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Journal Title: Travel Medicine and Infectious Disease
Volume: Volume 35
Publisher: Elsevier | 2020-05-01, Pages 101735-101735
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1016/j.tmaid.2020.101735
Permanent URL: https://pid.emory.edu/ark:/25593/vn7gt

Final published version: http://dx.doi.org/10.1016/j.tmaid.2020.101735

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Accessed September 29, 2023 11:12 AM EDT
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Therapeutic use of chloroquine and hydroxychloroquine in COVID-19 and other viral infections: A narrative review

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**ARTICLE INFO**

**Keywords:**
SARS-CoV-2
COVID-19
Chloroquine
Hydroxychloroquine

**ABSTRACT**

The rapidly spreading Coronavirus Disease (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2), represents an unprecedented serious challenge to the global public health community. The extremely rapid international spread of the disease with significant morbidity and mortality made finding possible therapeutic interventions a global priority. While approved specific antiviral drugs against SARS-CoV-2 are still lacking, a large number of existing drugs are being explored as a possible treatment for COVID-19 infected patients. Recent publications have re-examined the use of Chloroquine (CQ) and/or Hydroxychloroquine (HCQ) as a potential therapeutic option for these patients. In an attempt to explore the evidence that supports their use in COVID-19 patients, we comprehensively reviewed the previous studies which used CQ or HCQ as an antiviral treatment. Both CQ and HCQ demonstrated promising in vitro results, however, such data have not yet been translated into meaningful in vivo studies. While few clinical trials have suggested some beneficial effects of CQ and HCQ in COVID-19 patients, most of the reported data are still preliminary. Given the current uncertainty, it is worth being mindful of the potential risks and strictly rationalise the use of these drugs in COVID-19 patients until further high quality randomized clinical trials are available to clarify their role in the treatment or prevention of COVID-19.

1. Coronaviruses and the COVID-19 pandemic

Coronaviruses (CoVs) are important human and animal pathogens that have the ability to emerge and cross the species barrier, causing novel and occasionally fatal diseases [1,2]. They belong to the subfamily Coronavirinae of the Coronaviridae family in the order Nidovirales [3]. According to the International Committee on the Taxonomy of Viruses (ICTV), coronaviruses are classified into four genera including, *alphacoronavirus*, *betacoronavirus* (contains 4 lineages A, B, C and D), *gammacoronavirus* and *deltacoronavirus* [4]. They are large enveloped viruses with a large single-stranded RNA, 5′-capped, non-segmented genome with positive polarity ranging from 26 to 32 kb in size [5]. While CoVs from all genera infect a large number of mammals and birds, bats are proposed to be their natural reservoir [6,7]. In humans, on the other hand, only alpha and beta CoVs have been associated with diseases ranging from mild common cold to fatal severe respiratory infections. Two human alpha CoVs (hCoV-229E and hCoV-NL63) and two beta CoVs (hCoV-OC43 and hCoV-HKU1) are associated with...
common cold [8–11]. In 2002 and 2012, two novel highly pathogenic beta CoVs known as the severe acute respiratory syndrome-CoV (SARS-CoV) and the Middle East respiratory syndrome-CoV (MERS-CoV) emerged in China and Saudi Arabia, respectively [12–15]. These two viruses have spread widely and were associated with severe respiratory diseases with mild to severe and fatal outcomes. More recently, a novel human CoV known as severe acute respiratory syndrome-CoV-2 (SARS-CoV-2) emerged in December 2019 in Wuhan, the capital city of Hubei province in China as the third known highly pathogenic human beta CoV [16].

Since its emergence, SARS-CoV-2, which causes the Coronavirus Disease (COVID-19), has rapidly spread to more than 214 countries around the world, causing a large-scale global pandemic. Until April 10th, more than 1.6 million COVID-19 confirmed cases have been reported globally, including more than 100,000 deaths. There are currently no vaccines or specific antiviral drugs for SARS-CoV-2 [17]. The rapid global spread of this virus and the worrisome associated mortality rate encouraged the medical community and policy makers to expedite the process of exploring all available and potential interventions to control and mitigate this outbreak [18]. Several interventional treatment options for COVID-19 have been suggested with unclear efficacy and safety considerations [19]. Recent publications have suggested using chloroquine (CQ), a broadly used antimalarial drug, and its derivative hydroxychloroquine (HCQ) as a treatment for COVID-19 patients [20–22]. In this review, we explore the antiviral activities of CQ and HCQ against CoVs and non-CoVs in the majority of previously published in vitro, in vivo and clinical trial studies with an aim to find evidence that supports their use in COVID-19 patients.

### 2. Possible mechanisms of CQ and HCQ antiviral activities

Both CQ and HCQ, known antimalarial and antirheumatic drugs, have closely related chemical structures [22]. However, their mechanisms of action are still not fully elucidated. Several studies have revealed that both drugs have antiviral activity in vitro through different mechanisms [23–25]. In particular, CQ has been shown to interfere with different stages of the viral life cycle as shown in Fig. 1 [26–29]. Different studies have reported the ability of CQ to inhibit viral entry [30–32], uncoating [33], assembly and budding [34,35]. One of the suggested mechanisms by which CQ can affect the entry step of viruses is by inhibiting quinone reductase 2 [36], which is required for the biosynthesis of sialic acid [37]. Sialic acid was found to be involved in virus attachment and entry into host cells by several viruses including hCoV-OC43 and MERS-CoV [38,39]. Moreover, CQ was
shown to potently inhibit entry of SARS-CoV into cells by interfering with the glycosylation of its cellular receptor angiotensin converting enzyme 2 receptor (ACE2). SARS-CoV-2 also uses ACE2 as a receptor for entry with the glycosylation of its cellular receptor angiotensin converting enzyme 2 receptor (ACE2). SARS-CoV-2 also uses ACE2 as a receptor for entry with the glycosylation of its cellular receptor angiotensin converting enzyme 2 receptor (ACE2).

Table 2

| Drug | Virus | Cells | EC_{50} (μM) | SI | Main findings | Year | Ref |
|------|-------|-------|--------------|----|---------------|------|-----|
| CQ  | SARS-CoV | Vero E6 | 8.8 ± 1.2 | 30 | ↓ viral replication | 2004 | [104] |
| CQ  | SARS-CoV | Vero E6 | 4.4 ± 1.0 | – | ↓ viral replication | 2005 | [37] |
| CQ  | SARS-CoV | Vero 76 | 1–5 | 2–20 | ↓ viral replication | 2006 | [105] |
| CQ  | SARS-CoV | Vero | 6.5 ± 3.2 | > 15 | ↓ viral replication | 2006 | [106] |
| CQ  | SARS-CoV | Vero E6 | 4.1 ± 1.0 | > 31 | ↓ viral replication | 2014 | [107] |
| CQ-MP | SARS-CoV | Vero 76 | 4–6 | 3–8 | ↓ viral replication | 2006 | [105] |
| CQ-DP | SARS-CoV | Vero 76 | 3–8 | 2–10 | ↓ viral replication | 2006 | [105] |
| AMQ | SARS-CoV | Vero 76 | 3–10 | 2–10 | ↓ viral replication | 2006 | [105] |
| HCO | SARS-CoV | Vero | 34 ± 5 | > 3 | ↓ viral replication | 2006 | [106] |
| HFCQ | SARS-CoV | Vero | 1.4 ± 0.1 | 15 | ↓ viral replication | 2006 | [106] |
| HFCQ | MERS-CoV | Vero | 1.9–4.9 | 4–17 | ↓ viral replication | 2006 | [106] |
| CQ  | MERS-CoV | Huh7 | 3.0 ± 1.1 | 19.4 | ↓ viral replication | 2014 | [107] |
| CQ  | MERS-CoV | Vero E6 | 6.3 | – | Ineffective | 2018 | [116] |
| CQ  | SARS-CoV-2 | Vero E6 | 1.13 | > 88.5 | ↓ viral replication | 2020 | [113] |
| CQ  | SARS-CoV-2 | Vero | 5.47 | – | ↓ viral replication | 2020 | [112] |
| CQ  | SARS-CoV-2 | Vero E6 | 2.71–7.36 | 37.12–100.81 | ↓ viral replication | 2020 | [114] |
| HCQ | SARS-CoV-2 | Vero | 0.72 | – | ↓ viral replication | 2020 | [112] |
| HFCQ | SARS-CoV-2 | Vero E6 | 4.06–17.31 | 14.41–61.45 | ↓ viral replication | 2020 | [114] |
| CQ  | FCoV-229E | L132 | – | – | ↓ viral replication | 2008 | [109] |
| CQ  | FCoV-229E | Huh7 | 3.3 ± 1.2 | > 15 | ↓ viral replication | 2014 | [107] |
| CQ  | HCoV-OC43 | HRT-18 | 0.3 ± 0.0 | 1369 | ↓ viral replication | 2009 | [108] |
| CQ  | MHV4 | Murine cells | – | – | Ineffective | 1991 | [117] |
| CQ  | MHV3 | Murine Mφ | – | – | ↓ viral replication | 1966 | [115] |
| CQ  | F-CoV | CRFK | > 0.8 | – | ↓ viral replication | 2006 | [106] |
| HCQ | F-CoV | CRFK | 28 ± 27 | – | Ineffective | 2006 | [106] |
| FQ  | F-CoV | CRFK | 2.9 ± 1.2 | – | ↓ viral replication | 2006 | [106] |
| HFCQ | F-CoV | CRFK | > 4 | – | Weak effect | 2006 | [106] |
| CQ  | FIPV | fowl-4 | – | – | ↓ viral replication | 2013 | [110] |
| CQ  | PHEV | Neuro-2a | – | – | ↓ viral replication | 2017 | [111] |

* Tested at different multiplicities of infections (MOIs) of 0.01–0.8.

CQ: Chloroquine; CQ-MP: Chloroquine monophosphate; CQ-DP: Chloroquine diphosphate; AMQ: Amodiaquine; HCQ: Hydroxychloroquine; FQ: Ferroquine; HFQ: Hydroxy ferroquine; SARS-CoV: Severe acute respiratory syndrome-coronavirus; MERS-CoV: Middle East respiratory syndrome-coronavirus; SARS-CoV-2: Severe acute respiratory syndrome-coronavirus 2; MHV4: Mouse hepatitis virus Type 4; F-CoV: Feline coronavirus; FIPV: Feline infectious peritonitis virus; PHEV: Porcine hemagglutinating encephalomyelitis virus; Vero cells: African green monkey kidney epithelial cells; Huh7 cells: Human hepatocyte-derived carcinoma cells; L132: human epithelial lung cells; HRT-18: Human ileocecal colorectal adenocarcinoma cells; Mφ: macrophages; CRFK cells: Crandell-Reese feline kidney cells; fowl-4 cells: Felis catus whole fetus-4 cells; Neuro-2a: murine neuroblastoma cells; EC_{50}: 50% Effective concentration; SI: Selectivity index defined as the ratio of drug efficacy to cytotoxicity.

3. CQ and HCQ pharmacokinetics

The fact that both CQ and HCQ are considered for the management of COVID-19 patients clearly highlights the need to better understand their pharmacokinetics (PK) parameters. However, a full understanding of these parameters has been challenging despite the numerous reported studies. Generally, PK parameters for CQ and HCQ are comparable (Table 1) [54,55]. Following oral administration of CQ and HCQ, their bioavailability can reach up to 80% with plasma peak time around 2–4 h [56–58]. Thus, parenteral administration, if available, might be a better route especially that oral administration has shown huge interpatient variability [56,59,60]. The long half-life of both CQ and HCQ which could range from 30 to 60 days is likely attributed to their large volume of distribution (200–800 L/kg) and extensive tissue uptake [61–68]. CQ and HCQ are metabolized via CYP-450 enzymes to other active compounds, which are responsible for the extended pharmacological actions and increased toxicity [61,69]. Up to 60% of CQ and HCQ is primarily excreted renally as unchanged or metabolized forms, and the remaining (40%) is usually cleared through the liver, feces and skin or stored in other lean body tissues [54,69–74]. It’s important to note that CQ and HCQ have a chiral center, which produces two enantiomers R(−) or S(+) forms or isomers [75], in which little is known about the differences in their pharmacological activity and their corresponding metabolites. Most clinically used CQ and HCQ exist as a racemic mixture (50:50) of both isomers which complicates the

immunomodulatory properties of CQ and HCQ have raised the interest in using these drugs in COVID-19 patients at risk of cytokines release syndrome (CRS) [22].

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| Drug | Virus | Cells | EC_{50} (μM) | SI | Main findings | Year | Ref |
|------|-------|-------|-------------|----|---------------|------|-----|
| CQ   | HIV-1 | HL3Tl | –           | –  | ↑ viral replication | 1988 | [126] |
| CQ'  | HIV-1 | H-9   | –           | –  | low toxicity | 1990 | [118] |
| CQ'  | HIV-1 | U-937 | –           | –  | No toxicity | 1998 | [119] |
| CQ'  | HIV-1 | H-9   | 0.9         | –  | No toxicity | 1999 | [120] |
| CQ'  | HIV-1 | U-937 | 0.4         | –  | No toxicity | 1999 | [120] |
| CQ'  | HIV-1 | T cells | 0.9       | –  | No toxicity | 1999 | [120] |
| CQ'  | HIV-1 | Monocytes | 0.2       | –  | No toxicity | 1999 | [120] |
| CQ'  | HIV-1 | ACH-2 | 1          | –  | ↓ viral replication | 1999 | [120] |
| CQ'  | HIV-1 | U-1   | 0.1         | –  | No toxicity | 1999 | [120] |
| CQ'  | HIV-1 | ACH-2 | 1          | –  | No toxicity | 1999 | [120] |
| CQ'  | HIV-1 | MT-4  | 8.86 ± 1.18 | 6  | ↓ viral replication | 2006 | [106] |
| HCQ  | HIV-1 | U-937 | 1           | –  | low toxicity | 1993 | [123] |
| HCQ  | HIV-1 | CEM   | 10          | –  | low toxicity | 1993 | [123] |
| HCQ  | HIV-1 | 63    | 0.01        | –  | No toxicity | 1996 | [124] |
| HCQ  | HIV-1 | SP    | 0.1         | –  | No toxicity | 1996 | [124] |
| HCQ  | HIV-1 | G356  | –           | –  | –             | 1996 | [124] |
| HCQ  | HIV-1 | SPH   | –           | –  | –             | 1996 | [124] |
| HCQ  | HIV-1 | MT-4  | > 2.4       | –  | Ineffective | 2006 | [106] |
| FQ   | HIV-1 | MT-4  | 2.9 ± 1.1   | 3  | ↓ viral replication | 2006 | [106] |
| HCQ  | HIV-2 | MT-4  | 1–10        | –  | No toxicity | 2004 | [122] |
| CQ   | IAV H1N1 | MDCK | –           | –  | viral replication | 1981 | [127] |
| CQ   | IAV H1N1 | MDCK | 3.60        | –  | –             | 2006 | [128] |
| CQ   | IAV H1N1 | A549 | –           | –  | –             | 2007 | [129] |
| CQ   | IAV H1N1 | MDCK | 1.26        | –  | –             | 2007 | [130] |
| CQ   | IAV H1N2 | MDCK | 0.84        | –  | –             | 2006 | [128] |
| CQ   | IAV H1N2 | MDCK | 1.53        | –  | –             | 2007 | [130] |
| CQ   | IAV H1N2 | A549 | –           | –  | –             | 2007 | [129] |
| CQ   | IAV H5N1 | A549 | –           | –  | –             | 2013 | [20]  |
| CQ   | IAV H5N1 | MDCK | 14.38       | –  | –             | 2007 | [130] |
| CQ   | IAV H7N3 | MDCK | > 20        | –  | –             | 2007 | [130] |
| CQ   | IAV H7N3 | MDCK | 14.39       | –  | –             | 2007 | [130] |
| CQ   | Feo B | MDCK | –           | –  | –             | 1983 | [131] |
| CQ   | DENV-2 | BHK   | –           | –  | –             | 1990 | [43]  |
| CQ   | DENV-2 | Vero  | –           | –  | No toxicity | 2013 | [141] |
| CQ   | DENV-2 | C6/36 | –           | –  | Ineffective | 2013 | [141] |
| CQ   | DENV-2 | U-937 | –           | –  | –             | 2014 | [140] |
| CQ   | ZIKV | Vero | 9.82        | –  | No toxicity | 2016 | [135] |
| CQ   | ZIKV | bMECs | 14.20       | –  | No toxicity | 2016 | [135] |
| CQ   | ZIKV | MDCK | 12.36       | –  | No toxicity | 2016 | [135] |
| CQ   | ZIKV | NSCs | 4.15        | –  | –             | 2017 | [134] |
| CQ   | ZIKV | NSs | 4.15        | –  | –             | 2017 | [134] |
| CQ   | ZIKV | HuH7 | 1.72–2.72   | –  | –             | 2017 | [134] |
| CQ   | ZIKV | NSs | 10          | –  | –             | 2017 | [134] |
| AMD  | ZIKV | Vero | –           | –  | –             | 2017 | [134] |
| AMD  | ZIKV | HeLa | –           | –  | –             | 2007 | [132] |
| CQ   | CHIKV | Vero | 7.0 ± 1.5   | 37.14 | ↓ viral replication | 2010 | [41]  |
| CQ   | CHIKV | Vero | 17.2 ± 2.1  | 15.29 | ↓ viral replication | 2010 | [41]  |
| CQ   | CHIKV | Vero | 10.0 ± 1.2  | 26  | ↓ viral replication | 2010 | [41]  |
| CQ   | CHIKV | MDMM | –           | low toxicity | ↓ viral replication | 2018 | [133] |
| CQ   | CHIKV | Fibroblasts | –       | high toxicity | ↓ viral replication | 2018 | [133] |
| CQ   | EBOV | HEK 293T | 4.7       | –  | –             | 2013 | [139] |
| CQ   | EBOV | Vero | 16          | –  | –             | 2013 | [139] |
| HCQ  | EBOV | HEK 293T | 9.5       | –  | –             | 2013 | [139] |
| HCQ  | EBOV | Vero | 22          | –  | –             | 2013 | [139] |
| AMD  | EBOV | HEK 293T | 2.6       | –  | –             | 2013 | [139] |
| AMD  | EBOV | Vero | 8.4         | –  | –             | 2013 | [139] |
| AQ   | EBOV | HEK 293T | 4.3       | –  | –             | 2013 | [139] |
| AQ   | EBOV | Vero | 21          | –  | –             | 2013 | [139] |
| AQ   | EBOV | MRC-5 | –           | low toxicity | ↓ viral replication | 2015 | [137] |
| CQ   | EBOV | Vero | 1.77        | –  | –             | 2015 | [136] |
| CQ   | SIN  | BHK-21 | –           | –  | –             | 1981 | [142] |
| CQ   | VSV | BHK-21 | –           | –  | –             | 1981 | [142] |
| CQ   | Rabies | NS-20 | –           | –  | –             | 2010 | [149] |
| CQ   | PIV  | BHK-21 | –           | –  | –             | 1984 | [143] |
| CQ   | Poliovirus | HeLa | –           | –  | Ineffective | 1991 | [151] |
understanding of their PK and associated toxicity as they could behave differently inside the body [57,75–77].

4. CQ and HCQ adverse effects and related toxicities

The most common CQ and HCQ adverse effects are gastrointestinal symptoms such as nausea, vomiting and abdominal discomfort [78], and uncommonly worrisome fulminant hepatic failure [79], toxic epidermal necrolysis (TEN) [80] and cardiotoxicity that could manifest with QT abnormality [81–83]. Nevertheless, over the years CQ and HCQ have maintained a good safety profile when used in several chronic diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Despite some animal experiments suggesting that HCQ is probably less toxic than CQ, there is a lack of high quality evidence from clinical trials supporting this claim [74,84–87]. These toxicities could be related to the very long half-life and the large volume of distribution of both drugs. One of the significant toxic effects of CQ and HCQ is the possible ocular pigmentation due to their binding to melanin, which could lead to damage in different parts of the eye including the cornea, ciliary body and retina [88]. Notably, the incidence of such ocular toxicity is usually rare. For instance, it was shown that only 0.5% out of ~400 patients treated with HCQ (<6.5 mg/kg/day) for 6 years due to RA or SLE had developed ocular related complications [89]. Most studies have shown that such complications might only

Table 3 (continued)

| Drug | Virus | Cells | EC50 (μM) | SI | Main findings | Year | Ref |
|------|-------|-------|-----------|----|---------------|------|-----|
| CQ   | SLE   | BHK   | –         | –  | ↓ viral replication^ | 1990 | [43] |
| CQ   | POW   | BHK   | –         | –  | ↓ viral replication^ | 1990 | [43] |
| CQ   | NIV   | Vero  | –         | –  | ↓ viral replication | 2009 | [150] |
| CQ   | NIV   | HeLa  | 0.62      | –  | ↓ viral replication | 2010 | [148] |
| CQ   | HeV   | Vero  | –         | –  | ↓ viral replication | 2009 | [150] |
| CQ   | HeV   | HeLa  | 161       | –  | ↓ viral replication | 2010 | [146] |
| CQ   | EBV   | HHS14-16 | –     | –  | ↑ viral replication | 2017 | [125] |
| CQ   | HCV   | Huh-7 | 0.22      | –  | ↓ viral replication | 2010 | [144] |
| CQ   | DHBV  | PDH   | –         | –  | ↓ viral replication | 1990 | [145] |
| CQ   | DHBV  | PDH   | –         | No toxicity | ↓ viral replication | 1991 | [146] |
| CQ   | JEV   | B104  | 0.3       | –  | ↓ viral replication | 2010 | [149] |
| CQ   | MARV  | HEK 293T | 5.5      | –  | ↓ viral replication | 2013 | [139] |
| CQ   | MARV  | Vero 76 | 15       | –  | ↓ viral replication | 2013 | [139] |
| HCQ  | MARV  | Vero 76 | 18       | –  | ↓ viral replication | 2013 | [139] |
| AMD  | MARV  | HEK 293T | 2.3      | –  | ↓ viral replication | 2013 | [139] |
| AMD  | MARV  | Vero 76 | 8.3      | –  | ↓ viral replication | 2013 | [139] |
| AQ   | MARV  | Vero 76 | 42       | –  | ↓ viral replication | 2013 | [139] |
| AQ   | MARV  | Vero E6 | –        | –  | ↓ viral replication | 2015 | [150] |
| CQ   | CCHFV | Hub7  | –         | 21.3 | ↓ viral replication | 2015 | [150] |

CQ: Chloroquine; HCQ: Hydroxychloroquine; FQ: Ferroquine; HQ: Hydroxy ferroquine; AMD: Amodiaquine; Pre: pre-treatment; Post: post-treatment; Con: concurrent; AQ: Aminoquinoline; HIV: Human immunodeficiency viruses; IAV: Influenza A virus; Flu B: Influenza B virus DENV-2: Dengue virus 2; ZIKV: Zika virus; CHIKV: Chikungunya virus; EBOV: Ebola virus; SIV: Simian; VSV: Vesicular stomatitis viruses; PICV: Pichinde virus; SLE: St. Louis encephalitis virus; POW: Powassan virus; NIV: Nipah virus; HeV: Hendra virus; EBV: Epstein-Barr virus; HCV: Hepatitis C virus; DHBV: Duck hepatitis B virus; JEV: Japanese encephalitis virus; MARV: Marburg virus; CCHFV: Crimean-Congo hemorrhagic virus; HL3T1: HeLa derivative cells; H-9: Human T lymphocytic cells; U-937: Human promonocytic cells; U-1: Human promonocytic cells; ACH-2: Human T lymphocytic cells; MFO: macrophages; MT-4 cells: HTLV-I-transformed T-cell line; CEM: Human T lymphoblast cells; 63: Human macrophage hybridoma; SP: T-cell line derived from the pleural fluid of an HIV-1 infected individual; 63HIV: 63 cells infected by HIV; SPH: SP cells infected by HIV; MDCK: Madin Darby canine kidney; A549 cells: Human adenocarcinomic alveolar basal epithelial cells; BHK/BHK-21 cells: Syrian golden Syrian golden fibroblast cells; Vero cells: African green monkey kidney epithelial cells; C6/36: Aedes albopictus cell line; hBMEC: Human brain microvascular endothelial cells; NSCs: Neural stem cells; NS: Neurospheres; Hub7: Human hepatocyte-derived carcinoma cells; HeLa: Human epithelial cell line; MDMD: Monocyte-derived macrophages; HEK 293T: Human embryonic kidney cells; MRC-5: Human normal lung fibroblasts; MRC-5: Medical Research Council cell strain 5; B104: Rat neuroblastoma cell; NS-20: Murine neuroblastoma; HHS14-16: Burkitt lymphoma cell line; PDH: Primary duct hepatocytes; EC50 50% Effective concentration; SI: selectivity index defined as the ratio of drug efficacy to cytotoxicity (when no SI value was reported, level of toxicity was indicated if available).

^ Either alone or combined with hydroxyurea (HU1) + didanosine (ddI).

^ In combination with hydroxyurea (HU1) + didanosine (ddI).

^ Enhanced inhibition against HIV-1 and HIV-2 in combination with HCQ in H9 and MT-4 cells; and against HIV-1 in combination with indinavir (IDV), saquinavir (SQV) or ritonavir (RTV) in MT-4 cells or peripheral blood mononuclear cells (PBMCs).

^ Synergistic inhibitory effect of CQ with IFN-α.

^ A/Mallard/It/43/01 (H7N3).

^ A/Ty/It/220158/02 (H7N3).

^ The haemagglutinins (HAs) of the two avian H7N3 strains differ in two amino acid residues (261 in the H1A subunit and 161 in H2A subunit) and display different pH requirements.

^ Viral entry (viral pseudotype assay).

^ Viral replication.

^ Primary cells.

^ Cells stimulated with LPS.

^ Cells stimulated with PMCA.

^ EC50 in μg/mL.

^ Suggested enhanced replication and protection of taf from proteolytic degradation with CQ.

^ Suggested enhanced replication based on increased prM protein in progeny virions rather than M protein due to inhibition of proteolytic process.

^ CQ, HCQ and FQ showed no significant activity against parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxackie virus, Punta Toro virus, respiratory syncytial virus (RSV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), vaccinia virus, vesicular stomatitis virus (VSV), and influenza A virus (HIN2).
occur with long term treatment of chronic diseases which extends for more than 5 years with doses above or equal to 6.5 mg/kg/day [90,91]. However, ocular toxicity and changes could still occur under shorter treatments. Other complications such as development of proximal myopathy associated with respiratory failure have also been reported in patients treated with either CQ or HCQ [92-95]. Nonetheless, most of these complications were seen in elderly patients with an average age of 70 years suffering from chronic RA or autoimmune diseases. Both CQ and HCQ were also shown to be associated with rare but life-threatening cardiomyopathy [96-98]. Other less reported CQ and HCQ toxicities include urticaria [99], otoxicity [100,101] and some neurological effects [102,103].

5. In vitro antiviral activity of CQ and HCQ

The antiviral effects of CQ were suggested at least 50 years ago [23,25]. Since then, several studies have tested the ability of CQ and HCQ to inhibit the replication of a wide range of CoVs and non-CoV viruses in vitro as shown in Tables 2 and 3, respectively. The majority of these studies have revealed a substantial ability of CQ and HCQ as well as some of their derivatives to inhibit viral replication with no to low toxicity. Specifically, CQ has been shown to inhibit the replication of different CoVs including SARS-CoV, MERS-CoV and SARS-CoV-2 among others in several studies (Table 2) [37,104-115]. Only two studies showed no significant inhibitory effects of CQ on MERS-CoV and mouse hepatitis virus (MHV4) [116,117]. Other CoV derivatives such as amodiaquine (AMD), ferroquine (FQ), hydroxy ferroquine (HFQ) have been also shown to exert some antiviral activity [105,106]. Interestingly enough, while HCQ does not seem to have a significant effect in reducing SARS-CoV and Feline CoV replication [106], it was recently shown to have a potent in vitro inhibitory effects against SARS-CoV-2 replication [112,116]. Similarly, these compounds have shown excellent in vitro antiviral activity against several non CoV (mostly RNA viruses) with low toxicity in most cases (Table 3). For instance, HIV was shown to be inhibited by CQ alone or in combination with HCQ, hydroxyurea (HU1), didanosine (ddi), zidovudine (ZDV), indinavir (IDV), saquinavir (SQV) or ritonavir (RTV) [106,118-122]. While other derivatives such as HCQ and FQ have been also shown to inhibit HIV replication [106,123,124], one study showed no effect of HCQ and FQ on HIV [106]. Similarly, it was found that CQ could enhance Epstein-Barr virus replication [125]. Furthermore, another study has suggested possible enhanced HIV replication with CQ treatment through protection of tat protein from proteolytic degradation [126]. Influenza A and B viruses have also been shown to be inhibited by CQ [27,127-131] although contradicting results have been seen for some subtypes and strains such as avian H7N3 strains (A/Mallard/It/43/01 and A/Ty/It/220158/02) [106,130]. Several other studies have also reported in vitro inhibitory effect of CQ on multiple viruses such as chikungunya virus (CHIKV) [41,132,133], zika virus (ZIKV) [134-136], Ebola virus (EBOV) [137-139], dengue viruses (DENV) in mammalian cells [43,140,141] but not insect cells [141] as well as several others [43,139,142-150]. Nonetheless, some reports failed to observe antiviral activity of CQ, HCQ and FQ on several other viruses including polio virus, reovirus, respiratory syncytial virus (RSV), herpes simplex viruses, coxsackie virus, vesicular stomatitis virus (VSV), vaccinia virus, sindbis virus, parainfluenza-3 virus and Punta Toro virus [106,151].

6. In vivo animal antiviral activity of CQ and HCQ

There are limited studies established to investigate the possible antiviral effects of CQ or HCQ in animal models (Table 4). In general, studies showed no significant effect of CQ on CoVs including SARS-CoV and feline infectious peritonitis virus (FIPV) replication or clinical antiviral effects. Since then, several studies have tested the ability of CQ and HCQ to inhibit the replication of a wide range of CoVs and non-CoV viruses in vitro as shown in Tables 2 and 3, respectively. The majority of these studies have revealed a substantial ability of CQ and HCQ as well as some of their derivatives to inhibit viral replication with no to low toxicity. Specifically, CQ has been shown to inhibit the replication of different CoVs including SARS-CoV, MERS-CoV and SARS-CoV-2 among others in several studies (Table 2) [37,104-115]. Only two studies showed no significant inhibitory effects of CQ on MERS-CoV and mouse hepatitis virus (MHV4) [116,117]. Other CoV derivatives such as amodiaquine (AMD), ferroquine (FQ), hydroxy ferroquine (HFQ) have been also shown to exert some antiviral activity [105,106]. Interestingly enough, while HCQ does not seem to have a significant effect in reducing SARS-CoV and Feline CoV replication [106], it was recently shown to have a potent in vitro inhibitory effects against SARS-CoV-2 replication [112,116]. Similarly, these compounds have shown excellent in vitro antiviral activity against several non CoV (mostly RNA viruses) with low toxicity in most cases (Table 3). For instance, HIV was shown to be inhibited by CQ alone or in combination with HCQ, hydroxyurea (HU1), didanosine (ddiv), zidovudine (ZDV), indinavir (IDV), saquinavir (SQV) or ritonavir (RTV) [106,118-122]. While other derivatives such as HCQ and FQ have been also shown to inhibit HIV replication [106,123,124], one study showed no effect of HCQ and FQ on HIV [106]. Similarly, it was found that CQ could enhance Epstein-Barr virus replication [125]. Furthermore, another study has suggested possible enhanced HIV replication with CQ treatment through protection of tat protein from proteolytic degradation [126]. Influenza A and B viruses have also been shown to be inhibited by CQ [27,127-131] although contradicting results have been seen for some subtypes and strains such as avian H7N3 strains (A/Mallard/It/43/01 and A/Ty/It/220158/02) [106,130]. Several other studies have also reported in vitro inhibitory effect of CQ on multiple viruses such as chikungunya virus (CHIKV) [41,132,133], zika virus (ZIKV) [134-136], Ebola virus (EBOV) [137-139], dengue viruses (DENV) in mammalian cells [43,140,141] but not insect cells [141] as well as several others [43,139,142-150]. Nonetheless, some reports failed to observe antiviral activity of CQ, HCQ and FQ on several other viruses including polio virus, reovirus, respiratory syncytial virus (RSV), herpes simplex viruses, coxsackie virus, vesicular stomatitis virus (VSV), vaccinia virus, sindbis virus, parainfluenza-3 virus and Punta Toro virus [106,151].

6. In vivo animal antiviral activity of CQ and HCQ

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There are very limited published clinical trials that studied the possible antiviral effects of CQ or HCQ in CoV and non-CoV infected patients (Table 5). These published clinical trials have clearly shown no significant benefit of using CQ in the prevention or treatment against influenza, DENV or CHIKV infections in patients [133,155–158]. In fact, in one study, patients treated with CQ were more likely to develop adverse effects such as arthralgia at day 200 post-treatment [157]. On the other hand, few studies have reported that HCQ could decrease HIV-1 viremia, stabilize CD4+ T cell count and reduce IL-6 and IgG levels in infected patients [159], although others showed contradicting finding of increased HIV RNAemia in HCQ treated patients [160,161]. Interestingly, while few clinical studies have suggested that the use of HCQ alone or with azithromycin (AZT) could be beneficial for COVID-19 patients as it reduces viral shedding and time to clinical recovery [162–164], others have reported no effect in infected patients [165,166]. However, it is important to note that most of these studies have several limitations in study designs with small sample sizes. Nonetheless, around 104 clinical trials are ongoing in different countries to asses and evaluate the therapeutic and prophylactic effects of both CQ and/or HCQ in COVID-19 patients (Table 6).

8. Conclusion

The COVID-19 pandemic has spread out of control and has caused considerable morbidity and mortality in several countries. In this unprecedented situation, clinicians have tried all kinds of treatments in an effort to stem the progression of this disease. One treatment that has received huge attention was the empirical use of anti-malarial CQ/HCQ. While there is no strong and enough scientific and clinical data to support their use, several countries have already included CQ/HCQ in COVID-19 treatment protocols [167,168], not only as a treatment option for severely ill patients but also as a prophylactic measure.

Table 5
Main findings of clinical trials on the antiviral activity of CQ and its derivatives on CoVs and non-CoVs.

| Drug          | Virus                  | Design    | Dose mg/day | Total No. | Main Findings            | Year | Ref |
|---------------|------------------------|-----------|-------------|-----------|--------------------------|------|-----|
| HCQ + AZT     | SARS-CoV-2             | SAOLS     | 600 mg/day  (10 days) | 42        | ↓ viral load\(^b\)       | 2020 | [162]|
| HCQ           | SARS-CoV-2             | RCT       | 400 mg/day  (5 days)  | 62        | ↓ Recovery time          | 2020 | [163]|
| HCQ + AZT     | SARS-CoV-2             | Pilot     | 400 mg/day  (5 days)  | 30        | Ineffective\(^c\)       | 2020 | [165]|
| HCQ           | SARS-CoV-2             | OS        | 600 mg/day  (10 days) | 80        | ↓ viral load             | 2020 | [164]|
| HCQ + AZT     | SARS-CoV-2             | SAOLS     | 600 mg/day  (10 days) | 11        | Ineffective\(^d\)       | 2020 | [166]|
| CQ            | Influenza A/B          | RDBPCS    | 500 mg/day  (1 week)  | 1516      | Ineffective              | 2011 | [155]|
| CQ            | DENV                   | RDBPCS    | Once a week (11 weeks) | 307       | Ineffective\(^e\)       | 2010 | [156]|
| CQ            | DENV                   | RDBPCS    | 500 mg/day  (day 1 and 2) | 30        | Ineffective\(^e\)       | 2013 | [158]|
| CQ            | CHIKV                  | RDBPCS    | 600 mg/day  (day 1)   | 54        | Ineffective\(^e\)       | 2008 | [157]|
| HCQ           | HIV 1                  | Case report | 600 mg/day  | 2         | ↓ viral load\(^f\)       | 1996 | [169]|
| HCQ           | HIV 1                  | RDBPCS    | 800 mg/day  (8 weeks) | 40        | ↓ viral load\(^f\)       | 1995 | [170]|
| HCQ           | HIV 1                  | RDBS      | 800 mg/day  (16 weeks) | 72        | ↓ serum IL-6 & IgG       | 1997 | [159]|
| HCQ           | HIV 1                  | RDBPCS    | 400 mg/day  (42 weeks) | 83        | ↓ serum IL-6 & IgG       | 2012 | [161]|
| CQ + ART      | HIV                    | RDBPCS    | 250 mg/day  (12 weeks) | 33        | ↑ viral replication      | 2016 | [171]|
| CQ + ART      | HIV                    | RDBPCS    | 250 mg/day  (12 weeks) | 37        | ↓ Immune cell activation | 2016 | [171]|

HCQ: Hydroxychloroquine; AZT: Azithromycin; CQ: Chloroquine; ART: Antiretroviral therapy; SARS-CoV-2: Sever acute respiratory syndrome-coronavirus 2; DENV: Dengue Virus; CHIKV: Chikungunya virus; HIV: Human immunodeficiency virus; SAOLS: Single arm open labelled study; RCT: Randomized clinical trial; OS: Observational study; RDBPCS: Randomized double blind placebo controlled study; RDBS: Randomized double blinded study; BID: Twice per day.

\(^a\) Small sample size study, 1 death and 3 transferred to ICU among 26 patients treated with HCQ + AZT.

\(^b\) 1 patient developed to sever stage.

\(^c\) 1 death, 2 transferred to ICU, 1 complained of QT interval prolongation among 11 patients treated with HCQ + AZT.

\(^d\) Longer duration of DENV viremia, CQ was associated with a significant reduction in fever clearance time.

\(^e\) Temporary improvement in the quality of life.

\(^f\) Delayed immune response and more frequent arthralgia in treated group.

\(^g\) In one patient.

Scores in mice and cats, respectively [105,110]. However, it has been found that CQ significantly reduced HCoV-OC43 dissemination and replication in mice central nervous system (CNS) [152] and increased the survival rate of HCoV-OC43 infected newborn mice when their mothers treated by CQ most probably through placental and maternal milk transfer [108].

On the other hand, CQ administration has shown contradicting outcomes when used against non-CoVs RNA viruses in different animal models. Some studies have demonstrated antiviral efficacy of CQ in influenza A virus H5N1, ZIKV and EBOV infected mice [29,134,139]. Interestingly, CQ was effective against ZIKV in both wild type and IFNAR deficient mice, and protected infected suckling pups from infection and microcephaly when given to their mothers [29,134,136]. However, several other studies showed no significant antiviral effect of CQ against influenza A H1N1 and H3N2 viruses in mice and Ferrets, respectively [129]. Similarly, CQ was ineffective against EBOV in guinea pigs, mice and hamsters [137,138]. Nipah virus (NIV) in Ferrets and hamsters [148,153], Hendra virus (HeV) in hamsters [148], CHIKV in cytomolgous macaques [133], Lassa virus (LASV) in mice [139] and Semliki Forest Virus (SFV) in mice [154]. Importantly, most of these previous in vivo studies showed toxicity in animals [129,133,137,138,154]. Furthermore, it was shown that CQ could lead to disease exacerbation correlating with increased type I IFN response and delayed immune responses in CHIKV infected macaques [133], increased mortality rate of SFV-infected mice [154] and NIV or HeV infected hamsters [148].
| Drug  | Design  | Status      | Group(s)                  | Total No | Primary outcomes          | Country          | Registration No. |
|-------|---------|-------------|---------------------------|----------|---------------------------|------------------|-----------------|
| HCQ   | Interv.  | Completed   | Conventional treatment    | 360      | Viral clearance            | China            | ChiCTR2000029868 |
| HCQ   | Interv.  | Recruiting  | Conventional treatment    | 78       | Clinical status            | China            | ChiCTR2000029740 |
| HCQ   | Interv.  | Recruiting  | Placebo                   | 300      | Viral clearance            | China            | ChiCTR2000029559 |
|       | Retros.  | Recruiting  | Conventional treatment    | 1200     | T cell recovery time       | China            | ChiCTR2000031782 |
| CQ    | Interv.  | Recruiting  | Conventional treatment    | 30       | Viral clearance            | China            | NCT04261517     |
| CQ    | Interv.  | Recruiting  | Placebo/FAV               | 150      | Improvement or recovery    | China            | ChiCTR2000030978 |
| CQ    | Interv.  | Recruiting  | Placebo                   | 300      | Viral clearance            | China            | ChiCTR2000031204 |
| CQ    | Interv.  | Recruiting  | Conventional/CQ           | 100      | Length of hospital stay    | China            | ChiCTR2000029939 |
| CQ    | Interv.  | Recruiting  | Conventional/CQ           | 100      | Mortality                  | China            | ChiCTR2000029935 |
| CQ    | Interv.  | Recruiting  | Placebo                   | 205      | Viral clearance            | China            | NCT04286503     |
| CQ    | Interv.  | Recruiting  | Conventional treatment    | 20       | Viral clearance            | China            | ChiCTR2000029542 |
| CQ    | Interv.  | Recruiting  | Placebo                   | 112      | Clinical status            | China            | ChiCTR2000029741 |
| CQ    | Interv.  | Recruiting  | Control                   | 80       | Clinical recovery time     | China            | ChiCTR2000029988 |
| CQ    | Interv.  | Recruiting  | CQ                        | 10       | Viral clearance            | China            | NCT04319900     |
| CQ    | Interv.  | Recruiting  | Placebo/FAV               | 150      | Time to and frequency of   | China            | ChiCTR2000029975 |
| CQ    | Interv.  | Recruiting  | Cartimycin/CQ/LPV/RTV     | 520      | Improvement or recovery    | China            | NCT04286503     |
| CQ    | Interv.  | Recruiting  | HCQ                        | 100      | Clinical recovery time     | China            | ChiCTR2000029899 |
| CQ    | Interv.  | Recruiting  | CQ                        | 100      | Clinical recovery time     | China            | ChiCTR2000029898 |
| CQ    | Interv.  | Recruiting  | Conventional treatment    | 100      | Clinical recovery time     | China            | ChiCTR2000030054 |
| CQ    | Interv.  | Recruiting  | Placebo                   | 1600     | No. symptomatic confirmed  | USA              | NCT04318444     |
| CQ    | Interv.  | Recruiting  | Standard of care          | 500      | Clinical status            | USA              | NCT04335552     |
| CQ    | Interv.  | Recruiting  | HCQ/Vit C/D/Zinc          | 600      | Viral clearance            | USA              | NCT04335084     |
| CQ    | Interv.  | Recruiting  | HCQ/Vit C/D/Zinc          | 1250     | Hospitalization            | USA              | NCT04334967     |
| CQ    | Interv.  | Recruiting  | HCQ/Vit C/D/Zinc          | 60       | Symptoms resolution        | USA              | NCT04334512     |
| CQ    | Interv.  | Recruiting  | HCQ/AZT                   | 1550     | Hospital admission         | USA              | NCT04334382     |
| CQ    | Interv.  | Recruiting  | HCQ/AZT/HPD               | 210      | Viral clearance            | USA              | NCT04336554     |
| CQ    | Interv.  | Recruiting  | HCQ                        | 360      | Rate of positivity         | USA              | NCT04332225     |

(continued on next page)
| Drug | Design | Status | Group(s) | Total No | Primary outcomes | Country | Registration No. |
|------|--------|--------|----------|----------|------------------|---------|-----------------|
| HCQ | Interventionsal ROLS | Not yet recruiting | Standard of care | 160 | Viral clearance | USA | NCT04336332 |
| HCQ | Interventionsal RDBPCS | Recruiting | Placebo | 510 | Clinical status | USA | NCT04332991 |
| HCQ | Interventionsal RDBPCS | Not yet recruiting | Placebo | 400 | Quarantine release rate Hospital discharge rate Infection rate | USA | NCT04329923 |
| HCQ | Interventionsal RCT | Recruiting | Placebo | 3500 | Survival/recovery | USA | NCT04328467 |
| HCQ | Interventionsal RDBPCS | Recruiting | Placebo | 4000 | Clinical status | USA | NCT04328012 |
| HCQ | Interventionsal ROLS | Recruiting | Placebo | 300 | Clinical status | USA | NCT04329832 |
| HCQ | Interventionsal RSBS | Not yet recruiting | Ascorbic Acid | 2000 | Viral clearance | USA | NCT04328961 |
| HCQ | Interventionsal ROLCS | Recruiting | Placebo | 500 | Recovery | USA | NCT04341727 |
| HCQ | Interventionsal RCT | Recruiting | Placebo | 3000 | Incidence in asymptomatic Severity | USA/Canada | NCT04308668 |
| HCQ | Interventionsal RDBPCS | Not yet recruiting | Placebo | 55000 | Disease severity | USA, Australia, Canada, Ireland, South Africa, UK | NCT04333732 |
| HCQ | Interventionsal ROLS | Not yet recruiting | Placebo | 1000 | Clinical status | Canada | NCT04321993 |
| HCQ | Interventionsal RDBPCS | Not yet recruiting | Placebo | 1660 | Hospitalization IMV Mortality | Canada | NCT04329611 |
| CQ  | Interventionsal ROLCS | Not yet recruiting | Placebo | 1500 | Outpatients: admission or death Inpatients: IMV or death Confirmed infection in HCW | Canada | NCT04342463 |
| HCQ | Interventionsal RDBPCS | Not yet recruiting | Placebo | 1200 | Confirmed infection in HCW | France | NCT04328285 |
| HCQ | Interventionsal RDBPCS | Recruiting | Placebo | 1300 | Mortality IMV | France | NCT04325893 |
| HCQ | Interventionsal ROLS | Recruiting | Placebo | 3100 | Clinical status | France | NCT04315948 |
| HCQ | Interventionsal ROLCS | Recruiting | Placebo | 3100 | Clinical status | France | EudraCT 2020-000936-23 |
| CQ  | Interventionsal ROLCS | Recruiting | Placebo | 25 | Viral clearance | France | EudraCT 2020-000890-25 |
| HCQ | Interventionsal ROLS | Recruiting | Placebo | 1000 | Incidence Mortality Death | France | EudraCT 2020-001250-21 |
| HCQ | Interventionsal RDBPCS | Recruiting | Placebo | 1300 | Mortality IMV | France | EudraCT 2020-001271-33 |
| CQ  | Interventionsal CSS | Recruiting | Placebo | 50 | HCQ pharmacokinetics | France | EudraCT 2020-001281-11 |
| CQ  | Interventionsal ROLS | Recruiting | Placebo | 1000 | Renal failure | France | NCT04314817 |
| CQ  | Interventionsal ROLCS | Recruiting | Placebo | 273 | Survival rate | France | NCT04333914 |
| HCQ | Interventionsal RDBS | Not yet recruiting | Placebo | 30 | Chest CT-scan Viral clearance Clinical status | Iran | NCT04331470 |
| HCQ | Interventionsal ROLCS | Recruiting | Placebo | 30 | Chest CT-scan Viral clearance Clinical status | Iran | IRTC201002280003449N27 |
| HCQ | Interventionsal ROLCS | Recruiting | Placebo | 30 | Chest CT-scan Viral clearance Clinical status | Iran | IRTC201002280003449N28 |

(continued on next page)
| Drug | Design | Status | Group(s) | Total No | Primary outcomes | Country | Registration No. |
|------|--------|--------|----------|----------|------------------|---------|-----------------|
| HCQ  | Interventional ROLCS | Recruiting | HCQ/LPV/RTV | 50 | Clinical status | Iran | IRCT20100228003449N29 |
|      |                     |          | HCQ/LPV/RTV/SOF/LDV |           | Lab/radiological findings | Iran | IRCT20151227025726N12 |
|      |                     |          | HCQ/LPV/RTV | 20 | Clinical status | Iran | IRCT20100228003449N30 |
| HCQ  | Interventional SAOLS | Recruiting completed | HCQ/OTV/LPV/RTV/IFβ-1a | 50 | Clinical status | Iran | IRCT20151227025726N12 |
|      |                     |          | HCQ/ATV/RTV | 630 | Clinical status | Brazil | NCT043222123 |
| HCQ  | Interventional ROLCS | Not yet recruiting | Standard of care HCQ | 440 | Mortality | Spain | NCT043253257 |
| HCQ  | Interventional ROLCS | Recruiting | HCQ | 440 | No. confirmed cases | Spain | NCT04331834 |
| HCQ  | Interventional OLCS | Recruiting | RDV | 400 | Incidence of secondary cases | Spain | NCT04304053 |
| HCQ  | Interventional OLCS | Not yet recruiting | RDV | 700 | Mortality | Norway | NCT04321616 |
| HCQ  | Interventional OLCS | Recruiting | HCQ/RTV | 20 | Evolution of ARS, SpO2, hemodynamic stability | Norway | NCT04329572 |
| CQ   | Interventional RDBS | Recruiting Low Dose CQ | 20 | Mortality | Norway | NCT043253257 |
| HCQ  | Interventional ROLCS | Recruiting | HCQ/AZT | 440 | No. confirmed cases | Spain | NCT04331834 |
| CQ   | Interventional RDBS | Recruiting Placebo | 40,000 | No. symptoms severity | UK | EudraCT 2020-001113-21 |
| HCQ  | Interventional ROLCS | Recruiting Placebo | 150 | No. patients not admitted to ICU | Korea | NCT04307693 |
| HCQ  | Interventional ROLCS | Recruiting | No intervention control HCQ | 141 | Viral clearance | Korea | NCT04330586 |
| HCQ  | Interventional ROLCS | Recruiting | HCQ | 2486 | Incidence | Korea | NCT04330144 |
| HCQ  | Interventional ROLCS | Recruiting | Placebo | 2700 | Clinical status | Germany | NCT04340544 |
| HCQ  | Interventional ROLCS | Recruiting | Placebo | 220 | Viral clearance | Germany | NCT04322221 |
| HCQ  | Interventional ROLCS | Recruiting | Placebo | 334 | Hospital admission | Germany | NCT04338906 |
| HCQ  | Interventional ROLCS | Recruiting | Placebo | 220 | Viral clearance | Germany | EudraCT 2020-001224-33 |
| HCQ  | Interventional ROLCS | Recruiting | Placebo | 150 | Dose optimization | Australia | ACTRN12620000447954 |
| HCQ  | Interventional RTBPCS | Recruiting | Placebo | 680 | Sick days of HCW | Australia | ACTRN12620000447954 |
| HCQ  | Interventional ROLCS | Recruiting | Placebo | 400 | Infection rate | Mexico | NCT04318015 |
| HCQ  | Interventional ROLCS | Recruiting | Placebo | 500 | Mortality | Mexico | NCT04315896 |
| Drug     | Design          | Status                        | Group(s)                               | Total No | Primary outcomes                                           | Country Registration No. |
|----------|-----------------|-------------------------------|----------------------------------------|----------|-----------------------------------------------------------|--------------------------|
| HCQ      | Interventional ROLCS | Not yet recruiting          | Control HCQ                           | 1116     | Development of severe infection or death                 | Israel NCT04323631        |
|          | ROLCS: Randomized open label controlled study; |                    | Standard of care HCQ                  | 210      | Viral clearance                                           | Israel NCT04333628        |
|          | RDBS: Randomized double blind study; |                    | CQ                                     | 80       | Clinical status                                          | Turkey NCT04326725       |
| CQ       | Interventional ROLCS | Not yet recruiting          | Convalescent Plasma/HCQ/AZT           | 80       | Viral clearance (mg)                                      | Colombia NCT04332835     |
|          | Observation CCS   | Recruiting                  | HCQ                                    | 80       | Viral clearance                                          | Thailand NCT04303299      |
| HCQ      | Interventional ROLS | Not yet recruiting          | Quarantine/no treatment                | 50       | CRP level                                                | Japan JRCTe031190227      |
|          | OLS: Open label study; |                    | HCQ/LPV/RTV ± OTV                      | 60       | Symptoms reduction (mg)                                  | Greece EudraCT 2020-001345-38 |
| CQ       | Interventional ROLS | Not yet recruiting          | Natural Honey HCQ/LPV/RTV Arbidol HCQ | 250      | Viral clearance                                          | Vietnam NCT04328493       |
|          | ROLCS: Randomized placebo controlled study; |                    | CQ OTV ± AZT                           | 1000     | Viral clearance Fever                                    | Egypt NCT04323345         |
| HCQ      | Interventional ROLCS | Not yet recruiting          | Placebo HCQ/HCQ/AZT                   | 75       | Clinical status                                          | Pakistan NCT04328272      |
|          | GS: Open label study; |                    | Control HCQ/AZT OTV/HQ OTV AZT/HQ AZT/OTV HCQ/AZT/OTV | 500      | Viral clearance                                          | Pakistan NCT04338698      |
| CQ       | Interventional ROLCS | Not yet recruiting          | Standard of care HCQ                  | 950      | Disease progression Admission to ICU or death           | Netherlands Trial NL8490   |
|          | RDPCS: Randomized placebo controlled study; |                    | Placebo HCQ/AZT                        | 226      | Survival Hospitalization                                 | Denmark NCT04322396       |
| HCQ      | Interventional ROLCS | Recruiting                  | Control HCQ/LPV/RTV Wide range of drugs | 6800     | Mortality Days alive and outside ICU                     | New Zealand NCT02735707    |
|          | ROLCS: Randomized placebo controlled study; |                    | Placebo HCQ                           | 440      | Viral clearance                                          | Austria NCT0436748        |
|          | ROLCS: Randomized placebo controlled study; |                    | CQ/OTV RTV/DRV/OTV LPV/RTV FAV/LP/RTV OTV/CQ/DRV/OTV | 440      | Viral clearance                                          | Austria NCT04303299       |
|          | ROLCS: Randomized placebo controlled study; |                    | Quarantine                             | 400      | Hospitalization or all causes of death                  | Poland NCT04331600        |

**Table 6 (continued)**

**ROLCS**: Randomized open label controlled study; **RDPCS**: Randomized double blind study; **ROLS**: Randomized open label controlled study; **RROLCS**: Retrospective randomized open label controlled study; **RCT**: Randomized clinical trial; **RSBGS**: Retrospective randomized single blind controlled study; **RSBS**: Randomized single blind controlled study; **SAOLS**: Single arm open label study; **OLS**: Open label study; **RDBPCS**: Randomized double blind placebo controlled study; **RSBS**: Randomized single blind study; **OLCS**: Open label controlled study; **CSS**: Cross-sectional study; **RPCS**: Randomized placebo controlled study; **RTBSC**: Randomized triple blind controlled study; **CCPS**: Case-control prospective study.

**HCQ**: Hydroxychloroquine; **CQ**: Chloroquine; **FAV**: Favipiravir; **LPV**: Lopinavir; **RTV**: Ritonavir; **AZT**: Azithromycin; **CQ**: Chloroquine; **Vit C**: Vitamin C; **Vit D**: Vitamin D; **LST**: Losartan; **RDV**: Remdesivir; **IFβ-1a**: Interferon β-1a; **NIVO**: Nivolumab; **TCZ**: Tocilizumab; **LEV**: Levamisole; **BUD**: Budesonide; **FORM**: Formoterol; **SOF**: Sofosbuvir; **LDV**: Ledipasvir; **OTV**: Oseltamivir; **ATV**: Atazanavir; **COBI**: Cobicistat; **TDF**: Tenofovir disoproxil fumarate; **FTC**: Emtricitabine; **CIC**: Ciclesonide; **DRV**: Darunavir; **HCW**: Healthcare workers; **ARS**: Atazanavir; **TCZ**: Tocilizumab; **Vit D**: Vitamin D; **SOF**: Sofosbuvir; **RTV**: Ritonavir; **Č**: Ciclesonide; **FAV**: Favipiravir.

Data were obtained from NIH, U.S. National Library of Medicine (https://wwwclinicaltrials.gov/); the Chinese Clinical Trial Registry (http://www.chictr.org.cn/); the European Union Clinical Trials Registry (https://www.clinicaltrialsregister.eu); ISRCTN registry (http://www.isrctn.com/); Netherlands Trial Registry (https://www.trialregister.nl/); Iranian Registry for Clinical Trials (IRCT) (https://en.irct.ir/); Japanese Registry for Clinical Trials (JRCT) (https://jRCT.niph.go.jp/); and the Australian New Zealand Clinical trial Registry (ANZCTR) (https://www.anzctr.org.au/).

[a] The same study was registered in ISRCTN registry (registration no. ISRCTN50189673) with a total number of 5000 patients.

[b] Hydrocortisone, Ceftriaxone, Moxifloxacin or Levofloxacin, Piperacillin-tazobactam, Ceftaroline, Amoxicillin-clavulanate, Macrolide, OTV, IFβ-1a, and Anakinra.
In this comprehensive review of the antiviral effects of CQ and HCQ on SARS-CoV-2 as well as other viruses, we show a broad variation in the research outcomes. Both CQ and HCQ demonstrated promising in vitro results, however, such data have not yet been translated into meaningful in vivo studies. While few clinical trials have suggested some beneficial effects of CQ and HCQ in COVID-19 patients, most of the reported data are still preliminary [20,162,163]. Furthermore, at least 7 of the ongoing trials were canceled or stopped and it is not yet clear if this was due to possible adverse effects, ineffectiveness or other reasons.

There are several toxicities associated with these drugs [78–80], the one that is foremost concerning is the possibility of QT prolongation and the risk of Torsades de pointes, which is a potentially life-threatening arrhythmia [81–83]. Nevertheless, while our literature review showed that this is quite rare, it is not yet evident whether there would be any additive or possible synergistic risk when these drugs are combined with other medications such as AZT [83]. In fact, it is challenging to base a treatment decision in the absence of a complete research cycle and a clear vision of drug efficacy and safety. Given the current uncertainty, it is worth being mindful of the potential risks and strictly rational the use of these drugs in COVID-19 patients until further high quality randomized clinical trials are available to clarify their role in the treatment or prevention of COVID-19.

Funding

This work was supported by King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia, grant number 09-1, which is a part of the Targeted Research Program (TRP).

Declaration of competing interest

None declared.

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