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

Imad R. Musa*
Department of Internal Medicine, Royal Commission Hospital, Jubail, Saudi Arabia

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 (33–42%) [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

---

**Figure 1: Flow chart of the study selection**
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 intervention trials), 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. The studies were grouped according to the primary aims, focusing on viral replication inhibition, chloroquine's action on ACE2, alkalinization at the cellular level, chloroquine immunomodulatory effects, effects on sialic acid, therapeutic trials and studies that addressed more than one target. This enabled grouping of articles that focused specific targets and issues relevant to the study objectives and facilitated the retrieval of information.

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], [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-α (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-α 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-α 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-19 infection [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.

References

1. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 2020;14(1):72-3. https://doi.org/10.5582/bst.2020.01047 PMid:32074550.
2. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of Coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708-20.
Musa. Potential Antiviral Effect of Chloroquine Therapy Against Sars-Cov-2 Infection

3. Chowell G, Abdiritzak F, Lee S, Lee J, Jung E, Nishiura H, et al. Transmission characteristics of MERS and SARS in the healthcare setting: A comparative study. BMC Med. 2015;13(1):210. https://doi.org/10.1186/s12916-015-0450-0 PMid:26336062

4. Kang CK, Song KH, Choe PG, Park WB, Bang JH, Kim ES, et al. Clinical and epidemiologic characteristics of spreaders of middle east respiratory syndrome Coronavirus during the 2015 outbreak in Korea. J Korean Med Sci. 2017;32(6):744-9. https://doi.org/10.3346/jkms.2017.32.5.744 PMid:28378546

5. Nicostriti E, Petrosillo N, Ippolito G, D’Offizi G, Marchioni L, Bartoli TA, et al. National institute for the infectious diseases “L. Spallanzani”, IRCCS. Recommendations for COVID-19 clinical management. Infect Dis Rep. 2020;12(1):8543. https://doi.org/10.4081/idr.2020.8543 PMid:32218915

6. Remuzzi A, Remuzzi G. COVID-19 and Italy: What next? Lancet. 2020;395(10231):1225-8. https://doi.org/10.1016/s0140-6736(20)30627-9 PMid:32178769

7. Baud D, Qi X, Nielsen-Saines K, Musso D, Pomar L, Favre G. Real estimates of mortality following COVID-19 infection. Lancet Infect Dis. 2020;20(7):773. https://doi.org/10.1016/s1473-3099(20)30195-x PMid:32171390

8. Miller DK, Lenard J. Antihistaminics, local anesthetics, and other amines as antiviral agents. Proci Natl Acad Sci USA. 1981;78(6):3605-9. https://doi.org/10.1073/pnas.78.6.3605 PMid:6115382

9. Choudhary R, Sharma AK, Choudhary R. Potential use of hydroxychloroquine, ivermectin and azithromycin drugs in fighting COVID-19: Trends, scope and relevance. New Microbes New Infect. 2020;35:100684. https://doi.org/10.1016/jnmni.2020.100684 PMid:32322397

10. Meo SA, Klonoff DC, Akram J. Efficacy of chloroquine and hydroxychloroquine in the treatment of COVID-19. Eur Rev Med Pharmacol Sci. 2020;24(8):4539-47. PMid:32379993

11. Sarma P, Kaur H, Kumar H, Mahendru D, Avti P, Bhattacharyya A, et al. Virological and clinical cure in COVID-19 patients treated with hydroxychloroquine: A systematic review and meta-analysis. J Med Virol. 2020;92(7):776-85. https://doi.org/10.1002/jmv.25898 PMid:32297988

12. Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents. 2020;55(4):105932. https://doi.org/10.1016/j.ijantimicag.2020.105932 PMid:32145363

13. Abdul M, Al-Bari A. Chloroquine analogues in drug discovery: New directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. J Antimicrob Chemother. 2015;70(6):1608-21. https://doi.org/10.1093/jac/dkv018 PMid:25693996

14. Keyaerts E, Vigen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome Coronavirus by chloroquine. Biochem Biophys Res Commun. 2004;323(1):264-8. https://doi.org/10.1016/j.bbrc.2004.08.085 PMid:15351731

15. Keyaerts E, Li S, Vigen L, Rysman E, Verbeeck J, Van Ranst M, et al. Antiviral activity of chloroquine against human Coronavirus OC43 infection in newborn mice. Antimicrob Agents Chemother. 2009;53(8):3416-21. https://doi.org/10.1128/aac.01509-08 PMid:19506054

16. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel Coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269-71. https://doi.org/10.1038/s41422-020-0282-0 PMid:32020029

17. Savarino A. Use of chloroquine in viral diseases. Lancet Infect Dis. 2011;11(9):653-4. PMid:21550312

18. Al-Bari MA, Al-Bari CA. Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. Pharmal Res Perspect. 2017;5(1):e00293. https://doi.org/10.1002/prp2.289 PMid:28596841

19. Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against Coronavirus: What to expect for COVID-19? Int J Antimicrob Agents. 2020;55(5):105938. https://doi.org/10.1016/j.ijantimicag.2020.105938 PMid:32171470

20. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care. 2020;57:279-83. https://doi.org/10.1016/j.jcrc.2020.03.005 PMid:19621072

21. Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA Group. Preferred reporting item for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6(7):e1000097. https://doi.org/10.1371/journal.pmed.1000097 PMid:19621072

22. McArthur A, Klugárová J, Yan H, Florescu S. Innovations in fighting COVID-19: Trends, scope and relevance. New Dis. 2020;5(1):e00293. https://doi.org/10.1002/prp2.293 PMid:32145363

23. Savarino A, di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. Lancet Infect Dis. 2011;11(9):653-4. https://doi.org/10.1016/j.iijantimicag.2020.105938 PMid:32145363

24. Yan Y, Zou Z, Sun Y, Li X, Xu KF, Wei Y, et al. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. Cell Res. 2013;23(2):300-2. https://doi.org/10.1038/cr.2012.165 PMid:23208422

25. Blau DM, Holmes KV. Human Coronavirus HCoV-229E enters susceptible cells via the endocytic pathway. Adv Exp Med Biol. 2001;494:193-8. https://doi.org/10.1007/1-4615-1325-4_31 PMid:11774468

26. Kono M, Tatsumi K, Imai AM, Saito K, Kuriyama T, Shirasawa H. Inhibition of human Coronavirus 229E infection in an animal model. Cell Res. 2011;21(9):653-4. https://doi.org/10.1038/cr.2012.165 PMid:23208422

27. Shen L, Yang Y, Ye F, Liu G, Desforges M, Talbot PJ, et al. Safe and sensitive antiviral screening platform based on recombinant human Coronavirus OC43 expressing the luciferase reporter gene. Antimicrob Agents Chemother. 2016;60(9):5492-503 https://doi.org/10.1128/aac.00814-16 PMid:27381385

28. Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al. ACE2 receptor expression and severe acute respiratory
prophylactic and therapeutic efficacy. Vaccine. 2013;31(13):1717-24. https://doi.org/10.1016/j.vaccine.2012.02.003

30. Zhou P, Xing-Lou Y, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new Coronavirus of probable bat origin. Nature. 2020;579(7798):270-3. PMid:32015507

31. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS Coronavirus infection and spread. Virol J. 2005;2(1):69. PMid:16115318

32. Simmons G, Reeves JD, Rennekamp AJ, Amberg SM, Piefer AJ, Bates P. Characterization of severe acute respiratory syndrome-associated Coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. Proc Natl Acad Sci USA. 2004;101(12):4240-5. https://doi.org/10.1073/pnas.0306464101 PMid:15010527

33. Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, Schwartz O, et al. pH-dependent entry of severe acute respiratory syndrome Coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol. 2004;78(11):5642-50. https://doi.org/10.1128/jvi.78.11.5642-5650.2004 PMid:15140961

34. Danser AH, Epstein M, Battle D. Renin-angiotensin system blockers and the COVID-19 pandemic: At Present there is no evidence to abandon renin-angiotensin system blockers. Hypertension. 2020;75(6):1382-5. https://doi.org/10.1161/hypertensionaha.120.15082 PMid:32208987

35. Li W, Moore MJ, Vasllieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS Coronavirus. Nature. 2003;426(6965):450-4. https://doi.org/10.1038/nature02145 PMid:14647384

36. Burkard C, Verheije MH, Wicht O, van Kasteren SI, van Kuppeveld FJ, Haagmans BL, et al. Coronavirus M proteins accumulate in the 21st century. Int J Antimicrob Agents. 2007;30(4):297-308. https://doi.org/10.1016/j.ijantimicag.2007.05.015 PMid:17629679

37. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: An old drug against today’s diseases. J Virol. 2005;79(23):14614-21. https://doi.org/10.1128/jvi.79.23.14614-14621.2005 PMid:16282461

38. Marzi A, Reinheckel T, Feldmann H, Cathepsin B and L are not required for ebola virus replication. PLoS Negl Trop Dis. 2012;6(12):e1923. https://doi.org/10.1371/journal.pntd.0001923 PMid:23236527

39. Fox RI. Mechanism of action of hydroxychloroquine as an antiinfective drug. Semin Arthritis Rheumat. 1993;23(2):82-91. PMid:8278823

40. Tricou V, Minh NN, Pham T, Lee SJ, Farrar J, Wills B, et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. PLoS Negl Trop Dis. 2010;4(8):e785. https://doi.org/10.1371/journal.pntd.0000785 PMid:20706626

41. Gay B, Bernard E, Solignat M, Chazal N, Devaux C, Briant L. pH-dependent entry of chikungunya virus into Aedes albopictus cells. Infect Genet Evol. 2012;12(6):1275-81. https://doi.org/10.1016/j.meegid.2012.02.003 PMid:22386853

42. Khan M, Santhosh SR, Tiwari M, Rao PV, Parida M. Assessment of in vitro prophylactic and therapeutic efficacy of chloroquine against Chikungunya virus in vero cells. J Med Virol. 2010;82(5):817-24. https://doi.org/10.1002/jmv.21663 PMid:20336760

43. Chiang G, Sassaroli M, Louie M, Chen H, Stecher VJ, Speker K. Inhibition of HIV-1 replication by hydroxychloroquine: Mechanism of action and comparison with zidovudine. Clin Ther. 1996;18(6):1080-92. https://doi.org/10.1016/s0149-2918(96)80063-4 PMid:9001825

44. Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. Int J Antimicrob Agents. 2007;30(4):297-308. https://doi.org/10.1016/j.ijantimicag.2007.05.015 PMid:17629679

45. Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, et al. SARS Coronavirus entry into host cells through a novel clathrin- and caveolea-independent endocytic pathway. Cell Res. 2008;18(2):290-301. https://doi.org/10.1038/cr.2008.15 PMid:18227861

46. Klumpperman J, Locker JK, Meijer A, Horzinek MC, Geuze HJ, Rotter PJ. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. J Virol. 1994;68(10):6523-34. https://doi.org/10.1128/jvi.68.10.6523-6534.1994 PMid:8083990

47. Perrier A, Bonnin A, Desmarets L, Danneels A, Goffard A, Rouillé Y, et al. The C-terminal domain of the MERS Coronavirus M protein contains a trans-Golgi network localization signal. J Biol Chem. 2019;294(39):14406-21. https://doi.org/10.1074/jbc.ra119.008964 PMid:31399512

48. Randolph VB, Winkler G, Stollar V. Acidotropic amines inhibit proteolytic processing of flavivirus prM protein. Virology. 1990;174(2):450-4. https://doi.org/10.1016/0042-6822(90)90099-d PMid:2154882

49. Accapezzato D, Visco V, Francavilla V, Molette C, Donato T, Paroli M, et al. Chloroquine enhances human CD8+ T cell responses against soluble antigens in vivo. J Exp Med. 2005;202(6):817-28. https://doi.org/10.1084/jem.20051106 PMid:16157687

50. Garulli B, di Mario G, Sciarraffa E, Accapezzato D, Barnaba V, Castrucci MR. Enhancement of T cell-mediated immune responses to whole inactivated influenza virus by chloroquine treatment in vivo. Vaccine. 2013;31(13):1717-24. https://doi.org/10.1016/j.vaccine.2013.01.037 PMid:23380456

51. Pellet J, Busatto S, Ferrari M, Thompson EA, Mody K, Wolfiram J. Chloroquine and nanoparticle drug delivery: A promising combination. Pharmacol Ther. 2018;191:43-9. https://doi.org/10.1016/j.pharmthera.2018.06.007 PMid:29932886

52. Wolfiram J, Nizzero S, Liu H, Li F, Zhang G, Li Z, et al. A chloroquine-induced macrophage-preconditioning strategy for improved nanodelivery. Sci Rep. 2017;7(1):13738. https://doi.org/10.1038/s41598-017-14221-2 PMid:29062065
Potential Antiviral Effect of Chloroquine Therapy Against Sars-Cov2 Infection

Evaluation of immunomodulators, Reduction of immune activation with Assessment of chloroquine as a modulator

66. Routy JP, Angel JB, Patel M, Kanagaratham C, Radzioch D, et al. Assessment of chloroquine as a modulator of immune activation to improve CD4 recovery in immune nonresponding HIV-infected patients receiving antiretroviral therapy. HIV Med. 2015;16(1):48-56. https://doi.org/10.1111/hiv.12171

PMID:24889179

67. Briani L, Robert-Hebmann V, Acquaviva C, Pelchen-Matthews A, Marsh M, Devaux C. The protein tyrosine kinase p56lck is required for triggering NF-kappB activation upon interaction of human immunodeficiency virus type 1 envelope glycoprotein gp120 with cell surface CD4. J Virol. 1998;72(7):6207-14. https://doi.org/10.1128/VIJ.72.7.6207-6214.1998

PMID:9621091

68. Martinson JA, Montoya CJ,Usuga X, Ronquillo R, Landay AL, Desai SN. Chloroquine modulates HIV-1-induced plasmacytoid dendritic cell alpha interferon: Implication for T-cell activation. Antimicrob Agents Chemother. 2010;54(2):871-81. https://doi.org/10.1128/aac.01246-09

PMID:19949061

69. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: Implications for rheumatology. Nat Rev Rheumatol. 2020;(6):155-66. https://doi.org/10.1038/s41584-020-0372-x

PMID:32034323

70. Kwiek JJ, Haystead TA, Rudolph J. Kinetic mechanism of quinine oxidoeductase 2 and its inhibition by the antimalarial quinolines. Biochemistry. 2004;43(15):4538-47. https://doi.org/10.1021/bi030922w

PMID:15078100

71. Olofsson S, Kumlin U, Dimock K, Amberg N. Avian influenza cytokine and therapeutic target. Annu Rev Med. 1994;45(1):491-503. https://doi.org/10.1146/annurev.med.45.1.491

PMID:8198398

72. Barnard DL, Day CW, Bailey K, Heiner M, Montgomery R, Desai SN. MERS-CoV pathogenesis and antiviral efficacy of licensed drugs in human monocyte-derived antigen-presenting cells. Nat Biotechnol. 2020;38(4):379-81. https://doi.org/10.1038/s41587-020-00003-1

PMID:32160870

73. Multicenter Collaboration Group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for Chloroquine in the Treatment of Novel Coronavirus Pneumonia. Expert consensus on chloroquine phosphate for the treatment of novel Coronavirus pneumonia. Zhonghua Jie He He Hu Xi Za Zhi. 2020;43(3):185-8. https://doi.org/10.3760/cma.j.issn.0956-4000.2020061700505

PMID:31716632

74. Nonresponding HIV-infected patients receiving antiretroviral therapy. HIV Med. 2015;16(1):48-56. https://doi.org/10.1111/hiv.12171

PMID:24889179

75. Huang J. Efficacy of Chloroquine and Lopinavir/Ritonavir in Mild/General Novel Coronavirus (CoVID-19) Infections: A Prospective, Open-Label, Multicenter Randomized Controlled Clinical Study; 2020. https://doi.org/10.1186/s13063-020-04478-w

PMID:32147628

76. Touret F, de Lamballerie X. Of chloroquine and COVID-19. Antiviral Res. 2020;177:104762. https://doi.org/10.1016/j.antiviral.2020.104762

PMID:32147496

77. Administration D. EUA Hydroxychloroquine Sulphate Health

Open Access Maced J Med Sci. 2020 Sep 20; 8(1):184-192. 191
79. Duan YJ, Liu Q, Zhao SQ, Huang F, Ren L, Liu L, et al. Trial of chloroquines in the treatment of COVID-19 and its research progress in forensic toxicology. Fa Yi Xue Za Zhi. 2020;36(2):157-63. PMid:32212512

80. Singh AK, Singh A, Shaik A, Singh R, Misra A. Chloroquine and hydroxychloroquine in the treatment of COVID-19 with or without diabetes: A systematic search and a narrative review with a special reference to India and other developing countries. Diabetes Metab Syndr. 2020;14(3):241-6. https://doi.org/10.1016/j.dsx.2020.03.011 PMid:32247211

81. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020;56(1):105949. https://doi.org/10.1016/j.ijantimicag.2020.105949 PMid:32205204

82. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study. Travel Med Infect Dis. 2020;34:101663. https://doi.org/10.1016/j.tmaid.2020.101663 PMid:32289548

83. Mercuro NJ, Yen CF, Shim DJ, Maher TR, McCoy CM, Zimetbaum PJ, et al. Risk of QT interval prolongation associated with use of hydroxychloroquine with or without concomitant azithromycin among hospitalized patients testing positive for Coronavirus Disease 2019 (COVID-19). JAMA Cardiol. 2020;5(9):1036-41. https://doi.org/10.1001/jamacardio.2020.1834 PMid:32356863

84. Andreani J, Le Bideau M, Duflot I, Jardot P, Rolland C, Boxberger M, et al. In vitro testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. Microb Pathog. 2020;145:104228. https://doi.org/10.1016/j.micpath.2020.104228 PMid:32344177

85. Bessiere F, Roccia H, Deliniere A, Charriere R, Chevalier P, Argue L, et al. Assessment of QT intervals in a case series of patients with Coronavirus disease 2019 (COVID-19) infection treated with hydroxychloroquine alone or in combination with azithromycin in an intensive care Unit. JAMA Cardiol. 2020;5(9):1067-69. https://doi.org/10.1001/jamacardio.2020.1787 PMid:32356858

86. de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, et al. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of middle east respiratory syndrome Coronavirus replication in cell culture. Antimicrob Agents Chemother. 2014;58(8):4875-84. https://doi.org/10.1128/aac.03011-14 PMid:24841269

87. Mo Y, Fisher D. A review of treatment modalities for middle east respiratory syndrome. J Antimicrob Chemother. 2016;71(12):3340-50. https://doi.org/10.1093/jac/dkw338 PMid:27585965

88. Tétu P, Hamelin A, Lebrun-Vignes B, Soria A, Barbaud A, Francès C, et al. Prevalence of hydroxychloroquine-induced side-effects in dermatology patients: A retrospective survey of 102 patients. Ann Dermatol Venereol. 2018;145(6-7):395-404. https://doi.org/10.1016/j.annder.2018.03.168 PMid:29792286

89. Naksuk N, Lazar S, Peeraphatdit TB. Cardiac safety of off-label COVID-19 drug therapy: A review and proposed monitoring protocol. Eur Heart J Acute Cardiovasc Care. 2020;9(3):215-21. https://doi.org/10.1177/2048872620922784 PMid:32372695