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Chloroquine and hydroxychloroquine for COVID-19 treatment

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is an emerging disease caused bysevere acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that has been causing many people around the world affected. There is no approved treatment for COVID-19. Meanwhile, vaccine development still needs a long time before it becomes available to protect people from contracting COVID-19. Repurposing the available drugs is one of the fastest ways to get COVID-19 treatment. Studies have been conducted to discover for COVID-19 treatment that results in the finding of potential medication for COVID-19. Chloroquine and hydroxychloroquine are some of the available medication that shows potential for COVID-19 treatment. Preclinical study showed that the both drugs are active against SARS-CoV-2 *in vitro*. A pilot clinical study also showed their efficacy in COVID-19 treatment. Many clinical trials are now being conducted to prove their safety and efficacy for the prevention and treatment of COVID-19. However, until now there are not enough data to support the use of these drugs in COVID-19 management. Under the pressure to treat COVID-19 patients with chloroquine or hydroxychloroquine, clinicians shouldnot use these drugs for COVID-19 without considering the available information regarding theiruse for COVID-19. This review summarized the evidence regarding the potential of chloroquine and hydroxychloroquine in COVID-19 management.

ABSTRAK

Coronavirus disease 2019 (COVID-19) adalah penyakit akibat infeksi *severe acute respiratory syndrome coronavirus-2* (SARS-CoV-2) yang telah menyebabkan banyak orang di seluruh dunia terkena dampaknya. Sampai saat ini belum ada obat yang terbukti secara ilmiah untuk COVID-19. Sementara itu pengembangan vaksin masih membutuhkan waktu yang lama untuk melindungi orang dari tertular COVID-19. Penggunaan obat yang telah beredar menjadi salah satu cara tercepat dalam mendapatkan obat untuk COVID-19. Penelitian sebelum ini berhasil menemukan beberapa obat yang potensial untuk COVID-19. Klorokuin dan hidroksi klorokuin merupakan salah satu diantara obat yang potensial. Penelitian preklinik menunjukkan kedua obat ini aktif secara *in vitro* terhadap SARS-CoV-2. Penelitian klinik skala pilot juga menunjukkan kemanjurannya dalam pengobatan COVID-19. Saat ini sedang dilakukan uji klinik untuk membuktikan keamanan dan kemanjuran kedua obat ini untuk pencegahan dan pengobatan COVID-19. Namun demikian, belum ada data yang cukup untuk mendukung penggunaan kedua obat untuk pengobatan COVID-19. Di bawah tekanan untuk mengobati pasien COVID-19 dengan klorokuin atau hidroksiklorokuin, dokter tidak boleh menggunakan kedua obat iniuntuk COVID-19 tanpa mempertimbangkan informasi yang tersedia tentang penggunaannya untuk COVID-19. Ulasan ini akan merangkum bukti mengenai klorokuin dan hidroksi klorokuin dalam pengobatan COVID-19.

Keywords:
chloroquine;
hydroxychloroquine;
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INTRODUCTION

Chloroquine and hydroxychloroquine have been used for malaria prevention and treatment for a long time ago. Chloroquine is synthesized based on the structure of the active compound found in the bark of the Cinchona tree or fever tree.[1] Hydroxychloroquine was synthesized almost 20 years after chloroquine was synthesized. It is known as a safer drug compared to chloroquine even though they have an almost similar structure except for the hydroxyl (OH) moiety in one terminal of hydroxychloroquine structure.[2] Both drugs belong to 4-aminoquinoline groups which are among the first drugs used to treat malaria. However, the intensive use of these drugs caused *Plasmodium* resistance towards these drugs that lead to a significant drop of the use of these drugs as anti-malaria.[3] Besides its well-known antimalarial action, chloroquine and hydroxychloroquine are also being used for rheumatoid arthritis (RA), systematic lupus erythematosus (SLE) and other inflammatory diseases.[4]

During coronavirus disease-19 (COVID-19) pandemic in whichthe decisive treatment guidelines are not yet available, repurposing some currently available medications becomes one of the fastest ways to provide treatment for the patient. Chloroquine and its derivate hydroxychloroquine are proven to have activity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) *in vitro*.[5] The result of the *in vitro* study becomes the foundation of the use of chloroquine and hydroxychloroquine for COVID-19. Readily available, long-term experience of usage and low cost support the use of chloroquine and hydroxychloroquine in COVID-19. However, consideration to use chloroquine and hydroxychloroquine for COVID-19 should take into account the safety of the medication and the fact that there is no valid data about its efficacy in COVID-19.

This review was made to summarize the current state of understanding of chloroquine and hydroxychloroquine use for COVID-19. Articles were searched using several keywords namely chloroquine, hydroxychloroquine, SARS-CoV-2, and COVID-19. The related articles were reviewed and summarized in this manuscript. Until this review was made on 27 May 2020, there were only a few published research data related to the use of chloroquine and hydroxychloroquine as COVID-19.

DISCUSSION

Chloroquine and hydroxychloroquine as antimalarial

In the blood, *Plasmodium* enters erythrocytes andgrows by consuming hemoglobin and processing them in the food vacuole. In the food vacuole of the *Plasmodium*, hemoglobin is degraded into peptide and heme. Free heme accumulation as a result of hemoglobin digestion in the food vacuole can cause membrane lysis that can lead to the generation of reactive oxygen species (ROS) and other destructive consequences. Therefore, the heme is detoxified in the *Plasmodium* food vacuole by changing it into hemozoin, a large insoluble crystal containing heme.[6]

Chloroquine and hydroxychloroquine is a weak base. Chloroquine can be found in un-protonated, mono-protonated, and di-protonated forms in the physiological condition. The un-protonated forms of chloroquine can easily enter the cell membrane and food vacuole membrane in which chloroquine will be accumulated.[7] The accumulation of chloroquine into the food vacuole is selective which suggested being the result of chloroquine protonation due to the low pH of the food vacuole, active uptake by parasite transporter,

and chloroquine binding to a specific receptor in the food vacuole.[8] In the food vacuole, chloroquine interferes with the heme detoxification process which results in heme accumulation and subsequently toxic consequences willhappen.[9] Their ability to accumulate in the lysosome seems to be crucial for most of their activity including their immunomodulatory and antiviral activity.

Chloroquine and hydroxychloroquine as immunomodulator

Chloroquine and hydroxychloroquine are also used to treat autoimmune diseases such as RA and SLE. Several *in vitro* studies reveal its immunomodulatory activity. It has been known that chloroquine and hydroxychloroquine are accumulated in lysosome inside the lymphocytes and interfere with lysosomal and autophagosome activity in the lymphocyte. As a result, the lymphocyte function is inhibited which results in chloroquine and hydroxychloroquine immunomodulatory effect.[10] Lysosomes and autophagosome is known to have a role in processing and presenting antigen which then promoting immune activation.[11-12] As a weak base, chloroquine and hydroxychloroquine increase the pH in the lysosomes and autophagosomes which causes inhibition of their maturation. Inhibition of lysosomes and autophagosomes function results in inhibition of immune activation.[2,13] Besides, chloroquine and hydroxychloroquine also suggested to be able to prevent Toll-like receptor (TLR) activation through their ability to increase endosomal pH.[14] *In vitro* study also shows chloroquine and hydroxychloroquine ability to inhibit cytokines production by mononuclear cells.[15] The ability of chloroquine and hydroxychloroquine to modulate the immune system might be important in COVID-19 in which over-activity of immune response is suggested to worsen the disease. FIGURE 1 shows the chloroquine and hydroxychloroquine mechanism of action as an immunomodulator.

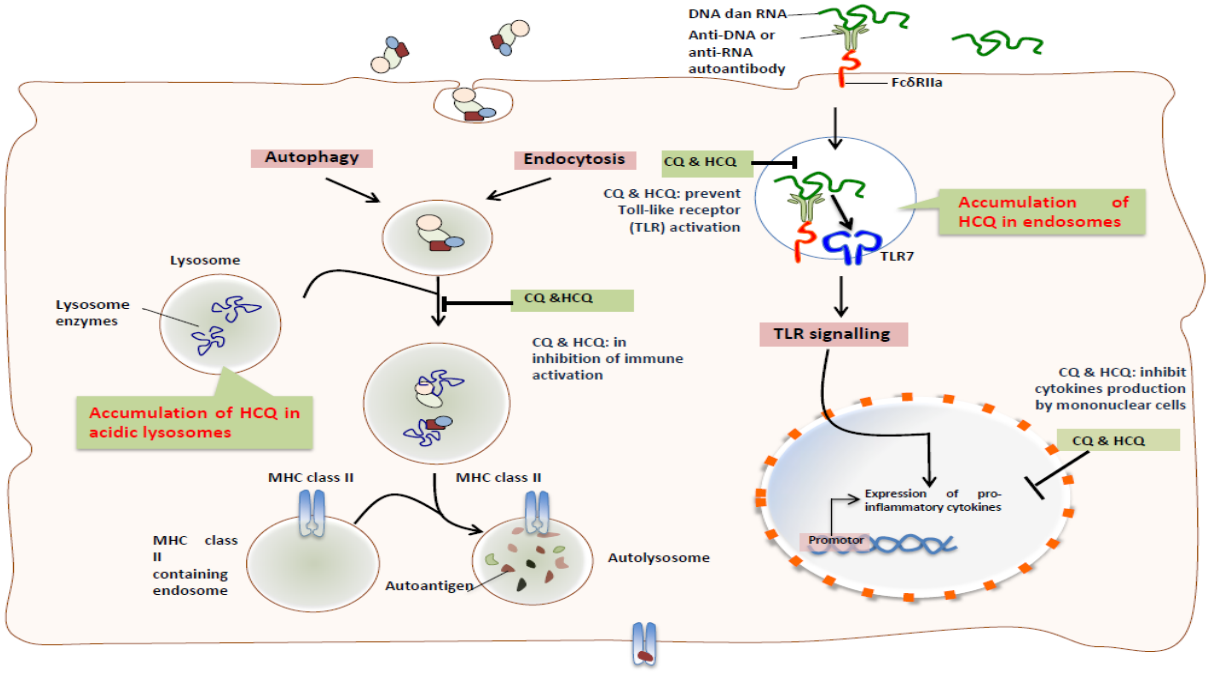

FIGURE 1. Chloroquine and hydroxychloroquine mechanism of action as immunomodulator.

Chloroquine and hydroxychloroquine as antiviral

The study regarding the antiviral activity of chloroquine was first reported decades ago. Since then, many studies have been performed to explore its potential as antiviral.[16] Study in Vero cells infected with Chikungunya virus (CHIKV) showed that chloroquine could reduce virus yield and viral RNA copy number.[17] However, a clinical trial in 2006 failed to prove the efficacy of chloroquine in chikungunya virus infection. Compare to the placebo-treated group, the chloroquine treated group showed the same mean duration of febrile arthralgia and rate of viremia decrease in CHIKV infected patients.[18] Chloroquine also has *in vitro* activity against Ebola virus.[19] In contrast, a study in guinea pig suggested that chloroquine did not protect the animal against the Ebola disease.[19] Based on chloroquine activity to inhibit H1N1 and H3N2 Influenza A virus (IAV) strain replication *in vitro*, clinical trial have been done in Singapore to evaluate chloroquine efficacy to prevent IAV infection.[20-21] Several *in vitro* studies also suggested chloroquine and hydroxychloroquine antiviral effect against other viruses such as human immunodeficiency virus (HIV), hepatitis C virus (HCV),and dengue virus (DENV).[22-24] Chloroquine and hydroxychloroquine also have been studied in coronavirus, in which SARS-CoV-2 belongs to. *In vitro* studies also have been done to evaluate chloroquine activity against coronaviruses, SARS-Cov and MERS-CoV.[25-26] The broad spectrum of chloroquine and its analog hydroxychloroquine as antiviral make them attractive to repurpose it for treatment of viral infection including the current coronavirus infection, SARS-CoV-2, the culprit that cause COVID-19.

There are several explanations of chloroquine and hydroxychloroquine mechanism of action as an agent for the prevention and treatment of viral infection. Chloroquine and hydroxychloroquine can interrupt the virus replication by intervening endosome-mediated viral entry. Some viruses infect the target cells by endocytosis. In the lysosome of the cells, the virus is exposed to low pH and action of several enzymes that will degrade the virus and liberate the infectious nucleic acid and sometimes enzymes necessary for viral replication.[27] Chloroquine is also known to inhibit budding of enveloped virus particles since chloroquine could increase pH which will interrupt pH-dependent post-translational modification of the new virus in the endoplasmic and trans-Golgi network.[28] In vitro chloroquine administration before SARS-CoV infection in Vero cells causes angiotensin-converting enzyme 2 (ACE2) terminal glycosylation impairment that results in reduced binding affinities between ACE2 and SARS-CoV spike protein and inhibits the initiation of SARS-CoV infection.[29] FIGURE 2 shows the antiviral mechanism of chloroquine and hydroxychloroquine.

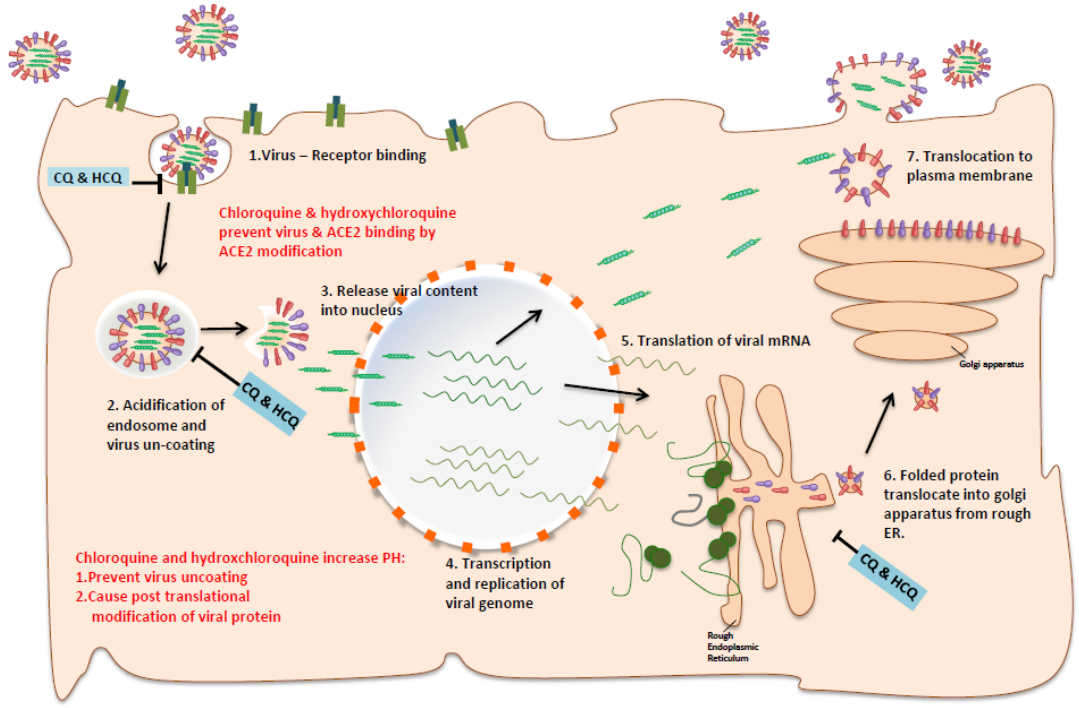

FIGURE 2. Chloroquineand hydroxychloroquinemechanism of action as antiviral.

Chloroquine and hydroxychloroquine for COVID-19

Chloroquine and hydroxychloroquine show *in vitro* activity against SARS-CoV-2. Chloroquine administration before SARS-CoV-2 inoculation to Vero6 cells shows a greater inhibition of viral replication than simultaneous or later administration of chloroquine.[30] This might important to its potential for SARs-CoV-2 infection prevention. Meanwhile, another *in vitro* study shows that hydroxychloroquine is more effective than chloroquine in terms of inhibiting SARS-COV-2. The effective concentration of 50% (EC50) of hydroxychloroquine is 0.72µM. Meanwhile, the EC_{50} of chloroquine is 5.47µM.[31]

Some studies report that chloroquine phosphate is superior in controlling exacerbation of pneumonia, improving lung imaging findings, triggering negative virus conversion, and shortening illness duration in COVID-19 patient but no data was included in the report.[32] In contrast, a new study in Brazilian Amazon showed that hospitalized COVID-19 patients receiving chloroquine are failed to show substantial viral clearance by day four even when it co-administered with azithromycin and or oseltamivir.[33] However, several countries have already included chloroquine and hydroxychloroquine in their COVID-19 treatment guideline i.e China, Korea, Belgia, Italy, etc.[34-37] Clinical trials have been recorded at https://clinicaltrials.gov to evaluate the safety and efficacy of chloroquine as an agent for the prevention and treatment of COVID-19.[38]

A study by Gautret *et al.*[39] in France have shown hydroxychloroquine efficacy as COVID-19 treatment. The study involved 36 COVID-19 patients with asymptomatic disease (six people), upper respiratory tract infection (22 people), and lower respiratory tract infection (eight people). The presence of the virus on the patient's nasal swab at day sixpost-inclusion of the study was

examined. The result showed that the administration of hydroxychloroquine decreased viral carriage on the 6th day after obtaining the drug.[39] However, there are some limitations of the clinical study including the small number of samples andthe comparator group is not homogeneous.

Safety of chloroquine and hydroxychloroquine

The most common adverse effect of chloroquine and hydroxychloroquineis a gastrointestinal disturbance that includes nausea, and vomiting.[40] Several studies also have reported the occurrence of chloroquine or hydroxychloroquine-mediated cardiotoxicity that commonly occurs as rhythm disorders, i.e. prolonged QT interval on the electrocardiogram.[41] In addition, chloroquine and hydroxychloroquine are related to retinopathy. The retinal damage related to chloroquine and hydroxychloroquine is caused bythe disturbance of photoreceptor outer segments lysosomal degradation by the retinal pigment epithelium that leads to an increase in lipofuscin in retinal pigment epithelial cells and photoreceptor degradation.[42] There are several risk factors for the development of retinopathy during hydroxychloroquine treatment. These factors are a drug dose of more than 5 mg/kg body weight/day, hydroxychloroquine treatment for more than 5 years, cumulative dose above 600–1,000g, chronic kidney disease, and hydroxychloroquine co-treatment with tamoxifen for more than six months. Hydroxychloroquine with its *N*-hydroxyethyl side chain that makes it more soluble than chloroquine is considered to be less toxic than chloroquine.[42] Therefore, it might be preferable to focus research effort on hydroxychloroquine than chloroquine.

Chloroquine and hydroxychloroquine dosing consideration in COVID-19 treatment

Chloroquine phosphate 250 mg is equivalent to 150 mg base and hydroxychloroquine 200 mg is equivalent to 155 mg base.[43-44] Chloroquine dose for Malaria acute attack is started with one g and followed by 500 mg after 6-8 hours and 500 mg single dose for two days. The total chloroquine dose for the whole treatment course is 2.5 g.[43] Hydroxychloroquine dose for uncomplicated malaria is 800 mg and followed by 400 mg after six hours, 24 hours, and 48 hours of the initial dose. The total hydroxychloroquine dose for the whole treatment course is two g.[44] Meanwhile, hydroxychloroquine dose for SLE and RA is 200-400 mg/dayand 400-600 mg/day respectively as a single dose or in two divided doses. The chloroquine and hydroxychloroquine dose for malaria, RA, and SLE are well tolerated. However, higher doses and longer duration of treatment with chloroquine and hydroxychloroquine are often related to side effects i.e cardiac rhythm disturbance and retinopathy

The dose of chloroquine and hydroxychloroquine in COVID-19 management is not conclusive yet. Based on a pharmacokinetic simulation study, the recommended dosage of hydroxychloroquine sulfate is 400 mg twice a day on day one, followed by 200 mg twice a day on days 2-5. Total hydroxychloroquine for the whole treatment duration is 2.4 g.[31] The guideline from Belgia for mild to moderate COVID-19 also recommends the same dose with those calculated in the pharmacokinetic simulation study.[36] Meanwhile, COVID-19 treatment guidelines from China recommend a higher dose of chloroquine phosphate than the dose for malaria. They

recommend chloroquine phosphate 500 mg/dose twice daily until day seven for adults with bodyweight over 50 kg and 500 mg/dose twice daily for day one followed by 500 mg/dose/day until day seven for adults with bodyweight less than 50 kg. The total dose for the whole treatment is ranging from 4-7 g.[34] COVID-19 treatment Guideline from Korea recommends hydroxychloroquine instead of chloroquine since chloroquine is not available in Korea. The recommends hydroxychloroquine dose for COVID-19 is 400 mg/day for 7-10 days. The total dose is 2.8-4 g.[35] This showed that some guidelines recommend Chloroquine and hydroxychloroquine dose higher than those used for malaria treatment.

Chloroquine and hydroxychloroquine are known to havelarge volume distribution and long half-life (32-50 days).[32] Therefore, the duration of treatment should not more than fivedays to avoid the accumulation of the drugs in the plasma and tissue which is related to an increased risk of toxicity.[36] Clinical trial in Brazilian Amazone that compared high dose (600 mg twice a day for 10 days) versus low dose (450 mg twice a day on day one and once daily for four days) of chloroquine in hospitalized COVID-19 patient showed that more QTc interval prolongation and lethality in high dose chloroquine treated group. In this study, total Chloroquine administration during treatment duration is 12 g that the total dose is higher than the total recommended dose of chloroquine and hydroxychloroquine for malaria. The study finding suggests that high dose of chloroquine should not be used for severe or critically ill COVID-19 patients especially in patients who also receive azithromycin and oseltamivir.[33] Multinational registry analysis involved 96032 patients from 6 different continents showed that the use of chloroquine and hydroxychloroquine with or without macrolide for COVID-19 increased the risk of ventricular arrhythmia in hospitalized patients. However, the dose of chloroquine and hydroxychloroquine used was not mentioned in this study.[45] Since the study used data from many countries, the dose of chloroquine and hydroxychloroquine must be varied. Subgroup analysis or dose-response analysis should be done to differentiate the effect of chloroquine and hydroxychloroquine various doses. Nevertheless, this showed that chloroquine and hydroxychloroquine should not be used as routine drugs for COVID-19 without careful consideration of their risk and benefit until firm evidence of their use in COVID-19 is confirmed.

CONCLUSION

No solid clinical trial data available currently to support the use of these drugs for COVID-19 patients. Randomized controlled clinical trial is urgently needed to evaluate the efficacy and safety of chloroquine and hydroxychloroquine for COVID-19 prevention and treatment. It might also important to focus research on hydroxychloroquine rather than chloroquine by considering hydroxychloroquine safer profile than chloroquine.

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REFERENCES

1. Permin H, Norn S, Kruse E, Kruse PR. On the history of Cinchona bark in the treatment of Malaria. Dan Medicinhist Arbog 2016; 44:9-30.

2. Schrezenmeier E, Dorner T. Mechanism of action ofhydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol 2020; 16(3):155-66. https://doi.org/10.1038/s41584-020-0372-x
3. Al-Bari MA. Chloroquine analogues in drug discovery: New directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. J Antimicrob Chemother 2015; 70:1608–21. https://doi.org/10.1093/jac/dkv018
4. Rainsfors KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. Inflammopharmacology 2015; 23(5):231-69. https://doi.org/10.1007/s10787-015-0239-y
5. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivate of chloroquine, is as effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020; 6:16. https://doi.org/10.1038/s41421-020-0156-0
6. Ridley RG. Malaria: dissecting chloroquine resistance. Curr Biol 1998; 8(10):R346-9. https://doi.org/10.1016/s0960-9822(98)70218-0
7. Homewood CA, Warhurst DC, Peters W, Baggaley VC. Lysosomes, pH and the anti-malarial action of chloroquine. Nature 1972; 235(5332):50-2. https://doi.org/10.1038/235050a0
8. Yayon A, Cabantchik ZI, Ginsburg H. Identification of the acidic compartment of Plasmodium falciparum-infected human erythrocytes as the target of the antimalarial drug chloroquine. EMBO J 1984; 3(11):2695-700.
9. Sullivan DJ Jr, Matile H, Ridley RG, Goldberg DE. A common mechanism for blockade of heme polymerization by antimalarial quinolines. J Biol Chem 1998; 273(47):31103–7. https://doi.org/0.1074/jbc.273.47.31103
10. Circu M, Cardelli J, Barr MP, O'Byrne K, Mills G, El-Osta H. Modulating lysosomal function through lysosome membrane permeabilization or autophagy suppression restores sensitivity to cisplatin in refractory non-small-cell lung cancer cells. PLoS One 2017; 12(9):e0184922. https://doi.org/10.1371/journal.pone.0184922
11. Ballabio A, Bonifacino JS. Lysosomes as dynamic regulators of cell and organismal homeostasis. Nat Rev Mol Cell Biol 2019; 21(2):101-18. https://doi.org/10.1038/s41580-019-0185-4
12. Ghislat G, Lawrence T. Autophagy in dendritic cells. Cell Mol. Immunol 2018; 15:944-52. https://doi.org/10.1038/cmi.2018.2
13. Rebecca VW, Nicastri MC, Fennelly C, Chude CI, Barber-Rotenberg JS, Ronghe A, et al. PPT1 promotes tumor growth and is the molecular target of chloroquine derivatives in cancer. Cancer Discov 2019; 9(2):220-9. https://doi.org/10.1158/2159-8290.CD-18-0706
14. Ewald SE, Lee BL, Lau L, Wickliffe KE, Shi GP, Chapman HA, et al. The ectodomain of Toll-like receptor 9 is cleaved to generate a functional receptor. Nature 2008; 456(7222):658-62. https://doi.org/10.1038/nature07405
15. van den Borne BE, Dijkmans BA, de Rooij HH, le Cessie S, Verweij CL. Chloroquine and hydroxychloroquine equally affect tumor necrosis factor-alpha, interleukin 6, and interferon-gamma production by peripheral blood mononuclear cells. J Rheumatol 1997; 24(1):55-60.
16. D'Alessandro S, Scaccabarozzi D, Signorini L, Perego F, Ilboudo DP, Ferrante P, et al. Theuse of antimalarial drugs against

viralinfection. Microorganism 2020; 8(1):E85. https://doi.org/10.3390/microorganisms8010085
17. Khan M, Santhosh SR, Tiwari M, Lakshmana 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
18. De Lamballerie X, Boisson V, Reynier JC, Enault S, Charrel RN, Flahault A, et al. On chikungunya acute infection and chloroquine treatment. Vector Borne Zoonotic Dis 2008; 8(6):837-9. https://doi.org/10.1089/vbz.2008.0049
19. Dowall SD, Bosworth A, Watson R, Bewley K, Taylor I, Rayner E, et al. Chloroquine inhibited Ebola virus replication in vitro but failed to protect against infection and disease in the in vivo guinea pig model. J Gen Virol 2015; 96:3484-92. https://doi.org/10.1089/vbz.2008.0049
20. Ooi EE, Chew JS, Loh JP, Chua RC. In vitro inhibition of human influenza A virus replication by chloroquine. Virol J 2006; 3:39. https://doi.org/10.1186/1743-422X-3-39
21. Paton NI, Lee L, Xu Y, Ooi EE, Cheung YB, Archuleta S, et al. Chloroquine for influenza prevention: A randomised, double-blind, placebocontrolled trial. Lancet Infect Dis 2011; 11:677-83. https://doi.org/10.1016/s1473-3099(11)70065-2
22. Jacobson JM, Bosinger SE, Kang M, Belaunzaran-Zamudio P, Matining RM, Wilson CC, et al. The effect of chloroquine on immune activation and interferon signatures associated with HIV-1. AIDS Res Hum Retrovir 2016; 32(7):636-47. https://doi.org/10.1089/AID.2015.0336
23. Blanchard E, Belouzard S, Goueslain L, Wakita T, Dubuisson J, Wychowski C, et al. Hepatitis C virus entry depends on clathrin-mediated endocytosis. J Virol 2006; 80:6964-72. https://doi.org/10.1128/JVI.00024-06
24. Borges MC, Castro LA, Fonseca BA. Chloroquine use improves dengue-related symptoms. Mem Inst Oswaldo Cruz 2013; 108(5):596-9. https://doi.org/10.1590/s0074-02762013000500010
25. Keyaerts E, Vijgen 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
26. Dyall J, Coleman CM, Hart BJ, Venkataraman T, Holbrook MR, Kindrachuk J, et al. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob Agents Chemother 2014; 58(8):4885-93. https://doi.org/10.1128/AAC.03036-14
27. Cassell S, Edwards J, Brown DT. Effects of lysosomotropic weak bases on infection of BHK-21 cells by Sindbis virus. J Virol 1984; 52(3):857-64.
28. Savarino A, Gennero L, Sperber K, Boelaert JR. The anti-HIV-1 activity of chloroquine. J Clin Virol 2001; 20(3):131-5. https://doi.org/10.1016/s1386-6532(00)00139-6
29. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2005; 2:69. https://doi.org/ 10.1186/1743-422X-2-69
30. 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
31. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivate of chloroquine, is

as effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020; 6:16. https://doi.org/10.1038/s41421-020-0156-0
32. 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
33. Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, Brito M, et al. Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: arandomized clinical trial. JAMA Netw Open 2020; 3(4):e208857.
34. China National Health Comission. Chinese clinical guidance for COVID-19 pneumonia diagnosis and treatment, 7th ed. March 4th, 2020. Available from:http://kjfy.meetingchina.org/msite/news/show/cn/3337.html
35. Korea Biomedical Review. Physicians work out treatment guidelines for coronavirus. Available from: https://www.koreabiomed.com/news/articleView.html?idxno=7428.
36. Interim clinical guideline for adult with suspected or conformed COVID-19 in Belgium. Available from: https://epidemio.wiv-isp.be/ID/Documents/Covid19/COVID19_InterimGuidelines_Treatment_ENG.
37. Italian Society of Infectious and Tropical Diseases. Handbook for the care of people with disease-COVI 19. 2.0 ed., 2020.
38. Clinical trial. gov. Available from: https://clinicaltrials.gov/ct2/results?cond=COVID19&term=chloroquine&cntry=&state=&city=&dist.Accessed on April 30th, 2020.
39. 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; 105949. https://doi.org/10.1016/j.ijantimicag.2020.105949
40. Srinivasa A, Tosounidou S, Gordon C. Increased incidence of gastrointestinal side effects in patients taking hydroxychloroquine: a brand-related issue? J Rheumatol 2017; 44(3):398. https://doi.org/10.3899/jrheum.161063
41. Chatre C, Roubille F, Vernhet H, Jorgensen C, Pers YM. Cardiac complications attributed to chloroquine and hydroxychloroquine: a systematic reviewof the literature. Drug Saf 2018; 41(10):919-31. https://doi.org/10.1007/s40264-018-0689-4
42. Jorge A, Ung C, Young LH, Melles RB, Choi HK. Hydroxychloroquine retinopathy implications of research advances for rheumatology care. Nat Rev Rheumatol 2018; 14(12):693-703. https://doi.org/10.1038/s41584-018-0111-8
43. Rx List. Aralendrug description. Available from: https://www.rxlist.com/aralen-drug.htm. Accessed on April 30th, 2020.
44. Rx List. Plaquenil drug description. Available from: https://www.rxlist.com/ plaquenil-drug.htm. Accessed on April 30th, 2020.
45. Mehra MR, Desai SS, Ruschitzka F, Patel AN. Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet 2020; S0140-6736(20):31180-6. https://doi.org/10.1016/S0140-6736(20)31180-6