Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Sep 20; 8(T1):184-191.
https://doi.org/10.3889/oamjms.2020.4854
eISSN: 1857-9655
Category: *T1 - Thematic Issue "Coronavirus Disease (COVID-19)"*
Section: *Systematic Review Article*

Potential Antiviral Effect of Chloroquine Therapy against SARS-CoV-2 Infection

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Edited by: Mirko Spiroski
Citation: Musa IR. Potential Antiviral Effect of Chloroquine Therapy against SARS-CoV-2 Infection. Open Access Maced J Med Sci. 2020 Sep 20; 8(T1):184-191. https://doi.org/10.3889/oamjms.2020.4854
Keywords: Chloroquine; Hydroxychloroquine; Antiviral action; Mechanism; Safety; Efficacy; SARS-CoV-2 and coronavirus disease 2019
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Received: 28-Apr-2020
Revised: 27-May-2020
Accepted: 14-Sep-2020

Funding: Publication of this article was financially supported by the Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The coronavirus disease 2019 (COVID-19) pandemic has affected many countries with increasing morbidity and mortality. In the absence of an effective vaccine and medication, chloroquine may be a potential choice.

AIM: This study aims to explore the role of the possible antiviral effects of chloroquine against SARS-CoV-2.

MATERIALS AND METHODS: A systematic search of studies relating to the antiviral effects against coronaviruses was conducted between January 1, 1990, and up to May 26, 2020, for relevant studies using PubMed, Scopus, and Google Scholar.

RESULTS: A total of 174 articles were initially identified. Ninety-seven papers were removed for failing to address the aim of the study. Seventy-seven full-text articles were retrieved for eligibility analysis. Ten studies focused on general inhibition of viral replication, ten evaluated its effects on angiotensin-converting enzyme 2, 19 addressed the effects on alkalizing the cellular pH, 25 concentrated on the immunomodulatory effect, two assessed the potential effects on sialic acid, and 24 explored the therapeutic outcome.

CONCLUSION: Chloroquine has promising antiviral effects on SARS-CoV-2 at different levels.

Introduction

In December 2019, a cluster of reported chest infections among citizens in Wuhan, China, that were caused by a newly isolated β-coronavirus, which was initially named "2019 Novel Coronavirus" (2019-nCoV) on January 12, 2020, by the World Health Organization (WHO). While the WHO officially named the disease coronavirus disease 2019 (COVID-19) on February 11, 2020, the International Committee Coronavirus Study Group suggested naming it "Severe Acute Respiratory Syndrome Coronavirus 2" (SARS-CoV-2) on the same day [1]. Human-to-human transmission of SARS-CoV-2 was observed mainly in close direct contact, a recent history of travel to Wuhan (72.3%) and among healthcare workers (3.8%) [2]. In contrast to COVID-19, SARS infection was relatively high among healthcare workers (33–42%), and almost similar contact rate (62–79%) [3], [4]. On March 12, 2020, the WHO declared COVID-19 to be a global pandemic and Italy was identified as the second most affected country with a higher case fatality rate (CFR) [5]. A week later, over 100 countries reported positive cases of COVID-19 with increased morbidities and mortalities [6]. Surprisingly, a recent time-delay adjusted estimation indicates that the COVID-19 CFR reached 20% in Wuhan compared to the cumulative number of deaths (5.6%) [7]. The rapid spread of the disease to the pandemic level, higher rate of morbidity and mortality, exhaustion of health facilities in the affected countries, non-availability of a vaccine, non-availability of approved medications for COVID-19, and previous reports of antiviral effects of chloroquine suggest chloroquine as a potential treatment option to modify the nature of the disease. The *in vitro* antiviral activity of chloroquine was observed in the late 1960s [8]. Recently, there has been a growing body of evidence during the COVID-19 pandemic that shows the antiviral efficacy of hydroxychloroquine alone or in combination with other medications [1], [9], [10], [11], [12].

The anti-inflammatory and immunomodulatory actions of chloroquine analogs have been reported in the treatment of viral infections and their pathologies [13]. Both chloroquine and hydroxychloroquine can negatively affect the growth of many different members of human coronavirus [14], [15]. Recently, a higher efficacy was reported in an *in vitro* study, favoring the control of SARS-CoV-2 infection [16]. Chloroquine analog in combination with other antiviral drugs is considered an effective option for therapy for viral diseases to avoid the interaction of P-glycoprotein and multidrug-resistance associated proteins in these viruses, which extrude medications from the cells and cellular organelles [17]. The results of chloroquine use in various *in vitro* studies demonstrated its effect on cellular pH [18], and it inhibits replication of several DNA and RNA

viruses [19] and interferes with terminal glycosylation of the cellular receptor angiotensin-converting enzyme 2 (ACE2) [20]. Hence, chloroquine was recently used in the management of COVID-19 during the current pandemic outbreak [1], [9], [10], [11], [12]. Chloroquine has long been used as an antimalarial and anti-inflammatory agent. It has a reasonable degree of safety at a low price. For these reasons, we decided to conduct this study to explore the possible antiviral effects of chloroquine and the possible mechanism of action to improve our understanding of this drug and shed light on it for potential future studies.

Materials and Methods

A systematic search of studies relating to chloroquine's antiviral effect against coronavirus was performed between January 01, 1990 and up to May 26, 2020 using PubMed, Scopus, and Google Scholar. We used combinations of the following search terms: "Chloroquine," "hydroxychloroquine," "antiviral action," "mechanism" safety" efficacy" "COVID-19," and "SARS-CoV-2." The preferred reporting items for systematic review and meta-analysis guidelines were adopted, as illustrated in Figure 1 [21]. The electronic database search yielded 174 articles. Ninety-seven studies were removed for not addressing the aim of the study, duplication, lacking a proper citation, and not being within the period decided beforehand. Titles and abstracts were assessed to identify eligibility for full screening. Studies that employed acceptable quantitative and/or qualitative methods, including randomized controlled trials, observational studies (such as cross-sectional, experimental, and interventional studies), review articles, ideas, editorials, letters to the editor, and opinions were included in the study. All articles focusing on the potential possible antiviral effects of chloroquine, the mechanism of action and therapeutic outcomes were eligible for inclusion. Then, all relevant studies were selected and full-text manuscripts retrieved for assessment. The clinical opinions were critically appraised using the recommended checklist by McArthur *et al*. (2015) to focus on relevant articles [22]. The studies were grouped according to the primary aims, focusing on viral replication inhibition, chloroquine's action on ACE2, alkalization at the cellular level, chloroquine immunomodulatory effects, effects on sialic acid, therapeutic trials and studies that addressed more than one item. This enabled grouping of articles that focused specific targets and issues relevant to the study objectives and facilitated the retrieval of information.

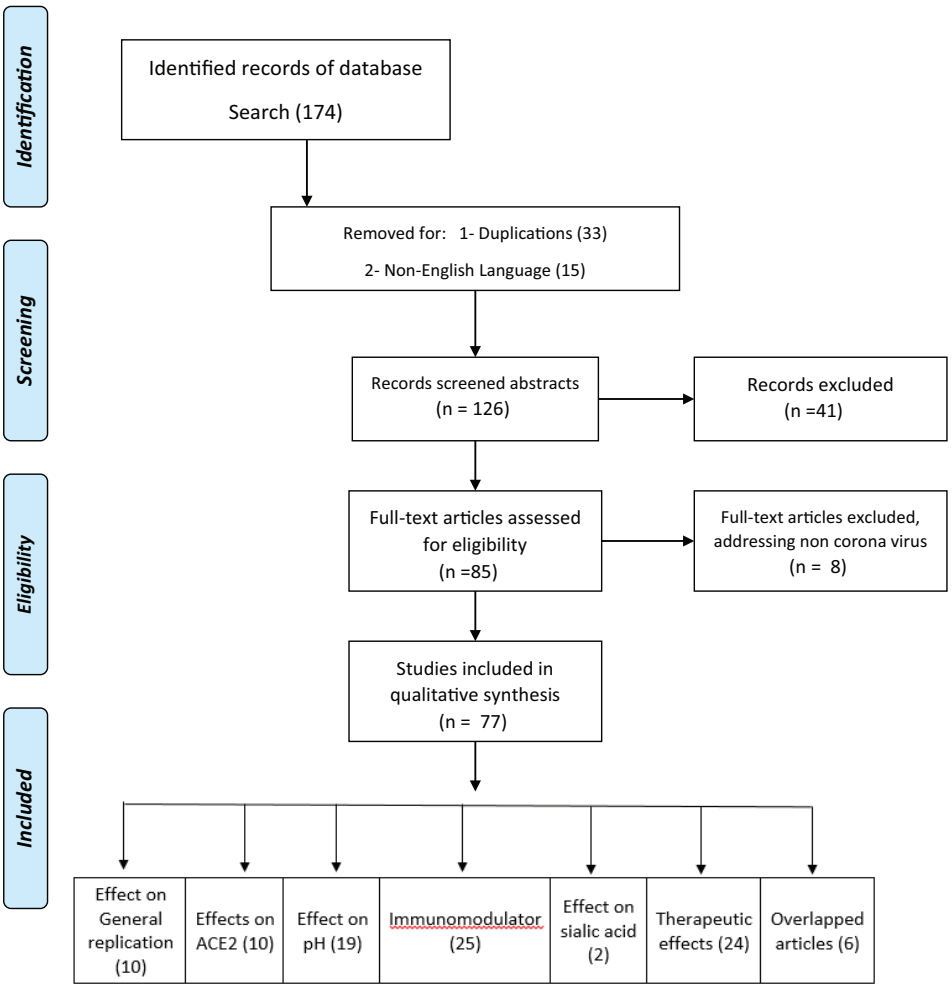

Figure 1: Flow chart of the study selection

Results

A total of 174 articles were initially identified. Ninety-seven studies were removed for not addressing the aim of the study, duplication, lack of proper citations, and poor use of language. After screening titles and abstracts, 77 full-text articles were retrieved for eligibility analysis. Ten studies focused on general viral replication inhibition [14], [19], [20], [21], [22], [23], [24], [25], [26], [27], ten evaluated its effects on ACE2 [13], [15], [28], [29], [30], [31], [32], [33], [34], 19 addressed the effects on alkalizing the cellular pH [13], [15], [31], [32], [33], [35], [36], [37], [38], [39], [40], [41], [42], [43], [44], [45], [46], [47], [48], 25 concentrated on chloroquine therapy as an immunomodulator [13], [16], [26], [37], [49], [50], [51], [52], [53], [54], [55], [56], [57], [58], [59], [60], [61], [62], [63], [64], [65], [66], [67], [68], [69], two assessed the potential effects on sialic acid [70], [71], 24 articles explored the therapeutic outcome [1], [5], [15], [16], [20], [35], [68], [69], [72], [73], [74], [75], [76], [77], [78], [80], [81], [82], [83], [84], [85], and nine addressed more than one target [13], [15], [16], [26], [31], [32], [35], [33], [37].

Discussion

The review of articles indicates that chloroquine has broad-spectrum antiviral activities at different sites and levels. These properties have caused many researchers to conduct studies and explore their potential effects. Some studies have focused on the general inhibition of viral cycle replication without illustrating details. Chloroquine, widely promoted as an antimalarial and autoimmune disease drug, was recently shown to have a potential broad-spectrum antiviral effect that interferes with the viral replication cycle [23], [24]. This was supported by the outcome of many *in vitro* studies that documented the inhibitory effect on the replication of some coronaviruses in epithelial lung cell cultures [25], [26], a recombinant HCoV-O43 coronavirus [27], and MERS-CoV [86]. A recently published study pointed to the extended inhibitory effect on several DNA and RNA viruses, including most human coronaviruses [19]. In addition, many experimental studies on coronavirus proved that chloroquine had a negative effect at the replication level [14], [19], [20]. However, one study reported ambiguous outcomes [87].

ACE2 is another target for chloroquine's antiviral effect. ACE2 is found in the lower respiratory tract of humans and is a cell receptor for SARS-CoV that is responsible for its replication and pathogenesis [28]. The virion glycoprotein on the surface of coronavirus uses the ACE2 receptor on the surface of human cells as a recognition site to gain access and facilitate both cross-species and human-to-human transmission [29], [35]. Bronchoalveolar lavage fluid is used to diagnose COVID-19 when the presence of ACE2 is indicated in the lower respiratory tract [30]. In *in vitro* studies, chloroquine appears to interfere with terminal glycosylation of the cellular receptor ACE2 to inhibit virus-receptor binding and ultimately abrogate the infection [13], [31]. Chloroquine's potent anti-SARS-CoV effects *in vitro* have been documented in many clinical trials [15], [35], [32], [33]. ACE2 as a site of recognition for coronavirus raises concerns about its interaction with ACE inhibitors and the outcome of coronavirus disease. However, a recently published study confirmed that ACE inhibitors do not inhibit ACE2 because ACE and ACE2 are different enzymes, and no data suggest that ACE inhibitor or Angiotensin II Type 1 receptor blocker therapy facilitates coronavirus entry by increasing ACE2 expression in both animal and human subjects [34].

Chloroquine can negatively affect a pre-entry step of the viral cycle by interfering with viral particles binding to their cellular cell surface receptor by blocking quinone reductase 2, which facilitates the biosynthesis of sialic acids. Sialic acids are present on cell transmembrane proteins as important components of ligand recognition [70], [71]. Interference with sialic acid biosynthesis might represent part of chloroquine's broad antiviral spectrum against coronaviruses that depend on sialic acid moieties as receptors [71].

Changing the intracellular pH is chloroquine's greatest potential antiviral effect because coronavirus replicates in acidic environments. In fact, coronavirus cell entry is achieved through the endolysosomal pathway that depends on a certain internal pH [36]. Increasing endosomal pH promotes chloroquine as a potential powerful antiviral agent. This will affect the transduction of pseudotype viruses decorated with SARS-CoV spike protein and will affect terminal glycosylation of the cellular receptor ACE2 [15], [32], [33], [35]. This may be explained by *chloroquine*'s ability to diffuse spontaneously and rapidly across the membranes of cells and organelles to acidic cytoplasmic vesicles such as endosomes, lysosomes, or Golgi vesicles to alter their pH [13]. This will disturb the activity of several enzymes, including those essential for proteolytic processing and post-translational modification of viral proteins, which will prevent the fusion of the virus to the cell membrane [37], [38], [39]. Its effect may extend to inhibit some vital steps, such as nucleic acid replication, glycosylation of viral proteins, new virus particle transport, virus assembly, virus release to achieve its antiviral effects [39], and other as-yet poorly understood antiviral activity mechanisms [31], [39].

Chloroquine analogs prevent viral entry and replication processes into the cytoplasm of susceptible cells by neutralizing acidic pH in endosomes to abrogate the infections [37], [40], [41], [42], [43], [44] because low pH is essential for fusion of the virus and endosomal membranes to release the viral SARS-CoV genome into the cytosol [45]. In non-human coronaviruses, the intracellular site of coronavirus budding is influenced by the localization of its membrane M proteins that accumulate in the Golgi complex beyond the site of virion budding [46]. This was supported by a recent report that showed that the C-terminal domain of the MERS-CoV M protein contains a trans-Golgi network localization signal [47]. In addition, it affects the virus maturation process by impairing the proper maturation of the viral protein [48].

Chloroquine is an antimalarial and autoimmune disease medication. Its immunomodulatory effects encourage scientists to evaluate its performance on viruses. It enhances the immune response by promoting the export of soluble antigens into the cytosol of dendritic cells and directing human cytotoxic CD8+ T cell responses against viral antigens [49]. Furthermore, it organizes the cross-presentation of non-replicating virus antigens by dendritic cells to CD8+ T-cells migrated to lymph nodes at the site of infection and ultimately establishes a broad protective immune response [50]. Chloroquine inhibits nanoparticle endocytosis by resident macrophages; this effect is dose related [51], [52]. Furthermore, chloroquine prevents the fusion of lysosomes, which is likely to interfere with upstream endocytic trafficking by blocking the effective transport between cellular organelles and the cell membrane [53]. However, one study reported no potential effect *of* chloroquine on primary human monocyte-derived macrophages and dendritic cells in MERS-CoV infection [53]. Chloroquine is a well-known immunomodulatory drug that can mediate an anti-inflammatory response [37]. This effect has been observed in the treatment of viral infections and associated pathologies [13], [16]. Consequently, chloroquine analogs block the release of several cytokines, chemokines, or mediators that are blamed for the severity of viral infections. Therefore, inhibition of endosomal acidification by chloroquine therapy may be promoted as a potential therapeutic target for viral infections and associated pathologies. Cytokines, chemokines, and the activities of several host endosomal proteases depend on endosomal-lysosomal acidification [54], [55].

One of the cytokines strongly implicated in viral pathologies is tumor necrosis factor-a (TNF-α), which activates macrophages to potentiate the production of mediators that facilitate both the permeability and infectivity of endothelial cells [56], [57]. Chloroquine's key effect is its prevention of macrophage activation and inhibition of TNF-a secretion from various cells at clinically relevant concentrations [13], [37], [58] inhibition of TNFα mRNA expression [59], [60], [61] and reduction of interleukin (IL-1 and IL-6) cytokines that are released from monocytes and macrophages [62]. Chloroquine also adopts another pathway to inhibit TNFα production by disrupting cellular iron metabolism [63]. Moreover, it blocks the conversion of pro-TNF into soluble mature TNFα molecules, which modifies the immune response [64]. Chloroquine analogs enhance immune activation in viral infection and reduce systemic T cell activation [65], [66]. Chloroquine inhibits IL-1β mRNA expression in T helper-1 (THP-1) cells and reduces IL-1β production [58]. Likewise, it affects the immune system through cell signaling and regulation of proinflammatory cytokines by inhibiting phosphorylation of p38 mitogen-activated protein kinase in THP-1 cells and caspase-1 [59]. Viruses frequently require the phosphorylation step to replicate [26], [67].

Chloroquine blocks toll-like receptor-mediated activation of plasmacytoid dendritic cells and myeloid differentiation primary response gene 88 signaling through three pathways. First, it decreases the levels of the downstream signaling molecules IL-1 receptor-associated kinase 4 and IFN regulatory factor 7. Second, it inhibits IFN-a synthesis and blocks the negative modulators of T-cells such as indoleamine 2,3-dioxygenase. Third, it promotes downstream signaling of programmed death-ligand 1 [68]. Clinically, both hydroxychloroquine and chloroquine have immunomodulatory effects that impair the increase in immune factors that cause a cytokine storm, which is followed by multiorgan failure and potentially death. Therefore, early treatment with chloroquine can abort or modify these serious complications [41], [69].

Many clinical trials have assessed the therapeutic efficacy of chloroquine against coronavirus. In an *in vitro* study, chloroquine had broad-spectrum antiviral effects in the control arm of SARS-CoV-2 infection [16]. Likewise, in a mouse model, it maintained a higher efficacy against coronavirus [15], [72]. Interestingly, chloroquine showed potent inhibitory effects on the treated primate cells before and after exposure to the virus, which shows both prophylactic and therapeutic advantages [31]. At present, many clinical trials are testing chloroquine as anti-COVID-19 therapy [73]. Chloroquine was recently promoted as a potential possible option for treating patients diagnosed with novel coronavirus pneumonia with a successful treatment rate, shortened hospital stay, and improved patient outcome. The recommended dose of chloroquine phosphate tablets was 500 mg twice per day for 10 days for mild, moderate, and severe cases of novel coronavirus pneumonia, providing that patients had no contraindications [74]. Preliminary reports from China suggest that approximately 100 infected patients treated with chloroquine experienced a more rapid symptomatic and radiological lung computed tomography improvement in addition to a shortened hospital stay and recovery period compared with control groups [1], [74], [75], [76]. This would reflect the first successful story for the use of chloroquine in humans to treat an acute viral disease and supports research into its potential as a therapy option during the current COVI-19 outbreak [77]. Based on this promising result, chloroquine has been included in the list of trial drugs in the guidelines for the diagnosis and treatment of COVID-19 released by the National Health Commission of the People's Republic of China[74], [76]. In addition, the Dutch Centre of Disease Control and the Italian Society of Infectious and Tropical Disease (Lombardy section) recommend chloroquine for patients with COVID-19 [5], [20]. In light of the urgency, the absence of a vaccine and effective medications and the pressure health-care systems face to save lives during the COVID-19 pandemic, many countries, including the United States and France, have suggested using chloroquine to manage patients with COVID-19 under certain circumstances [77], [78], [79], [80]. In a small sample size study that recruited 36 subjects, hydroxychloroquine therapy was significantly associated with a reduction in viral load and viral shedding period and worked synergistically with azithromycin against COVID-19 [81]. Likewise,

another study, evaluating 80 cases with a mild presentation, demonstrated rapid clearance of the virus and shortened the mean hospital stay to 5 days with combination therapy of hydroxychloroquine and azithromycin: Progressively negative results of nasopharyngeal PCR assay for the virus were documented at day 7 (83%) and day 8 (93%). In addition, 97.5% of virus cultures from patient respiratory samples were negative on day 5 [77]. Similarly, chloroquine prevented exacerbation of pneumonia with radiological improvement and shortened the course of the disease [1]. Interestingly, in an *in vitro* study on SARS-CoV-2, a similar synergistic effect was obtained in combination therapy of hydroxychloroquine and azithromycin, as both reduce the acidity of the lysosome to impair viral replication [83]. Chloroquine efficacy may support the observational thought that COVID-19 infections are highly pandemic in countries where malaria is the least pandemic and are the least pandemic in nations where malaria is highly pandemic [10]. On the other hand, hydroxychloroquine therapy for patients with COVID-19 infection was associated with a high risk of QT prolongation, and greater changes in QT were observed with concurrent treatment with azithromycin [84], [85] and drug-induced torsades de pointes [85]. Hydroxychloroquine should be avoided in patients with glucose-6-phosphate dehydrogenase deficiency to prevent hemolytic anemia. Both hydroxychloroquine and chloroquine have narrow therapeutic indices for chloroquine and are associated with gastrointestinal symptoms, retinopathy, deafness/tinnitus, and life-threatening toxicity (cardiomyopathy, arrhythmias, and methemoglobinemia) [88]. Recently published data, pointed to increase frequency of ventricular arrhythmias associated with chloroquine therapy for COVID -19infection [89]. Hence, vigilance and cardiac monitoring are recommended to balance the risks and benefits.

Limitations of the study

This study was conducted by one researcher and used only PubMed, Scopus, and Google Scholar databases and timeframes, and some valuable data were not included. Another limitation is related to the article selection criteria that were used.

Conclusion

Chloroquine has a broad-spectrum range of documented antiviral activities and immunomodulators, which is supported by recent limited fruitful clinical trials in humans. In addition, it has a long history of use, anti-inflammatory advantages, safety in reasonable dosages, and low price. Its antiviral effects should be further assessed in large clinical trials in the near future.

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