Letters in Applied NanoBioScience
Platinum Open Access Journal (ISSN: 2284-6808)

Review
Volume 9, Issue 4, 2020, 1705 - 1716
https://doi.org/10.33263/LIANBS94.17051716

A Review on Hydroxychloroquine Drug: to Combat COVID-19

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Received: 13.07.2020; Revised: 18.08.2020; Accepted: 20.08.2020; Published: 25.08.2020

Abstract: The drug hydroxychloroquine is an efficacious drug for the treatment of various ailments of the human. This drug is used to Malaria, Rheumatic Arthritis, Systemic lupus erythematosus, and other liver ailments. It increases the lysosomal pH of the molecule and produces an acidic environment. So as to reduce the toxicity of the cell. COVID-19 is a pandemic and emerging disease across the globe, which is devastating to the human population. As a remedial measure, hydroxychloroquine drug can be used as an effective measure for the treatment of COVID-19. The mechanism of hydroxychloroquine includes inhibition of cytokine production and signaling pathways, which also gives cellular and cardiovascular effects. The recent steps and procedures taken by the countries to use hydroxychloroquine as an effective drug against COVID-19 are also discussed. As hydroxychloroquine is a derivative of chloroquine shows a more efficacious effect on COVID-19, but as a traditional drug, chloroquine causes more damage as compared to its derivative. Thus we conclude that the emerging disease COVID-19 can be constrained by the use of this efficient drug.

Keywords: Hydroxychloroquine; efficacy; TLR signaling; autophagy; lysosomal activity; COVID-19.

Abbreviations: COVID-19-Corona virus disease 19; HCQ-Hydroxychloroquine; CQ-Chloroquine; TLR-Toll–like receptor; TTCR-Time to clinical recovery; pDCs-Plasmocytoid dendritic cells; MHC-Major histocompatibility complex; cGMP-Cyclic guanosine monophosphate; cAMP-Cyclic adenosine monophosphate; PPT 1-Palmitoyl–protein thioesterase; RA-Rheumatoid arthritis; IRF 3-Interferon regulatory factor 3; cGAS-Cyclic GMP-AMP synthase; IFN-Interferon; IL-Interleukin; APS-Antiphospholipid syndrome; WBC-White blood cells.

1. Introduction

Hydroxychloroquine is immune-suppressive, anti-autophagic in nature, and antimalarial characteristics, hydroxychloroquine may inhibit immune function by involving in the processing and presentation of Antigens which leads to cytokine production. As a lysosomotropic agent, hydroxychloroquine upsurge Intra-lysosomal pH, which impairs the autophagic protein degradation process, hydroxychloroquine-mediated accumulation of ineffective autophagosomes may result in cell death in tumor cells reliant on autophagy for survival. In addition to that, the above agent is very dominant against the Erythrocytic types of *plasmodium vivax* and *Malariae* and most of the strains of *Plasmodium falciparum* but not on their gametocytes. Hydroxychloroquine is present in only those individuals that have come into contact or have ingested it. Hydroxychloroquine is found to be present in the food vacuoles of the agent (parasite) in the erythrocyte. This raises the pH of the acidic vesicles, involving in

vesicle activities and stops phospholipid metabolism. In the suppressive treatment of plasmodium, hydroxychloroquine inhibits the erythrocytic phase of growth and development. In severe cases of malaria, it disturbs the erythrocytic schizogony of the parasite. Its capability to be collected in erythrocyte of the parasite, which accounts for their toxicity against the erythrocytic stages of the infection caused by plasmodia. As an anti-rheumatic agent, hydroxychloroquine acts as a mild Immune-suppressant, retarding the release of rheumatoid factor and acute-phase molecules. It accumulates in WBC, lysosomal membrane stabilization, and inhibits the activity of enzymes (like collagenase, proteases) that cause cartilage damage.

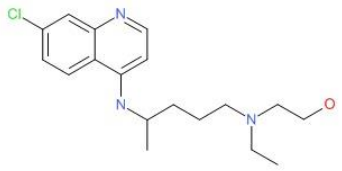

Figure 1. The general structure of hydroxychloroquine (*Source: generated by ChemSketch*).

2. Chemistry of hydroxychloroquine

Hydroxychloroquine has different chemical and physical properties that can define its nature effectively. These include weight, mass charge canonical structures, etc. The table below illustrates it better.

Table 1. Chemical and physical properties of hydroxychloroquine.

Property Name	Property Value
1.Molecular weight	335.9 g / mol
2.Molecular formula	C_{18} H $_{26}$ CL N_3 O
3.Appearance	White crystals
4.Water solubility	26 mg/L
5.Melting point	90 degree celsius
6.Hydrogen bond donor count	2
7.Hydrogen bond acceptor count	4
8.Heavy atom count	23
9.Formal charge	0
10. Complexity	331
11. Covalently bonded unit count	1
12. Compound is canonicalised	yes
13. Isotope atom count	0
14. Monoisotopic mass	335.17644 g/ mol
15. Rotatable bond count	9
16. Synonyms	Plaquenil, oxychloroquine

The below two figures are the structures of hydroxychloroquine in 2-D and 3-D configurational forms. The figures are made by PubChem and designed by pymol. The structures are necessary for their correct structural analysis. It gives accurate information about the bonding pattern, hydrogen bonds, atoms arrangement, etc.

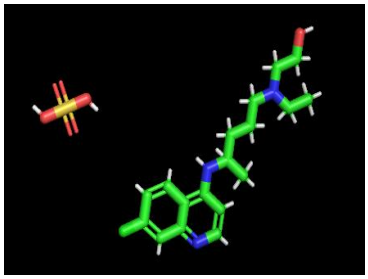

Figure 2. 2-Dimensional structure (*Source: Generated by PyMol software).*

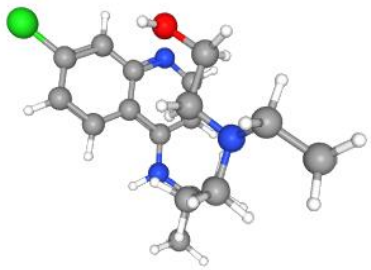

Figure 3. 3-Dimensional structure (*Source: www.pubchem.com*).

3. Efficacy of hydroxychloroquine on COVID-19

Researches prove that chloroquine (CQ) shows some antagonism against COVID-19 *in vitro.* However, evidence related to its symptoms in patients is restricted. The above study objects to estimate the efficacy of hydroxychloroquine (HCQ) in the curing of patients suffering from COVID-19. Main methods: From 4 February to 28 February 2020, 62 infectious patients of COVID-19 were detected and are admitted to RenMin Hospital of Wuhan University. All patients were in random divided into Parallel-group trial, 31 patients were set to receive an extra 5-day HCQ (400 mg/d) therapy, Time to clinical recovery or TTCR, clinical features, and Radiological outcomes were evaluated at standardization and five days after treatment to estimate the result of HCQ. Key results: For the 62 COVID-19 patients, 29 of 62 (46.8%)were male, and 33 of 62(53.2%) were female, the average age was 44.7 (15.3) years. No change in the sex distribution and age between the HCQ group and the control group. But for Time to clinical recovery, the cough reduction time and Body temperature recovery time were considerably reduced in the HCQ treatment group people. In addition, a large number of patients with recovered pneumonia in the HCQ treatment group 25 of 31(80.6%) paralleled with the control group 17 of 31(54.8%). Particularly, all four patients increased to severe illness in the control group. On the other hand, there were two patients with minor reactions in the HCQ group. The significant result is that patients suffering from COVID-19, the usage of HCQ could be used to reduce TTCR and stimulate the absorption of pneumonia.

4. Mode of action

Different mechanisms of action are assumed to elucidate the adverse effects or therapeutic effects of hydroxychloroquine and chloroquine; mostly, they are being used *in vitro* researches. Remarkably, the relationship between these mechanisms and the medical efficacy and safety detected *in vivo* has yet to be fully defined [71]. Drugs with antimalarial properties have undeviating molecular effects on autophagy, lysosomal activity, and signaling pathways. Analysis of the impact of these drugs on pDCs or Plasmacytoid dendritic cells, B cells, T cells, and antigen-presenting cells are also reachable. Such different therapeutic interferences of the immune system [70], the mode of action, are possibly context-dependent, i.e., dependent on the inflammatory conditions and/or affected tissues or organs.

4.1. Main mechanisms of actions by hydroxychloroquine and chloroquine.

Hydroxychloroquine and chloroquine can retard certain body functions at the cellular level and different molecular pathways involved in immune system activation, as written below, somewhat by collecting in lysosomes and auto-phagosomes of phagocytic cells and changing local pH concentrations [71, 70]:

- Inhibition of class II MHC expression, presentation of antigen and activate the immune system (lowering CD154 expression by T cells);
- Production of different pro-inflammatory cytokines, such as Interleukin-1, Interferon α, and Tumor Necrosis Factor, which can provide protection against cytokine-mediated cartilage resorption is inhibited;
- Intrusion with Toll-like receptor 7 or TLR7 and TLR9 signaling pathways;
- Interference with cyclic GMP-AMP (cGAMP) synthase (cGAS) activity.

4.2. Molecular effects.

4.2.1. Inhibition of autophagy and lysosomal activity.

A significant mechanism of action of chloroquine and hydroxychloroquine is the intervention of autophagy and lysosomal action. It is broadly accepted that CQ and HCQ get concentrated in lysosomes (lysosomotropic) and stop their function. *In vitro,* CQ can undermine the lysosomal membranal activity and stimulate the production of lysosomal enzymes inside the cells [23]. As proof of this latter mechanism is rare, the capability of these drugs to interfere with lysosomal activity has been frequently accepted [24, 25, and 26]. Interference of lysosomal activity may retard the function of lymphocytes and have anti-inflammatory effects or immunomodulatory.

The mechanism by which the drugs can have anti-inflammatory properties is the impairment of antigen presentation through the lysosomal pathway. Lysosomes consist of hydrolytic enzymes, and in cooperation with the vesicles, they engulf cargo (such as organelles and material from inside the cell (in a process known as autophagy) or substances from the outer side of the cell (through the endocytosis or phagocytosis pathway)). Lysosomes are indulged not only in recycling cellular substrates[27] but also in the processing of antigen and presentation of class II MHC, indirect immune activation also takes place[28]. The process Autophagy is also indulged in antigen presentation and immune system activation [29, 30]. For ex, data from a single analysis suggest that autophagy is essential for MHC class II-mediated auto-antigen presentation by antigen-presenting cells to $CD4^+$ T cells [31]. As the pH of lysosomes is optimum for lysosomal enzymes that take part in hydrolysis, by raising the pH of endosomal sections [32], CQ and HCQ might disable the maturation of lysosomes and autophagosomes and retard antigen presentation via the lysosomal pathway. As a result, recent researches suggest that HCQ and CQ inhibit lysosomal and autophagosome functions and promote immune activation.

Away from lysosomotropic, methods to detect exact molecular targets of hydroxychloroquine within the lysosome are also currently in progress. Research has been recognized on Palmitoyl-protein thioesterase 1 or PPT1, an enzyme used in the breakdown of lipid-altered proteins, as a possible lysosomal target of CQ and its derivative compounds [33]. HCQ can bind and stop the PPT1 activity [33], and remarkably, The Enzyme PPT1 shows its overexpression in the patient's synovial tissue with rheumatoid arthritis (RA) [34]. While an example of ongoing research, confirmatory functional studies and the identification of other molecular targets within the lysosomes are nevertheless warranted.

4.2.3. Inhibition of signaling pathways.

Hydroxychloroquine and chloroquine can also impede with TLR or toll-like receptor signaling. For example, modifications in endosomal pH can interfere with the processing of

TLR9 and TLR7 [35], and, hence, these antimalarial drugs prevent TLR activation upon extracellular stimuli by arbitrating changes in the pH [35]. CQ or HCQ can also bind to DNA or RNA and block TLR9 signaling at the Intracellular level by stopping the TLR–ligand interactions (steric blockade). This latter theory is supported by an analysis that is based on fluorescence spectroscopy and surface Plasmon resonance depicting that antimalarial drugs can directly inhibit CpG–TLR9 interactions [36, 37]. In addition to TLR9 signaling, CQ can also stop RNA-mediated activation of TLR7 signaling [38, 39]. As the exact mechanism by which these drugs restrict TLR7 and TLR9 requires more explanation at the molecule stage [40], inhibition of TLR binding and processing are 2 important mechanisms of action.

Another possible mode of action of HCQ and CQ is the interference with cyclic GMP-AMP (cAMP) synthase (cGAS) activity by retarding ligand binding [41]. The cGAS–stimulator of Interferon genes (STING) pathway is a most important source of the IFN type I response. Cytosolic DNA binds to cGAS and the second messenger cGAMP to facilitate STING-dependent transcription of type I IFNs through the transcription factor IFN regulatory factor 3 (IRF3)[41,42]. cGAS inhibitors are currently in development for the cure of inflammatory rheumatic diseases [44].

4.3. Cellular effects.

4.3.1. Cytokine production and immune activation.

Hydroxychloroquine and chloroquine can indirectly lower the release of anti-inflammatory cytokines by a different type of cell. *In vitro*, HCQ, and CQ stop the release of IL-1, IL-6, TNF, and IFN-γ by Mono-nucleated cells [45]. Moreover, therapy with HCQ inhibits the release of TNF, IFNα, IL-6, and CCL4 (also known as MIP1β) in pDCs and NK cells co-cultures stimulated with RNA-containing immune complexes [46,47].

4.4. Cardiovascular effects.

As hydroxychloroquine is not an anticoagulant, this drug is broadly said to have vascular defensive effects and prevent the growth of thrombotic complications. The discussed protective effect appears to be most appropriate for patients with a Secondary coagulopathy due to systemic inflammation [53] and in patients with primary Antiphospholipid syndrome or APS [54].

Patients suffering from inflammatory rheumatic diseases are at a high risk of developing cardiovascular problems in comparison to the general population [55,56,57,58]. This high risk is triggered by the underlying disease, drugs used in the treatment of the disease (such as NSAIDs, including COX-2 inhibitors [58] and high-dose glucocorticoids) and the existence of complications such as Arterial hypertension, Hyperlipidaemia, chronic kidney failure, and diabetes mellitus. In contrast to the treatment with HCQ, which seems to overcome these effects and long-term benefits are provided by lowering the risk of cardiovascular events, reducing glucose fasting levels [59], and lowering hyperlipidemia [60,61].

5. Recent advancements in COVID-19 treatment by the application of hydroxychloroquine

An occurrence of developing the disease (COVID-19) by the introduction of a novel coronavirus (named SARS-CoV-2), which takes its origin from Wuhan, China, and infects the

whole Chinese regions and other parts of the world, at the end of December 2019. The WHO confirmed the outbreak of COVID-19 as a pandemic on 12 March 2020 [3]. Approximately 80% of patients suffering from mild disease and the overall case-death rate is about 2.3% but extends to 8% in patients aged 70 to 79 years and 14.8% in those whose age is more than or equal to 80 years, the information given according to a recent Chinese study [4]. However, there is undoubtedly an important number of asymptomatic carriers in the population, and thus the mortality rate is probably overemphasized. France is now confronting the COVID-19 upsurge with more than 4500 cases, as of 14 March 2020 [4,5]. So, there is a crucial need for an operational treatment to treat symptomatic patients but also to reduce the duration of virus carriage in order to limit community transmission[5]. To cure COVID-19, transposition of old drugs to be used as antiviral therapy which can be a successful strategy because analysis of its side effects, safety profile, and drug reactions are well known [6,7]. A current paper stated an inhibitory effect of a new antiviral drug-remdesivir and chloroquine (an old antimalarial drug) on the developmental cycle of a novel coronavirus *in vitro* [8] and an initial medical trial conducted in COVID-19 Chinese patients, showed that CQ had a vital effect in relation to clinical results and viral clearance, as compared to controls groups [9,10]. Chinese scientists mentioned that patients identified as acute, moderate, and chronic cases of COVID-19 and without contraindications to CQ, be treated with 500 milligrams CQ two times a day for ten days [11].

Hydroxychloroquine (a Derivative of chloroquine) has been revealed to have an anti-SARS-CoV activity *in vitro* [12]. HCQ clinical safety profile is accepting good recovery results than CQ (for long-term usage), allows greater routine dose [13], and has lesser concerns about the drug to drug interactions [14]. Our group has an inclusive experience in effectively curing patients with chronic diseases due to intracellular bacteria (*Coxiella burnetii* causes Q fever and 'Whipple's disease due to *Tropheryma whipplei)* with long-term HCQ treatment or 600 mg per day for 12 to 18 months for more than twenty years [15,16]. So, we conducted a clinical trial that aims at the evaluation of the effect of HCQ on SARS-CoV-2-infected patients approved by the Ministry of Health at the French government. In this description, we discussed our initial test results, aiming at virological data in patients receiving HCQ as compared to CG or control group.

6. Full examination of hydroxychloroquine treatment

6.1. Setting.

This current research is organized by The Mediterranean Infection University Hospital Institute in Marseille. Patients who were given treatment with hydroxychloroquine were enlisted and managed in the Marseille center. Controls without hydroxychloroquine treatment were recruited in Marseille, Nice, Avignon, and Briançon centers, all located in the South France region [16].

6.2. Patients.

Patients admitted in hospitals with confirmed COVID-19 were added in this approach if they satisfy 2 initial norms: 1.) age >12 years 2.) PCR recognized the SARS-CoV-2 way of movement in the Nasopharyngeal sample at admission, whatever their clinical status [17].

Patients were excited if they had any kind of allergy with the treatment of hydroxychloroquine or chloroquine or had another known contraindication to treatment with

the study drug, includes deficiency of Glucose-6-Phosphate Dehydrogenase, Retinopathy, and QT prolongation. Pregnant patients were not included based on their affirmation and pregnancy test outcomes when they were required [18].

6.3. Informed consent.

Before involvement in the group, patients had to meet some inclusion criteria had to give their consent to participate in the study. Written permission and signed consent were taken from adult participants (more than18 years) or from guardians for minor patients (less than 18 years). A well-organized data related to the participation of the patients in the study group was done [17,18] in addition to their benefits and risks. Patients receive the information related to their clinical welfare during the period of their treatment, either they participate or not. In patient identification, a particular study number was given sequentially to the added participants in accordance with the series of patient no. Allotted to every study center [18]. The study was organized according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) list of guidelines of good clinical practice, the Helsinki Declaration, and applicable typical operating procedural methods.

6.4. Method.

At starting, patients were seen for enrollment, primary data collection, and therapy at day-0, and again for day-to-day follow-up for the duration of 14 days. At each day, patients were given high-level medical treatment, and at some possible times, a nasopharyngeal sample was collected. All medical data were collected via standardized questionnaires [4]. The patients in the Marseille center were given oral Hydroxychloroquine sulfate (200 mg), 3 times a day during 10 days (in this primary phase, we didn't enroll children in the treatment group on the basis of data indicating that children have mild symptoms of COVID-19[4]). Patients didn't agree to the treatment or had any doubts, are added to controls in Marseille center. Patients in other centers didn't receive HCQ and act as controls. Antibiotics and Symptomatic treatment as a method to protect from bacterial super-infection were given by investigators on the basis of clinical judgment. HCQ was given by the National Pharmacy of France on a required basis [4,5].

6.5. Clinical classification.

Patients were divided into 3 groups: first is URTI or upper respiratory tract infection when presenting with pharyngitis, rhinitis or isolated low-grade fever and myalgia, second is LRTI or lower respiratory tract infection with symptoms of pneumonia or bronchitis [5] and third is Asymptomatic with no symptoms.

6.6. PCR assay.

The virus was evaluated by real-time reverse transcription-PCR [17].

6.7. Hydroxychloroquine treatment.

Original HCQ has been taken from the patient's Serum samples by UHPLC-UV using a previous method [18]. The level of the chromatogram at 1.05 minutes of retention resembles HCQ metabolite. The serum amount of this metabolite is calculated from UV absorption, as

for HCQ concentration [18,19]. By considering the 2 concentrations, it provides an estimation of initial serum hydroxychloroquine concentration.

6.8. Culture.

Used for all patients, 500 µL of the sample collected from the nasopharyngeal swab was delivered through a pore size of 0.22 µm centrifugal filter; then they were transferred into the wells of 96-well culture microplates, of which 4 wells contained Vero E6 cells (ATCC CRL-1586) in less crucial culture medium with 1% glutamine and 4% fetal calf serum [19]. After centrifugation at 4,000 rpm, plates were in incubation at room temperature (37°C). Microplates were in observation daily for the confirmation of the cytopathogenic effect. Possible identification of the virus in supernatant was made using a Scanning electron microscope (SU5000 from Hitachi) then examined by specific RT-PCR [20].

Table 2. Benefits of hydroxychloroquine over chloroquine by the changes in the human organ.

Histopathological changes	Control group	Chloroquine group	Hydroxychloroquine
LIVER-	-	+++	+
1. Hepatoportal and sinusoidal Congestion	-	+++	+
2. Inflammatory cellular filtrate	-	+++	+/-
3. Cloudy swelling and hydropic degeneration	-	+++	-
4. Cellular necrosis			
KIDNEYS-	-	+++	+
1.vascular congestion			
2. inflammation	-	+++	+
3. hypercellularity of glomeruli	-	+++	+
4. interstitial and tubular hemorrhage	-	+++	-
5.focal tubular necrosis	-	++	-
HEART-			
1. Interstitial fibrosis	-	++	-
2. Hemorrhage	-	++	+
3. Cardiac muscle necrosis	-	++	-
4. Cloudy swelling of cardiac muscle and loss of striation	-	+++	+
Source: European scientific journal			

A comparison of the histopathological changes caused due to chloroquine and hydroxychloroquine in rat organs as chloroquine causes more damage than hydroxychloroquine.

7. Conclusion

Thus we conclude that the greater pandemic ever happens and which is more effective than any other. The world is facing a huge loss of global economy, infrastructure, and a number

of healthcare services. As a remedy number of scientists, organizations and universities are trying to produce vaccines and a drug that can combat the effect of COVID-19. Hydroxychloroquine can be effective in reducing the effect of the disease. As the chloroquine is more toxic and causes damage to the organs. So effective drug hydroxychloroquine which is less toxic and causes less damage to the body. As recent advancements, the trial for HCQ on COVID-19 is somehow successfully. For better results, scientists are examining the greater rate of recovery for COVID-19 patients by hydroxychloroquine to declare it as a universal medicine for the disease**.**

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

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