BRIEF REPORT

Treatment of COVID-19 with Chloroquine: Implication for Malaria Chemotherapy Using ACTs in Disease Endemic Countries

Neils Ben Quashie, PhD¹,²,³ and Nancy Odurowah Duah-Quashie, PhD²,³

¹Centre for Tropical Clinical Pharmacology and Therapeutics, University of Ghana Medical School, Accra, Ghana
²Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana
³West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana

Correspondence: Neils Ben Quashie, Centre for Tropical Clinical Pharmacology and Therapeutics, University of Ghana Medical School, University of Ghana, P.O. Box GP 4236, Accra, Ghana. Tel: +233 545507575. E-mail <nbquashie@ug.edu.gh/nquashie@noguchi.ug.edu.gh>.

ABSTRACT

Based on reports of parasite resistance and on World Health Organization recommendation, chloroquine was replaced with the artemisinin-based combination therapies (ACTs) as the first choice of drugs for the treatment of uncomplicated malaria. Disuse of chloroquine led to restoration of drug-sensitive parasite to some extent in certain countries. Ever since chloroquine and hydroxychloroquine were touted as potential treatment for coronavirus disease 2019 (COVID-19), there has been a dramatic surge in demand for the drugs. Even in areas where chloroquine is proscribed, there has been an unexpected increase in demand and supply of the drug. This situation is quite worrying as the indiscriminate use of chloroquine may produce drug-resistant parasites which may impact negatively on the efficacy of amodiaquine due to cross-resistance. Amodiaquine is a partner drug in one of the ACTs and in some of the drugs used for intermittent preventive treatment. We herein discuss the consequences of the escalated use of chloroquine in the management of COVID-19 on chemotherapy or chemoprevention of malaria and offer an advice. We speculate that parasite strains resistant to chloroquine will escalate due to the increased and indiscriminate use of the drug and consequently lead to cross-resistance with amodiaquine which is present in some drug schemes aforementioned. Under the circumstance, the anticipated hope of reverting to the use of the ‘resurrected chloroquine’ to manage malaria in future is likely to diminish. The use of chloroquine and its derivatives for the management of COVID-19 should be controlled.

KEYWORDS: COVID-19, chloroquine, cross-resistance, amodiaquine, hydroxychloroquine, treatment

THE COVID-19 PANDEMIC

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus causing coronavirus disease 2019 (COVID-19), emerged in December 2019 and spread rapidly across the world with a fast rate of infection, leaving a significant morbidity and mortality on it trail. The pandemic has been described severally: as a global health crisis of our time and the
greatest challenge faced globally since World War II. Indeed, the disease is unleashing an unforetold devastation on social, economic and political lives of many Nations which will take a long time to resolve.

The virus attacks the respiratory tract system of the host. Genetic analysis by sequencing indicates that the virus is a betacoronavirus closely linked to the SARS virus [1]. As at now, there is no known specific, effective, proven and pharmacological treatment for COVID-19. However, scientists are working hard to find antivirals specific to the virus. It must be emphasized that the long-term strategy to combat COVID-19 would be to develop a vaccine. This may take some time, as the vaccine must be rigorously tested and its safety confirmed through clinical trials before routine use in humans. In the meantime, drugs for the management of the symptoms of the disease are urgently needed.

Many different treatment options have been proposed including the use of drugs such as chloroquine, hydroxychloroquine, arbidol and remdesivir among others [2]. These drugs are currently undergoing clinical studies to test their efficacy and safety in the treatment of the COVID-19. With regard to chloroquine, in vitro studies indicate that the drug is effective in reducing viral replication in infections including the SARS-associated coronavirus (CoV) and MERS-CoV [3–5].

Although largely undocumented, it is speculated that substantial amount of chloroquine and hydroxychloroquine are already being used unofficially as prophylaxis or for the treatment of the symptoms associated with COVID-19. This situation could have a dire consequences on management of malaria, as chloroquine-resistant parasites may increase, with a possible cross resistance to amodiaquine. This article, therefore, seeks to discuss the possible effect of the increased indiscriminate use of the drug on malaria management with some artemisinin-based combination therapies (ACTs) as well as intermittent preventive treatment (IPT) containing amodiaquine as partner drug and give an advice.

USES OF CHLOROQUINE AND HYDROXYCHLOROQUINE
Chloroquine was developed in 1934 as an antimalarial drug, whilst hydroxychloroquine was developed a decade later. Hydroxychloroquine is generally used for the treatment of autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis in addition to malaria. It is known to regulate the activity of the immune system, which may be overly active in some conditions. It does so by modifying the underlying disease process, rather than treating the symptoms.

In the immediate past, chloroquine was the first-line drug for the treatment of uncomplicated malaria in disease endemic areas of the world and also used for the treatment of extra-intestinal amebiasis. However, towards the end of the 20th century, the malaria parasite, Plasmodium falciparum, developed serious resistance to chloroquine.

Due to the reported widespread parasite resistance to the drug, the World Health Organization (WHO) recommended its replacement with the ACTs as the first antimalarial drug of choice. This led to the dwindling use of chloroquine. Currently, chloroquine is used for mono-therapy of malaria only in countries such as Honduras where there are no reported parasite resistance to the drug.

Resistance to chloroquine is associated with specific genetic point mutation at various codons in the P. falciparum chloroquine resistance transporter (pfCRT) gene [6–8] and is modulated by mutations in the P. falciparum multidrug resistance locus 1 (pfmdr1) [7]. Substitution of adenylate with cytidy late at position 76 of pfCRT gene which changes lysine to threonine (K76 to T76) is a single molecular marker that strongly correlates with chloroquine drug resistance in P. falciparum [9, 10]. The mutations in codons 76 (pfCRT, K76T) and 86 (pfmdr1, N86Y) are therefore important regarding parasite resistance to chloroquine [8, 11]. It has also been reported that other mutations at different codons of the pfCRT gene are associated with chloroquine resistance [9, 11, 12]. Drug pressure driven by high chloroquine usage in an area is a major determinant of selection and spread of chloroquine-resistant genes among P. falciparum population [10, 11]. Interestingly, disuse of chloroquine as the first-line antimalarial drug for the treatment of uncomplicated malaria in certain endemic areas resulted in the re-appearance of strains of parasites sensitive to the drug [13–15].
than later, chloroquine, which is one of the safe and cheapest antimalarial drugs, may be re-considered in a combination-therapy rather than in monotherapeutic use in the management of malaria.

CHLOROQUINE AND HYDROXYCHLOROQUINE AS POTENTIAL DRUG IN THE MANAGEMENT OF COVID-19
Since the onset and spread of COVID-19, chloroquine and hydroxychloroquine have been touted as a possible cure or chemoprevention of the novel disease. The rationale for the choice of these drugs against COVID-19 is based on the reported increased in the endosomal pH which inhibits fusion of the SARS-CoV-2 and the host cell membranes [16]. In vitro, 'both drugs block the transport of SARS-CoV-2 from early endosomes to endolysosomes, which may be required for release of the viral genome' [17].

Reports touting chloroquine and hydroxychloroquine as potential treatment for COVID-19 mostly carried out by the international press have led to a dramatic increase in demand and supply of the drugs. Chloroquine, which has been banned in many malaria endemic countries, and hydroxychloroquine have suddenly surfaced and it is being used as treatment or prophylaxis against COVID-19.

It is important to state that, lately, the publicity given to these drugs for the treatment or chemoprophylaxis of COVID-19 has dwindled due to the reported side effect and efficacy of the drugs in the management of COVID-19. The FDA has released a report indicating serious problems with the treatment of COVID-19 with these drugs. Heart rhythm problems, blood system disorders and kidney injuries among others have been reported to be associated with hospitalized patients treated with the drugs [18]. In his media briefing on COVID-19 on 25 May 2020, the WHO Director-General announced the suspension of hydroxychloroquine and chloroquine from Solidarity trial because of their safety and efficacy concerns [19]. The WHO decision was based on reports from ongoing clinical trials. However, from news reports globally, this announcement seemed not to have abated the demand and use of the drug for the management of COVID-19, especially in economically poor settings where most of the burden of malaria happens to situate and the fear of contracting COVID-19 is likely to be high.

DOSE REGIME OF CHLOROQUINE FOR MALARIA OR COVID-19 MANAGEMENT
The usual chloroquine dose for the treatment of malaria for an adult weighing least 60 kg is: 1 g salt (600 mg base) orally as an initial dose, followed by 500 mg salt (300 mg base) orally after 6–8 h, then 500 mg salt (300 mg base) orally once a day on the next 2 consecutive days.

Treatment or chemoprophylaxis regime using chloroquine or hydroxychloroquine against COVID-19 varies. Usually, the adult dose used against the disease is 1 g salt (600 mg base) orally on Day 1, followed by 500 mg salt (300 mg base) orally once a day for a total duration of 4–7 days depending on clinical evaluation.

It is obvious from these dose regimes that quite a substantial amount of the drug is used in the management of COVID-19. It is speculated that, in some places, very high doses of chloroquine and its derivative are used indiscriminately for chemoprevention and treatment of COVID-19. Such practices must be discouraged through intense and sustained education as continuation may lead possibly to serious health implications.

INCREASED USE OF CHLOROQUINE TO TREAT COVID-19 AND ITS EFFECT ON MANAGEMENT OF MALARIA
It is important to state that the reason for the choice of ACTs to treat malaria is to slow down the development of resistance to the antimalarial drugs involved [21]. The principle behind the combination is that, the fast-acting drug, which is the artemisinin, quickly reduces the parasite load whilst the slow acting, partner antimalarial, gradually mob up the residue parasites [22]. The potency of the ACTs is therefore dependent on the efficacy of both the artemisinin component and the partner drug. Therefore, reduced susceptibility of parasites to the partner drugs in the ACTs can potentiate the development of resistance to the artemisinin with time. Amodiaquine is a partner drug in one of the ACTs (artesunate-amodiaquine, AS-AQ). This combination is one of the most popular choice of ACT for
the treatment of uncomplicated malaria because of cost and ease of administration. Amodiaquine is also a partner in combined drugs used in the management of malaria in interventional preventive measures, recommended for specific high-risk groups in areas of high malaria transmission. For instance, in 2013, WHO recommended the use of amodiaquine in combination with sulfadoxine and pyrimethamine (SP-AQ) for seasonal malaria chemoprevention (SMC) in children at high risk areas. SMC is defined as ‘the intermittent administration of full treatment courses of an antimalarial medicine during the malaria season to prevent malarial illness with the objective of maintaining therapeutic antimalarial drug concentrations in the blood throughout the period of greatest malarial risk’. This intervention has been shown to be effective, cost-effective, safe and feasible for preventing malaria among children younger than 5 years of age in areas with highly seasonal malaria transmission.

Amodiaquine is also used in combination with SP for intermittent preventive treatment during pregnancy (IPTp) in some disease endemic countries. IPTp reduces maternal malaria episodes, placental, maternal and foetal anaemia, parasitaemia, low birth weight and neonatal mortality.

Increased use of chloroquine to manage COVID-19 is likely to trigger the development and emergence of strains of malaria parasites resistant to chloroquine. In addition to the mechanism of parasites resistance to chloroquine described above, there could be subsequent selection of additional changes in genes regulating *P. falciparum* response to chloroquine [23, 24]. Such situations will favour cross-resistance between chloroquine and amodiaquine, which is part of some ACTs and IPT in many disease endemic areas [23–25].

It can therefore be conjecture that the indiscriminate and escalated use of chloroquine or it analogies to manage COVID-19 may impact negatively on amodiaquine efficacy due to structural similarities. Chloroquine, hydroxychloroquine and amodiaquine are all derivatives of the drug classified as *4-aminoquinoline*. *4-Aminoquinoline* have an amino group at 4-position of the quinoline moiety. Amodiaquine differ from chloroquine by only a side chain (Fig. 1). Therefore, the possibility of cross resistance between chloroquine or its derivatives and amodiaquine is very high.

Indeed, there are numerous reports in literature indicating cross resistance between chloroquine and amodiaquine. Various *in vitro* parasite sensitivity assays attest to this: work carried out in India by AnupKumar, *et al.* [26], in Philippines by Smrkovski, *et al.* [27], in Senegal by Diawara, *et al.* [28], and in Colombia by Fall, *et al.* [29], in South America by Young [30] and in Gabon by Pradines, *et al.* [31].

It must however be noted that a significant positive correlation suggesting *in vitro* cross-resistance may not necessarily be predictive of cross-resistance *in vivo*. Nevertheless, information on *in vitro* cross-resistance of one compound to an existing antimalarial is important as it becomes a potential indicator of future resistance: a marker or an early warning sign of an emerging parasite resistance, especially when chemical structures of the compounds involved are similar.

Therefore, as the use of chloroquine or hydroxychloroquine increases, the possibility of amodiaquine losing it current potency due to the aforementioned phenomenon is eminent.

Such an occurrence may lead to increased failure of the AS-AQ combination and a more serious consequences on the IPT used for pregnant women and children (SP-AQ), especially in areas where the efficacy of SP is already weakened. Again, under such circumstances, the planned future use of chloroquine

![Chemical structures of amodiaquine, chloroquine and hydroxychloroquine.](image-url)
in the management of malaria in the manner previously described [32] is likely to be jeopardized.

All put together, it must be said that the escalated use of chloroquine and its derivatives to manage COVID-19 is likely to be problematic for the control of malaria using some of the existing control drugs schemes. Measures must therefore be put in place to monitor and control the use of chloroquine and its derivatives to manage COVID-19 to avert these expectancies. There is the need to continuously monitor the efficacy of amodiaquine in disease endemic settings where it is used.

CONCLUSION
It is quite worrying, as the increased use of chloroquine or hydroxychloroquine to manage COVID-19 disease will lead to increased drug pressure and consequently the selection of drug-resistant parasite, as happened in the era preceding the introduction of the ACTs. Resistant strains of the malaria parasites to chloroquine or derivatives with cross-resistance to amodiaquine are likely to appear due to increased drug pressure as a consequence of the escalated use of the drug for the treatment of COVID-19. The use of chloroquine or it derivative to manage COVID-19 must therefore be strictly controlled. Education of the general populace on the issues associated with the use of the drugs must be done. There is also the need to constantly monitor the efficacy of amodiaquine in disease endemic countries.

REFERENCES
1. Team NCPERE. Vital surveillances: the epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) – China. China CDC Weekly 2020; 2:113–22.
2. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther 2020; 14:58–60.
3. Savarino A, Boelaert JR, Cassone A, et al. Effects of chloroquine on viral infections: an old drug against today’s diseases? Lancet Infect Dis 2003; 3:722–7.
4. Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. Int J Antimicrob Agents 2020;55:105923.
5. 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;14:72–3. 10.5582/bst.2020.01047.
6. Mayor AG, Gomez-Olive X, Aponte JJ, et al. Prevalence of the K76T mutation in the putative Plasmodium falciparum chloroquine resistance transmembrane protein (PfCRT) gene and its relation to chloroquine resistance in Mozambique. J Infect Dis 2001;183:1413–6.
7. Sidhu AB, Verdier-Pinard D, Fidock DA. Chloroquine resistance in Plasmodium falciparum malaria parasites conferred by Pf crt mutations. Science 2002;298:210–3.
8. Djimde A, Doumbo OK, Cortese JF, et al. A molecular marker for chloroquine-resistant falciparum malaria. N Engl J Med 2001;344:257–63.
9. Cooper RA, Hartwig CL, Ferdig MT. pfcrt is more than the Plasmodium falciparum chloroquine resistance gene: a functional and evolutionary perspective. Acta Trop 2005; 94:170–80.
10. Djimde AA, Doumbo OK, Traore O, et al. Clearance of drug-resistant parasites as a model for protective immunity in Plasmodium falciparum malaria. Am J Trop Med Hyg 2003;69:558–63.
11. Fidock DA, Nomura T, Talley AK, et al. Mutations in the P. falciparum digestive vacuole transmembrane protein Pf CRT and evidence for their role in chloroquine resistance. Mol Cell 2006;6:861–71.
12. Wootton JC, Feng X, Ferdig MT, et al. Genetic diversity and chloroquine selective sweeps in Plasmodium falciparum. Nature 2002;418:320–3.
13. Froesch AE, Lauper MK, Mathanga DP, et al. Return of widespread chloroquine-sensitive Plasmodium falciparum to Malawi. J Infect Dis 2014;210:1110–4.
14. Mekonnen SK, Aseffa A, Berhe N, et al. Return of chloroquine-sensitive Plasmodium falciparum parasites and emergence of chloroquine-resistant Plasmodium vivax in Ethiopia. Malar J 2014;13:244.
15. Balikagala B, Sakurai-Yatsushiro M, Tachibana S, et al. Recovery and stable persistence of chloroquine sensitivity in Plasmodium falciparum parasites after its discontinued use in Northern Uganda. Malar J 2020;19:76.
16. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30: 1–3.
17. Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020;6:16.
18. https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital-setting.or (10 September 2020, date last accessed).
19. WHO Director-General opening remarks at the media briefing on COVID-19 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—25-may-2020 (10 September 2020, date last accessed).
20. White NJ, Nosten F. Artemisinin-Based Combination Treatment of Falciparum Malaria. Am J Trop Med Hyg 2007;77:181–92.

21. Foote SJ, Kyle DE, Martin RK, et al. Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. Nature 1990; 345:255–8.

22. Dorsey G, Staedke S, Clark TD, et al. Combination therapy for uncomplicated falciparum malaria in Ugandan children: a randomized trial. JAMA 2007;297:2210–9.

23. Dorsey G, Zongo I, Ouedraogo J-B, et al. Roles of specific *Plasmodium falciparum* mutations in resistance to amodiaquine and sulfadoxine-pyrimethamine in Burkina Faso. Am J Trop Med Hyg 2006;75:162–5.

24. Ochong EO, van den Broek IVF, Keus K, et al. Association between chloroquine and amodiaquine resistance and allelic variation in the *Plasmodium falciparum* multiple drug resistance 1 gene and the chloroquine resistance transporter gene in isolates from the upper Nile in southern Sudan. Am J Trop Med Hyg 2003;69:184–7.

25. Holmgren G, Gil JP, Ferreira PM, et al. Amodiaquine resistant *Plasmodium falciparum* malaria in vivo is associated with selection of pfcr7T and pfmdr1 86Y. Infect Genet Evol 2006;6:309–14.

26. Anupkumar RA, Sharma SK, Ghosh RM, Bhatt SS, Mohanty CR, et al. In vitro assessment of drug resistance in *Plasmodium falciparum* in five States of India. Indian J Med Res 2012;135:494–9.

27. Smrkovski LL, Buck RL, Alcantara AK, et al. Studies of resistance to chloroquine, quinine, amodiaquine and mefloquine among Philippine strains of *Plasmodium falciparum*. Trans R Soc Trop Med Hyg 1985;79: 37–41.

28. Diawara S, Madamet M, Kounta MB, et al. Confirmation of *Plasmodium falciparum* in vitro resistance to monodethalamodiaquine and chloroquine in Dakar, Senegal, in 2015. Malar J 2017;16:118.

29. Fall B, Diawara S, Sow K, Baret E, et al. Ex vivo susceptibility of *Plasmodium falciparum* isolates from Dakar, Senegal, to seven standard anti-malarial drugs. Malar J 2011;10:310.

30. Young MD. Amodiaquine and hydroxychloroquine resistance in *Plasmodium falciparum*. Am J Trop Med Hyg 1961;10: 689–93.

31. Pradines B, Mabika Mamfoumbi M, Parzy D, et al. In vitro susceptibility of African isolates of *Plasmodium falciparum* from Gabon to pyronaridine. Am J Trop Med Hyg 1999; 60:105–8.

32. Sisowath C, Petersen I, Veiga MI, et al. In vivo selection of *Plasmodium falciparum* parasites carrying the chloroquine-susceptible pfcr7T allele after treatment with artemether-lumefantrine in Africa. J Infect Dis 2009;199:750–7.