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HYDROXYCHLOROQUINE ACTION ON SARS-CoV-2 AND DRUG-DRUG INTERACTION HAMPERING NORMAL PHYSIOLOGICAL HOMEOSTASIS

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a rapidly emerging viral infection causing coronavirus disease 2019 (COVID-19). Hydroxychloroquine and chloroquine have garnered unprecedented attention as potential therapeutic agents against COVID-19 following several small clinical trials, uncontrolled case series, and public figure endorsements. While there is a growing body of scientific data, there is also concern for harm, particularly QTc prolongation and cardiac arrhythmias. Here, we perform a rapid narrative review and discuss the strengths and limitations of existing *in-vitro* and clinical studies. We call for additional randomized controlled trial evidence prior to the widespread incorporation of hydroxychloroquine and chloroquine into national and international treatment guidelines.

KEYWORDS: Chloroquine, Clinical trials, COVID-19, Hydroxychloroquine, SARS-COV-2.

INTRODUCTION

Hydroxychloroquine (HCQ), sold under the brand name Plaquenil among others, is a medication used to prevent and treat malaria in areas where malaria remains sensitive to chloroquine. Other uses include treatment of rheumatoid arthritis, lupus, and porphyria cutanea tarda. It is taken by mouth. HCQ is being studied to prevent and treat coronavirus disease 2019 (COVID-19). Hydroxychloroquine [(RS)-2-[{4-[(7-chloroquinolin-4-yl)amino]pentyl}(ethyl)amino]ethanol] is in the antimalarial and 4-aminoquinoline families

of medication. Hydroxychloroquine was approved for medical use in the United States in 1955. It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. In 2017, it was the 128th most commonly prescribed medication in the United States, with more than five million prescriptions. The speculative use of hydroxychloroquine for COVID-19 threatens its availability for people with established indications.[1]

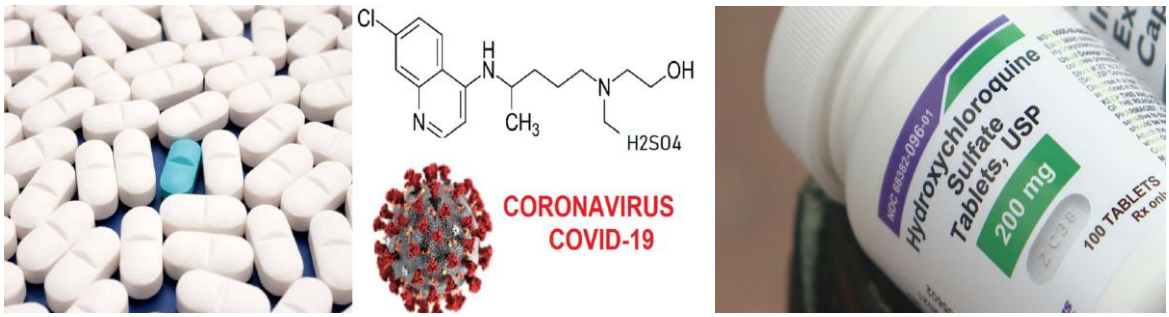

Figure 1: Hydroxychloroquine.

Risk factors

Chloroquine and hydroxychloroquine are known to potentially cause heart rhythm problems, and these could be exacerbated if treatment is combined with other medicines, such as the antibiotic azithromycin, that have similar effects on the heart. Recent studies have reported serious, in some cases fatal, heart rhythm problems with chloroquine or hydroxychloroquine, particularly when taken at high doses or in combination with the antibiotic azithromycin. In addition to side effects nerve cell damage that can lead to seizures (fits) and low blood sugar (hypoglycaemia), these medicines may cause neuropsychiatric disorders, including agitation, insomnia, confusion, psychosis and suicidal ideation.

Drug–drug interactions

The effect of antimalarial drugs on other drugs (and vice versa) is an important clinical consideration. Both chloroquine and hydroxychloroquine are substrates for cytochrome P450 (CYP) enzymes (enzymes responsible for the metabolism of multiple drugs) and hence can interfere with other drugs. CYP enzymes catalyze the dealkylation of chloroquine and hydroxychloroquine to pharmacologically active metabolites. The specific CYP enzymes responsible for the metabolism of various drugs have been investigated using microsomal stability assays or recombinant enzymes. CYP2C8, CYP3A4, CYP2D6 and CYP1A1 can metabolize chloroquine. However, the contribution of these isoforms might vary between individuals and indeed blood concentrations of hydroxychloroquine are reported to vary

among individuals. In terms of other drugs, concurrent use of chloroquine increases the plasma concentration of digitoxin (a cardiac glycoside) fourfold. Hence, levels of digitoxin require close monitoring during antimalarial therapy. Hydroxychloroquine influences the levels of metoprolol through prevention of its metabolism by competing for the same CYP enzyme, CYP2D. As a result, plasma concentrations and the bioavailability of metoprolol increases with hydroxychloroquine therapy. Interestingly, levels of other drugs that are also metabolized by CYP2D6, such dextromethorphan, are not increased during hydroxychloroquine therapy. As antimalarial drugs are thought to interfere with medications that influence the QT interval, patients on hydroxychloroquine therapy concurrently taking such drugs for the treatment of cardiac comorbidities should also be monitored for the potential risk of cardiac arrhythmia. According to ophthalmology recommendations, co-medication of tamoxifen (a selective estrogen receptor modulatory used to treat breast cancer) with hydroxychloroquine is associated with an increased risk of eye toxicity owing to synergistic inhibition of lysosomal enzymes in retinal epithelial cells. Thus, combined use of tamoxifen with hydroxychloroquine or chloroquine should be limited to 6 months. Another relevant drug interaction to consider is the interaction between antimalarial drugs and other DMARDs. Hydroxychloroquine can reduce the gastrointestinal absorption of methotrexate through local pH changes and hence lowers the bioavailability of methotrexate. This effect might explain the decreased risk of methotrexate-associated acute liver adverse effects during co-administration with hydroxychloroquine. No other interactions between hydroxychloroquine and methotrexate (such as interaction on the enzymatic level have been reported. Hydroxychloroquine can also increase levels of ciclosporin; hence, levels of ciclosporin should also be closely monitored during combined therapy. Some drugs can also interfere with the bioavailability of hydroxychloroquine and chloroquine. For example, agents that increase the pH of gastric acid (for example, proton-pump inhibitors) might interfere with the oral absorption and oral bioavailability of antimalarial drugs. However, in one study of patients with SLE, the plasma concentrations of hydroxychloroquine between patients taking proton-pump inhibitors and patients not taking proton-pump inhibitors did not differ. Finally, smoking has previously been suspected to interfere with the bioavailability of hydroxychloroquine; however, a study in 2017 found no correlation between hydroxychloroquine plasma concentration and smoking status.[2]

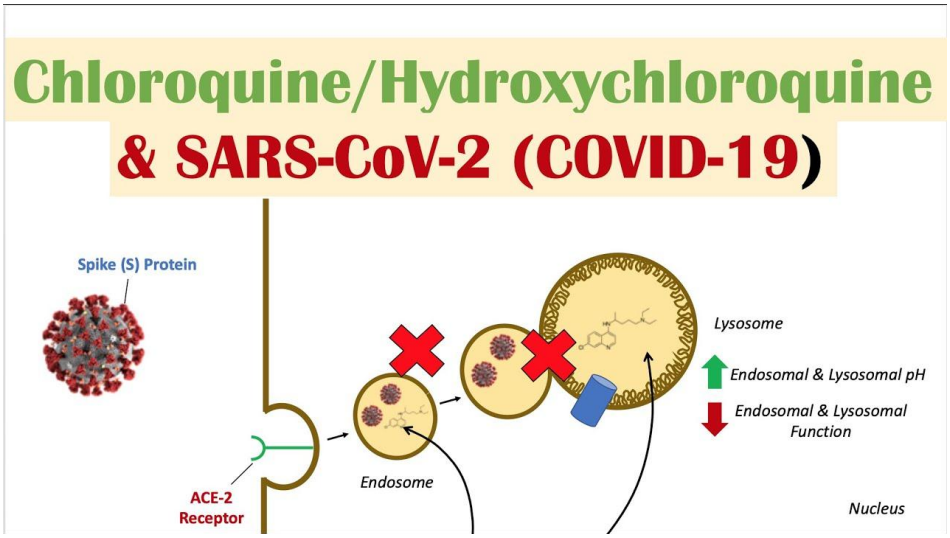

Figure 2: Hydroxychloroquine & Covid-19.

Hydroxychloroquine & COVID-19

As of April 6, 2020, the published evidence of the effectiveness of hydroxychloroquine or chloroquine for the prevention and treatment of COVID-19 in humans is limited to five small studies and one subjective report. In early March, Chen et al. published the results of the first hydroxychloroquine study in patients with COVID-19. In this small, 30-person inpatient, randomized controlled trial comparing hydroxychloroquine to the standard of care, researchers found no statistically significant differences in time to viral clearance by day seven between those who received hydroxychloroquine (87% clearance) versus those who did not (93%, $P > 0.05$). They also did not identify any difference in clinical outcomes (i.e., duration of fever, changes in lung imaging). While they did not comment on the severity of illness of those enrolled, those in the hydroxychloroquine and control arms had symptoms for approximately seven and six days respectively. At two weeks, all patients had negative viral nucleic acid tests. On March 16, 2020, Gao et al., extracted data from 100 patients with confirmed COVID-19 from ongoing inpatient studies in China and reported patient improvement with the use of chloroquine The authors claimed that chloroquine was superior to standard of care treatment in helping reduce time to clinical recovery and improving lung imaging findings; however, no data supporting these findings were published, and no clinical information, including the severity of illness and outcomes, nor statistical analyses were presented in this brief report.[3]

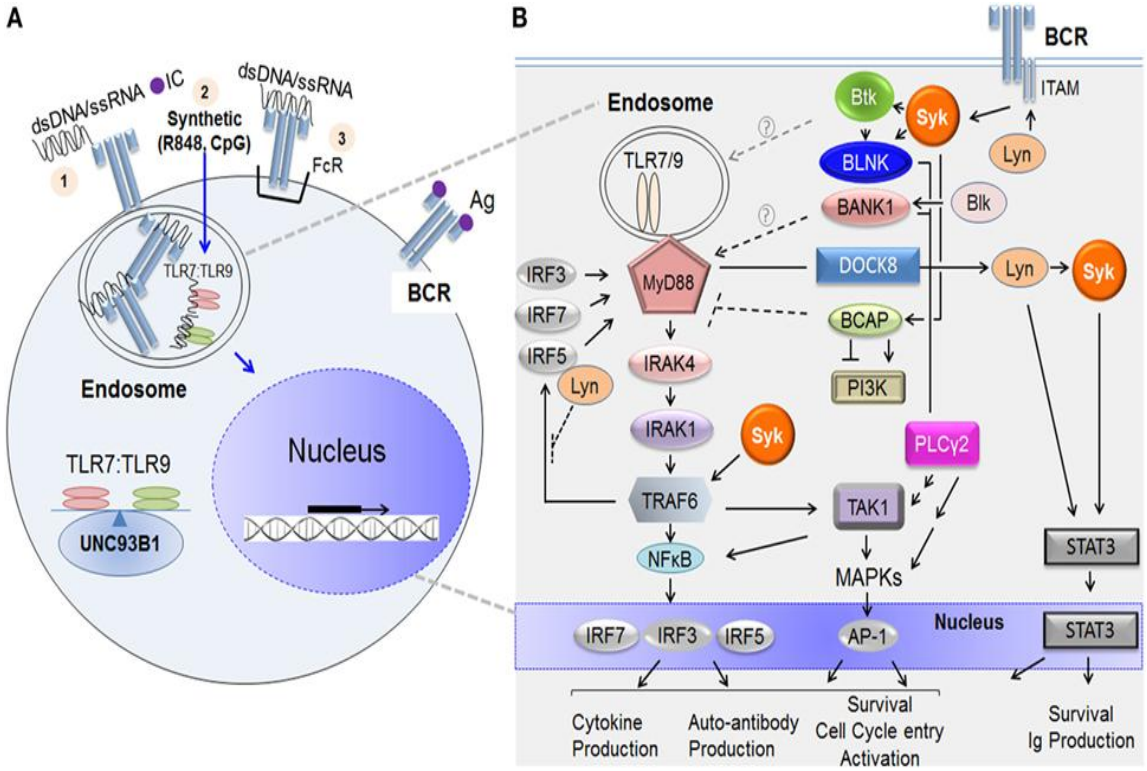

Figure 3: Signalling pathways.

Adverse effects

Molecular effects

Inhibition of lysosomal Activity and Autophagy: An important mode of action of chloroquine and hydroxychloroquine is the interference of lysosomal activity and autophagy. It is widely accepted that chloroquine and hydroxychloroquine accumulate in lysosomes (lysosomotropism) and inhibit their function. *In-vitro*, chloroquine can destabilize lysosomal membranes and promote the release of lysosomal enzymes inside cells. Although evidence of this latter mechanism is scarce, the ability of these drugs to interfere with lysosomal activity has been repeatedly documented. Interference of lysosomal activity might inhibit the function of lymphocytes and have immunomodulatory or even anti-inflammatory effects. One mechanism by which these drugs might have anti-inflammatory effects is by impairing antigen presentation via the lysosomal pathway. Lysosomes contain hydrolytic enzymes and cooperate with other vesicles to digest cargo (such as organelles and material from inside the cell (in a process known as autophagy) or material from outside the cell (via the endocytosis or phagocytosis pathway). Lysosomes are involved not only in recycling cellular substrates but also in antigen processing and MHC class II presentation, indirectly promoting immune activation. Autophagy is also involved in antigen presentation and immune activation. For example, data from one study suggest that autophagy is important for MHC

class II-mediated autoantigen presentation by antigen-presenting cells to $CD4^+$ T cells. As the pH in lysosomes is optimal for lysosomal enzymes involved in hydrolysis, by increasing the pH of endosomal compartments, chloroquine and hydroxychloroquine might impair the maturation of lysosomes and autophagosomes and inhibit antigen presentation along the lysosomal pathway. Overall, the available studies suggest that hydroxychloroquine and chloroquine impair or inhibit lysosomal and autophagosome functions and subsequently immune activation. Beyond lysosomotropism, efforts to identify exact molecular targets of hydroxychloroquine within the lysosome are also currently underway. One study has identified palmitoyl-protein thio-esterase 1 (PPT1), an enzyme involved in the catabolism of lipid-modified proteins, as a potential lysosomal target of chloroquine and chloroquine derivatives. Hydroxychloroquine can bind and inhibit PPT1 activity, and, notably, PPT1 is overexpressed in the synovial tissue of patients with RA. Although an interesting example of ongoing research, confirmatory functional studies and the identification of other molecular targets within the lysosomes are nevertheless warranted.[4]

Inhibition of signaling pathways: Hydroxychloroquine and chloroquine can also interfere with Toll-like receptor (TLR) Signaling. For example, changes in endosomal pH can interfere with TLR9 and TLR7 processing, and, hence, these antimalarial drugs might prevent TLR activation upon extracellular stimuli by mediating changes in the local pH. Chloroquine or hydroxychloroquine can also directly bind to nucleic acids and hence might block TLR9 Signaling at the intracellular level by inhibiting TLR–ligand interactions (steric blockade). This latter hypothesis is supported by an analysis based on surface plasmon resonance and fluorescence spectroscopy showing that antimalarial drugs can directly inhibit CpG–TLR9 interactions. In addition to TLR9 signaling, chloroquine can also inhibit RNA-mediated activation of TLR7 signaling. Although the exact modes of action by which these drugs inhibit TLR7 and TLR9 requires further delineation at the molecular level, inhibition of TLR processing and inhibition of TLR binding are likely central mechanisms of action. Another potential mode of action of hydroxychloroquine and chloroquine is interference with cyclic GMP-AMP (cGAMP) synthase (cGAS) activity by inhibiting ligand binding. The cGAS–stimulator of IFN genes (STING) pathway is a major source of the type I IFN response. Cytosolic DNA binds to cGAS and the second messenger cGAMP to mediate STING-dependent transcription of type I IFNs through the transcription factor IFN regulatory factor 3 (IRF3). Notably, cGAS inhibitors are currently in development for the treatment of inflammatory rheumatic diseases.[5]

Cellular effects

Cytokine Production and Immune activation: Hydroxychloroquine and chloroquine can indirectly reduce the production of anti-inflammatory cytokines by various cell types. *In-vitro*, hydroxychloroquine and chloroquine inhibit the production of IL-1, IL-6, TNF and IFNγ by mononuclear cells. Furthermore, treatment with hydroxychloroquine inhibits the production of TNF, IFNα, IL-6 and CCL4 (also known as MIP1β) in pDC and natural killer cell co-cultures stimulated with RNA-containing immune complexes. TLR signals stimulate the production of cytokines, and hence hydroxychloroquine and chloroquine might inhibit cytokine production by inhibiting TLR pathways. Notably, a small molecule inhibitor of IL-1 receptor-associated kinase 4 (IRAK4; a component of the TLR7 and TLR9 signaling pathways) could reduce production of cytokines by peripheral blood mononuclear cells (PBMCs) more strikingly than hydroxychloroquine. Upon stimulation, IRAK4 inhibition altered the expression of a larger number of RNA-induced and immune-complex-induced genes than hydroxychloroquine (492 versus 65 genes). This finding suggests that hydroxychloroquine is less effective at inhibiting the production of a wide range of cytokines than the IRAK4 inhibitor. Nevertheless, this study also convincingly shows that hydroxychloroquine has a notable effect on cytokine production and gene expression, including an inhibitory effect on TNF production by PBMCs from patients with SLE. Indeed, in other studies, treatment with hydroxychloroquine was associated with a reduction in serum levels of IFNα in patients with SLE. Furthermore, in patients with RA, long-term treatment with hydroxychloroquine (200–400 mg/day) can reduce circulating levels of IL-1 and IL-6 and is associated with improvement in erythrocyte sedimentation rate. The anti-inflammatory effects of hydroxychloroquine and chloroquine could be explained in part by the upstream interference of immune activation (including inhibition of lysosomal activity). Indeed, treatment with hydroxychloroquine is associated with a dose-dependent downregulation of the co-stimulatory molecule CD154 on $CD4^+$ T cells from patients with SLE, which is accompanied by a decrease in intracellular Ca^{2+} mobilization and translocation of nuclear factor of activated T cells cytoplasmic 1 (NFATc1) and NFATc2. However, the direct effect of antimalarial drugs on cytokine production requires further delineation.[6]

Cardiovascular effects: Although hydroxychloroquine is not an anticoagulant, this drug is widely believed to have vascular protective effects and prevent the development of thrombotic complications. This protective effect seems to be most relevant for patients with a secondary coagulopathy owing to systemic inflammation and in patients with primary APS.

Patients with inflammatory rheumatic diseases are at an increased risk of developing cardiovascular complications compared with the general population. This increased risk is caused by the underlying disease, drugs used to treat the disease (such as NSAIDs, including COX-2 inhibitors and high-dose glucocorticoids) and the presence of comorbidities, such as arterial hypertension, hyperlipidemia, chronic kidney failure and diabetes mellitus. By contrast, treatment with hydroxychloroquine seems to counter these effects and provide long-term benefits by reducing the risk of cardiovascular events, lowering fasting glucose levels and reducing hyperlipidemia. For example, in a study of patients with SLE, combined use of low-dose aspirin and hydroxychloroquine was superior to treatment with aspirin or hydroxychloroquine alone in terms of preventing cardiovascular complications. However, sufficiently large and controlled studies are needed to quantify the benefit-to-risk profile of hydroxychloroquine in the prevention of cardiovascular complications in patients with rheumatic diseases and other non-rheumatological cohorts. Potential mechanisms by which hydroxychloroquine and chloroquine reduce the procoagulatory state in autoinflammatory diseases include inhibition of antiphospholipid antibody binding or inhibition of platelet aggregation. Notably, in a mouse model of APS, hydroxychloroquine treatment was associated with improvement in endothelial function. The exact molecular mechanisms by which these drugs mediate their antithrombotic effects remain largely unknown.[7]

Effects on eye: One of the most serious side effects is retinopathy (generally with chronic use). People taking 400 mg of hydroxychloroquine or less per day generally have a negligible risk of macular toxicity, whereas the risk begins to increase when a person takes the medication over five years or has a cumulative dose of more than 1000 grams. The daily safe maximum dose for eye toxicity can be computed from a person's height and weight. Macular toxicity is related to the total cumulative dose rather than the daily dose. Regular eye screening, even in the absence of visual symptoms, is recommended to begin when either of these risk factors occurs. Toxicity from hydroxychloroquine may be seen in two distinct areas of the eye: the cornea and the macula. The cornea may become affected (relatively commonly) by an innocuous cornea verticillata or vortex keratopathy and is characterized by whorl-like corneal epithelial deposits. These changes bear no relationship to dosage and are usually reversible on cessation of hydroxychloroquine. The macular changes are potentially serious. Advanced retinopathy is characterized by reduction of visual acuity and a "bull's eye" macular lesion which is absent in early involvement.[8]

Overdose: Hydroxychloroquine and chloroquine are extremely toxic in overdose. Serious symptoms of overdose generally occur within an hour of ingestion. These symptoms may include sleepiness, vision changes, seizures, coma, stopping of breathing, and heart problems such as ventricular fibrillation and low blood pressure. Loss of vision may be permanent. Low blood potassium, to levels of 1 to 2 mmol/L, may also occur. Cardiovascular abnormalities such as QRS complex widening and QT interval prolongation may also occur. Chloroquine has a risk of death in overdose in adults of about 20%, while hydroxychloroquine is estimated to be two or threefold less toxic. While overdoses of hydroxychloroquine have historically been uncommon, one report documented three deaths out of eight cases. Treatment recommendations include early mechanical ventilation, heart monitoring, and activated charcoal. Supportive treatment with intravenous fluids and vasopressors may be required with epinephrine being the vasopressor of choice. Stomach pumping may also be used. Sodium bicarbonate and hypertonic saline may be used in cases of severe QRS complex widening. Seizures may be treated with benzodiazepines. Intravenous potassium chloride may be required; however, this may result in high blood potassium later in the course of the disease. Dialysis does not appear to be useful.

- **Child danger warning:** Accidentally swallowing just a few tablets has been fatal in some children. Keep this drug in a child-resistant bottle out of reach of children.
- **Worsened skin conditions warning:** Skin conditions, such as psoriasis or porphyria. This medication may make these conditions worse.
- **Eye damage:** This medication can damage eyes, leading to vision problems that can be permanent. This damage is more likely when the drug is used in high doses.
- **Heart damage:** This medication can cause heart disease. Although uncommon, some cases have been fatal.

More common side effects of it

- Headache
- Dizziness
- Diarrhea
- Stomach cramps
- Vomiting

* Mild side effects can go away within a few days and a couple of weeks

Serious side effects

- Blurred vision or other vision changes, which may be permanent in some cases
- Heart disease, including heart failure and issues with your heart rhythm; some cases have been fatal
- Ringing in your ears or hearing loss
- Angioedema (rapid swelling of your skin)
- Hives
- Mild or severe bronchospasm
- Sore throat
- Severe hypoglycemia
- Unusual bleeding or bruising
- Blue-black skin color
- Muscle weakness
- Hair loss or changes in hair color
- Abnormal mood changes
- Mental health effects, including suicidal thoughts

Hydroxychloroquine warnings

This drug comes with several warnings.[9]

- **Allergy warning:** Though rare, this drug may cause an allergic reaction. Symptoms can include:

✓ Hives

✓ Swelling

✓ Trouble breathing

- **Alcohol interaction warning:** Alcohol misuse can damage your liver, which can affect how hydroxychloroquine works in your body. If you drink alcohol, ask your doctor about whether it's safe for you to drink while taking hydroxychloroquine.
- **For people with liver problems or alcohol misuse:** Liver problems or a history of alcohol misuse can make this drug less effective.
- **For people with certain enzyme deficiencies:** This drug may cause red blood cells to rupture (break open) in people with low levels of glucose-6-phosphate dehydrogenase (G6PD). G6PD is an enzyme, which is a type of protein.
- **For pregnant women:** This drug should be avoided in pregnancy. Some studies show that the medication can be passed through the mother's bloodstream to the baby.

- **For women who are breastfeeding:** Small amounts of this drug pass through breast milk, but it's not known what effect this may have on a child who is breastfed. You and your doctor should decide whether you'll take this medication or breastfeed.
- **For seniors:** This drug is processed by your kidneys. Older adults with reduced kidney function may not be able to process this drug well, which can increase the risk of side effects, including vision damage. Older adults may require more frequent eye exams while taking this drug to monitor for signs of vision damage.[10]
- **For children:** This drug can be dangerous to children. Accidentally swallowing even just a few tablets can lead to death in a small child. Keep this drug in a child-resistant bottle out of reach of children. Children shouldn't use this drug for long periods. Children taking this medication for a long period of time may experience permanent damage to their vision and other side effects.

CONCLUSION

Additional studies examining hydroxychloroquine and chloroquine in preventing and treating COVID-19 are desperately needed. Given the weak evidence available, larger controlled trials are needed to more thoroughly assess if hydroxychloroquine/chloroquine have a clinical benefit in COVID-19. Several ongoing randomized clinical trials are actively recruiting participants to better address this question. These randomized trials are powered to show a reduction in meaningful clinical outcomes such as the development of COVID-19 in prevention trials or the need for hospitalization, critical care, or death in treatment trials. The results of these trials will be instrumental in determining whether or not these two antimalarial medications are at all efficacious, and if so, at what dose and for what duration they should be safely recommended in guidelines.

REFERENCES

1. Flint J, Panchal S, Hurrell A, van de Venne M, Gayed M, Schreiber K, et al. "BSR and BHPR guideline on prescribing drugs in pregnancy and breastfeeding—Part I: standard and biologic disease modifying anti-rheumatic drugs and corticosteroids". Rheumatology, 2016; 55(9): 1693–7.
2. Mohammad S, Clowse ME, Eudy AM, Criscione-Schreiber LG (March). "Examination of Hydroxychloroquine Use and Hemolytic Anemia in G6PDH-Deficient Patients". Arthritis Care & Research, 2018; 70.

3. Kaufmann AM, Krise JP (April). "Lysosomal sequestration of amine-containing drugs: analysis and therapeutic implications". Journal of Pharmaceutical Sciences, 2007; 96 (4): 729–46.
4. Hurst NP, French JK, Gorjatschko L, Betts WH (January). "Chloroquine and hydroxychloroquine inhibit multiple sites in metabolic pathways leading to neutrophil superoxide release". The Journal of Rheumatology, 1988; 15(1): 23–7.
5. Smolen, J. S. et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann. Rheum. Dis, 2014; 73: 492–509.
6. Fanouriakis, A. et al. update of the EULAR recommendations for the management of systemic lupus erythematosus. Ann. Rheum. Dis, 2019; 78: 736–745.
7. Pons-Estel, B. A. et al. First Latin American clinical practice guidelines for the treatment of systemic lupus erythematosus: Latin American Group for the Study of Lupus (GLADEL, Grupo Latino Americano de Estudio del Lupus)-Pan-American League of Associations of Rheumatology (PANLAR). Ann. Rheum. Dis, 2018; 77: 1549–1557.
8. Tektonidou, M. G. et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. Ann. Rheum. Dis, 2019; 78: 1296–1304.
9. Takeda K, Kaisho T, Akira S "Toll-like receptors". Annual Review of Immunology, 2003; 21: 335–76.
10. Kushal Nandi, Dr. Dhrubo Jyoti Sen, Arpita Biswas, Dr. Khokan Bera, Dr. Bankim Nandy, Dr. Falguni Patra and Dr. Beduin Mahanti Hydroxychloroquine: the one-man army protecting human civilization from the dangerous bite of malaria: Journal of Drug Discovery and Therapeutics, 2020; 8(5): 01-06.