found in the extensive literature available.17 The anti-inflammatory action may be an interesting side effect for repositioning molecules as antiviral drugs, since many viral infections are accompanied by inflammatory conditions, as it is the case of COVID-19.

ARE CQ AND HCQ SUITABLE FOR USE IN THE TREATMENT OF COVID-19?

In vitro (in Latin, “in glass”) assays are experiments where biological processes take place outside living systems, in properly controlled, closed environments and usually performed in glass containers. In turn, in vivo tests (in Latin, “within the living”) refer to experiments that occur or take place within a living organism. Based on the results of in vitro tests that demonstrated the inhibitory action of CQ and HCQ against infection with the new coronavirus SARS-Cov-2 (Table 1), it is natural to expect that these antimalarials would also be active in in vivo experiments. In other words, if these molecules were active against SARS-Cov-2 in assays outside the body, they are also likely to fight this coronavirus inside the human body when administered to sick patients.
### Table 1: Studies describing *in vitro* antiviral activity of chloroquine (CQ) and/or hydroxychloroquine (HCQ) against pathogenic viruses.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genome</th>
<th>Virus</th>
<th>Disease</th>
<th>Cultured cells</th>
<th>Compound</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunyaviridae</td>
<td>RNA, ss (-)</td>
<td>CCHFV</td>
<td>Hemorrhagic fever</td>
<td>Vero E6, Huh7</td>
<td>CQ, 0-50 µM</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 28.1-43.4 µM Selectivity Index = 21-36</td>
<td>21</td>
</tr>
<tr>
<td>Coronaviridae</td>
<td>RNA, ss (+)</td>
<td>MHV-3</td>
<td>Hepatitis (rodents)</td>
<td>Peritoneal macrophages</td>
<td>CQ, 0-60 µg/mL</td>
<td>Almost complete virus disappearance when CQ = 60 µg/mL</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OC43</td>
<td>Flu</td>
<td>HRT-18</td>
<td>C, 0.032-500 µM</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; = 0.302 µM Selectivity Index = 1369</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SARS-Cov</td>
<td>Respiratory syndrome</td>
<td>Vero E6</td>
<td>CQ, 0-100 µM</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 8.8 ± 1.2 µM Selectivity Index = 30</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SARS-Cov2</td>
<td>Respiratory syndrome</td>
<td>Vero E6</td>
<td>CQ, 0-50 µM HCQ, 0-50 µM</td>
<td>CQ, EC&lt;sub&gt;50&lt;/sub&gt; = 2.7-7.4 µM HCQ, EC&lt;sub&gt;50&lt;/sub&gt; = 4.5-13 µM</td>
<td>24</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>RNA, ss (-)</td>
<td>EBOV</td>
<td>Ebola</td>
<td>MRC-5</td>
<td>CQ, 0-10 µM</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 3.319 µM</td>
<td>25</td>
</tr>
<tr>
<td>Flaviviridae</td>
<td>RNA, ss (+)</td>
<td>HCV</td>
<td>Hepatitis C</td>
<td>Huh-5-2</td>
<td>CQ, 0-97 µg/mL HCQ, 0-33 µM</td>
<td>CQ (10.75 µM) e HCQ (6.6 µM): 93% inhibition</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZKV</td>
<td>Zika</td>
<td>BHK-21, Huh7, Vero</td>
<td>CQ, 0.01-10 µM</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 1.72 – 4.15 µM</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV</td>
<td>Dengue</td>
<td>U937</td>
<td>CQ, 0-50 µg/mL</td>
<td>Higher inhibition at 50 µg/mL</td>
<td>28</td>
</tr>
<tr>
<td>Herpesviridae</td>
<td>DNA, ds</td>
<td>HSV-1</td>
<td>Herpes</td>
<td>Lymphocytes B (mouse)</td>
<td>CQ (10 µM) + interferon</td>
<td>CQ increased antiviral activity of interferon</td>
<td>29</td>
</tr>
<tr>
<td>Orthomyxovirida</td>
<td>RNA, ss (-)</td>
<td>Influenza</td>
<td>Bird flu</td>
<td>CEC, HeLa</td>
<td>CQ, 0-50 µg/mL</td>
<td>Inhibition in all tested CQ concentrations</td>
<td>30</td>
</tr>
<tr>
<td>Paramyxovirida</td>
<td>RNA, ss (-)</td>
<td>NDV</td>
<td>Newcastle Disease</td>
<td>CEC, HeLa</td>
<td>CQ, 0-50 µg/mL</td>
<td>Virus inhibition only in CEC cells</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sendai</td>
<td>Respiratory (rodents)</td>
<td>CEC, HeLa</td>
<td>CQ, 0-50 µg/mL</td>
<td>Virus inhibition only in CEC cells</td>
<td>30</td>
</tr>
<tr>
<td>Picornaviridae</td>
<td>RNA, ss (+)</td>
<td>RV</td>
<td>Flu</td>
<td>epithelial trachea cells</td>
<td>HCQ, 50 µM</td>
<td>32 fold reduction in virus content</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EV-A71</td>
<td>Hand-Foot-Mouth</td>
<td>RD</td>
<td>CQ, 0-250 µM</td>
<td>Citotoxicity when CQ &gt; 100 µM</td>
<td>32</td>
</tr>
<tr>
<td>Retroviridae</td>
<td>RNA, ss (+)</td>
<td>HIV-1</td>
<td>AIDS</td>
<td>human lymphocytes</td>
<td>CQ, 0-100 µM</td>
<td>ED&lt;sub&gt;50&lt;/sub&gt; = 15 µM</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Macrophages and T cells</td>
<td>HCV, 1-1000 µM</td>
<td>ED&lt;sub&gt;50&lt;/sub&gt; = 10 µM</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Rhabdoviridae</td>
<td>RNA, ss (-)</td>
<td>VSV</td>
<td>Vesicular stomatitis</td>
<td>CEC, HeLa</td>
<td>CQ, 0-50 µg/mL</td>
<td>Inhibition in all tested CQ concentrations</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rabies</td>
<td>Rabies</td>
<td>NS-20</td>
<td>CQ, 0-250 µg/mL</td>
<td>CQ was more active when added after viral infection</td>
<td>35</td>
</tr>
<tr>
<td>Togaviridae</td>
<td>RNA, ss (+)</td>
<td>SFV</td>
<td>Sindbis Fever</td>
<td>BHK-21</td>
<td>CQ, 100 µM</td>
<td>Inhibition &gt; 90%</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CHIKV</td>
<td>Chikungunya</td>
<td>Vero</td>
<td>CQ, 5-20 µM</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 7.0 ± 1.5 µM Selectivity Index = 37</td>
<td>37</td>
</tr>
</tbody>
</table>

ss = single strand; ds = double strand.
However, it is worth remembering that the premise that an \textit{in vitro} active substance is also active in \textit{in vivo} tests is not always confirmed in practice.\textsuperscript{39} The dose, route of administration, absorption, distribution and/or bioavailability of the drug in the animal or human organism, among several other factors, may not guarantee that this substance effectively reaches its pharmaceutical target.\textsuperscript{40,41} A clear example was shown in the study by Dowall and colleagues:\textsuperscript{25} although CQ has been shown to be active against the EBOV virus (Ebola) in \textit{in vitro} tests (Table 1), it was harmless in experiments carried out on animals infected with this virus. The same happened against the Influenza virus (Table 1), where the antiviral action of CQ first appeared in \textit{in vitro} assays, but later \textit{in vivo} experiments demonstrated that CQ was not effective in preventing influenza infection in a clinical study in humans.\textsuperscript{42} Therefore, these and countless other examples easily found in the literature guide us in the sense that there is an evident and imperative need to confirm the \textit{in vivo} antiviral action of CQ and HCQ before these drugs are officially available for the treatment of COVID-19. The choice of not following this protocol can result in an ineffective treatment of the disease associated with a risk of unnecessary exposure of the patient to various side effects and serious adverse effects that these drugs bring with them. With the use of CQ / HCQ, there are real risks of blindness (retinopathy), cardiac arrhythmia, heart failure and arrest, deafness (ototoxicity), seizure and coma, among others, especially when the use of these drugs occurs in higher doses and for longer periods.\textsuperscript{17,24}

In addition, due to their lysosomal affinity, CQ and HCQ accumulate in cells of various tissues, including cardiac and skeletal muscles.\textsuperscript{17,20} Side effects of HCQ include irreversible effects such as cardiomyopathy, prolongation of the QT interval, proximal myopathy and neuropathy, seizures, especially in older adults, cardiovascular and epilepsy patients and patients with renal failure, all of them taking part of higher risk groups.\textsuperscript{12,17} Moreover, patients with CQ or HCQ-induced myopathy have muscle weakness, dyspnea, absence of reflexes in the legs and ventilatory failure in severe cases. It is clear, therefore, that the toxic effects of CQ and HCQ can further aggravate the health of patients with COVID-19, especially the elderly, obese and diabetics, as these unwanted effects can add up or even be potentiated.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Country} & \textbf{Sample size} & \textbf{Treatment} & \textbf{Outcome} & \textbf{C/QHCQ efficacy?} & \textbf{Reference} \\
\hline
China & N = 100 & CQ & Treated patients showed faster recovery than control & Yes & 43 \\
\hline
France & N = 36 & HCQ: 3x200mg.day\(^{-1}\), 10 days. Part of HCQ treated patients was also treated with azithromycin (AZTM). & After 6 days, 57.1% CQ treated patients were cured compared to 12.5% in the control group. HCQ + AZTM Group showed 100% cure. & Yes & 44 \\
\hline
France & N = 80 & HCQ: 3x200mg.day\(^{-1}\), 10 days. AZTM: 500mg (day1), 250mg.day\(^{-1}\), 4 days. (no control group) & > 90% patients were cured after the 5\textsuperscript{th} day. & Yes & 45 \\
\hline
China & N = 62 & HCQ: 2x200mg.day\(^{-1}\), 5 days. & Improvement: 80.7% of the treated group x 54.8% of the control group. & Partially confirmed. & 46 \\
\hline
Brazil & N = 81 & CQ: 2x600mg.day\(^{-1}\), 10 days (G1); 450mg.day\(^{-1}\), 5 days (G2). & 13.5% of death. G1 was interrupted due to toxic effects from CQ treatment. & No & 47 \\
\hline
China & N = 30 & HCQ: 400mg.day\(^{-1}\), 5 days. & Treated and control groups achieved similar results. & No & 48 \\
\hline
USA & N = 1376 & HCQ: 2x600mg (day1), 400mg. day\(^{-1}\), 5 days. & Treated and control groups achieved similar results. & No & 49 \\
\hline
France & N = 181 & HCQ: 600mg.day\(^{-1}\), 7 days. & Treated and control groups achieved similar results. & No & 50 \\
\hline
France & N = 11 & HCQ: 600mg.day\(^{-1}\), 10 days. AZTM: 500mg (day1), 250mg.day\(^{-1}\), 4 days. (no control group) & 80% patients remained infected after the 5\textsuperscript{th} day of treatment. & No & 51 \\
\hline
China & N = 150 & HCQ: 1200mg.day\(^{-1}\), 3 days; 800mg.day\(^{-1}\), 2-3 weeks. & Treatment did not improve patient recovery when compared to control. & No & 52 \\
\hline
\end{tabular}
\caption{\textit{In vivo} studies that investigated the use of chloroquine (CQ) and/or hydroxychloroquine (HCQ) in the treatment of COVID-19.}
\end{table}
At the best of knowledge, Table 2 lists all studies published so far that investigated the in vivo action of CQ/HCQ in the treatment of patients with COVID-19. Three of these\textsuperscript{12-14} came to the conclusion that CQ and HCQ proved effective as medication and, therefore, they should be recommended in the treatment of this disease. Only one study\textsuperscript{15} concluded that the results obtained indicate a partial efficacy of these antimalarials against COVID-19 and the remaining six studies\textsuperscript{16-21} indicated that such drugs are inefficient in the clinical treatment of COVID-19, in some cases accompanied by greater toxicity and adverse effects in relation to the control group. These negative results served to justify the recommendation of not using CQ or HCQ in the treatment of COVID-19 until there are more consistent experimental data that duly support this choice.

Therefore, there is a discrepancy in results from the studies presented in Table 2. How can we understand and deal with these different results and conclusions? The first point to be raised concerns the lack of standardization and randomization in the planning and execution of these studies. Many of them were unable to manage the experimental conditions and did not consider the numerous factors such as age ranges, gender, comorbidities, pre-infection health status and additional drug treatment received in hospitals during the administration of CQ or HCQ. Two studies carried out in France did not even use control groups over the course of the experiments, which precludes a consistent conclusion on effectiveness since there are no control data for comparison.

Another important factor to consider is the difference in the treatments performed in each study. While one study evaluates only CQ, another evaluates only HCQ and others include additional HCQ treatment with azithromycin (AZTM). In addition, there is a disparity in daily doses and treatment periods in each study, which compromises a safe comparison between studies with regard to the results achieved and conclusions drawn.

Final Comments

The present scenario remains, therefore, inconclusive. Care must be taken in the interpretations of the studies available so far. To date, the scientific community does not see any set of reliable scientific data that can convincingly support the clinical use of CQ or HCQ in the treatment of COVID-19. This understanding was also recently expressed by the World Health Organization when discontinuing the use of HCQ against COVID-19.\textsuperscript{22} More controlled and better-planned studies will certainly provide results enough to corroborate (or not) this scenario definitively.

ACKNOWLEDGEMENT

The author is grateful to the Federal University of Bahia (UFBA), Brazil, for providing general support and research facilities.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS


REFERENCES

Batista.: Chloroquine and Hydroxychloroquine for COVID-19?


