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Review Article

The Response of Hydroxychloroquine for Covid-19

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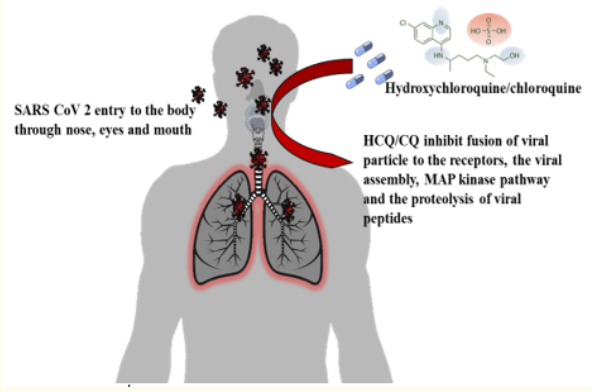
Abstract

Coronavirus 2 (SARS-CoV-2) is now becoming a serious threat to public health all over the world and it is responsible for severe acute respiratory syndrome called COVID-19. The lack of a proper vaccine or medication forced the researchers and public health agencies to repurpose drugs that are already approved for various other diseases and known to be safe. Drug Repurposing encourages the scientific community to look up into possible treatment methods for the infection in real-time until an effective vaccine is developed against the viral infection. In this context, Hydroxychloroquine (HCQ), a well-known antimalarial drug, is getting tremendous significance as a potential drug against novel coronavirus. The antimalarial activity of HCQ has been well studied in detail over the past many decades. These studies have helped in the identification of HCQ as potential antirheumatic as well as anticancer drugs.

This review highlights the multifaceted benefits of the drug hydroxychloroquine starting from its discovery. Apart from the therapeutic activity of HCQ, this review also focused on the recent results of clinical trials of HCQ against novel SARS-CoV-2 virus and their efficacy in treating COVID-19.

Keywords: *Covid-19; Hydroxychloroquine; Chloroquine; Anti-Viral; Anti-Malarial*

Graphical Abstract



Graphical Abstract: *Spreading of SARS-Cov2 and effect of hydroxychloroquine and chloroquine on SARS-CoV-2.*

Introduction

In the present scenario where the world is facing a pandemic with no known cure, hydroxychloroquine is the name that echoes all over the world due to its efficacy as a drug against the novel SARS-CoV-2 virus. SARS-COV-2 virus is the new strain of virus belonging to the coronavirus family that has undergone widespread transmission all over the world. The unavailability of an effective vaccine for this virus makes it difficult to control the spread of the disease. In this context, the World Health Organization (WHO) launched an international mega clinical trial named SOLIDARITY for finding an effective treatment for COVID-19. HCQ is one of the drugs suggested for clinical trials for COVID-19 patients worldwide by a panel of experts.

Hydroxychloroquine, a hydroxylated derivative of chloroquine, is famous around the globe for its antimalarial and anti-inflammatory properties. Both these drugs, hydroxychloroquine and chloroquine, have been in the medical industry since the middle of the 1900s in the prophylaxis of autoimmune diseases or to manage their symptoms. The drug is tagged as the most effective and safest medicine required in a health care system. Notably, several studies are being carried out to check their efficiency in fields like cancer therapy and antiviral therapies also, since they have proven efficiency in treating a wide range of diseases. Their unique pharmacokinetics profile intrigued the experts to repurpose this drug for treating the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

World leaders have also advocated the use of hydroxychloroquine, and it is considered as a game-changer in the fight against COVID-19 infection. Hydroxychloroquine is designated as an off-label drug by the US Food and Drug Administration for compassionate use along with WHO announcing to conduct trials to test the drug for various potential treatments. Doctors in China, the epicentre of the dreadful disease reported that the drug helped to speed the recovery of patients who were mildly infected with the virus. Although until now there exists only very little evidence to support that hydroxychloroquine work against coronavirus infection, these reports have ignited many preliminary studies. Chloroquine and hydroxychloroquine, both 4-aminoquinolines, are found to be active against a span of viruses *in vitro*. The exact mechanism of action is not known, but some studies show that it inhibits the enzymes required for viral replication [1]. In the case of (SARS-CoV-2), the 4-aminoquinolines may be responsible for the inhibition of glycosylation of angiotensin-converting enzyme-2, the receptor that SARS-CoV-2 uses to enter cells [2]. This review attempts to highlight the structural characteristics of HCQ, their mode of action, and finally, comprehend the recent reports that have used HCQ as a drug candidate against SARS-CoV-2 virus.

Origin of hydroxychloroquine

The use of HCQ has started in the early 17th century, where the people of Peru used the bark of cinchona trees for treating fever and other malarial symptoms. The Jesuit priest missionaries transported the cinchona bark to Europe for treating deadly malaria that shook Europe that period. The extraction of pure quinine from the cinchona bark and the use of that in the treatment of malaria paved the way for a golden era in the discovery of new antimalarial compounds. With the advent of Organic Chemistry, the scenario slowly changed from extraction of quinine to the synthesis of quinine related compounds. Bayer pharmaceutical company in Germany was the first to synthesize quinine-associated compounds. They introduced Chloroquine, but later it was prevented in human trials due to toxicity issues. The need for a less toxic variety of chloroquine led to the discovery of Hydroxychloroquine (HCQ) by slightly modifying the chemical structure of Chloroquine. In 1950, chemists Alexander R. Surrey and Henry F. Hammer reported the first synthesis hydroxychloroquine by introducing a hydroxyl group into chloroquine [3].

The parent company, Sterling Drug, obtained a US patent of the compound in the same year. HCQ showed the same mode of action against the malarial parasite like its ancestor molecule chloroquine by inhibiting polymerization of heme. The intense research on the mode of action of this in the prevention of malaria led to the use of HCQ as an antirheumatic drug. Nowadays, HCQ is widely used for the treatment of various rheumatological conditions, including arthritis and systemic lupus erythematosus. In the virtue of their autophagy properties, HCQ is even used in the treatment of cancer. Since HCQ is found to be an excellent drug against various autoimmune diseases,

the scientific community believes that it can also be useful in the treatment of the new viral pandemic COVID-19 leading to the repurposing of HCQ. The milestones in the history of hydroxychloroquine are shown as a timeline in figure 1.

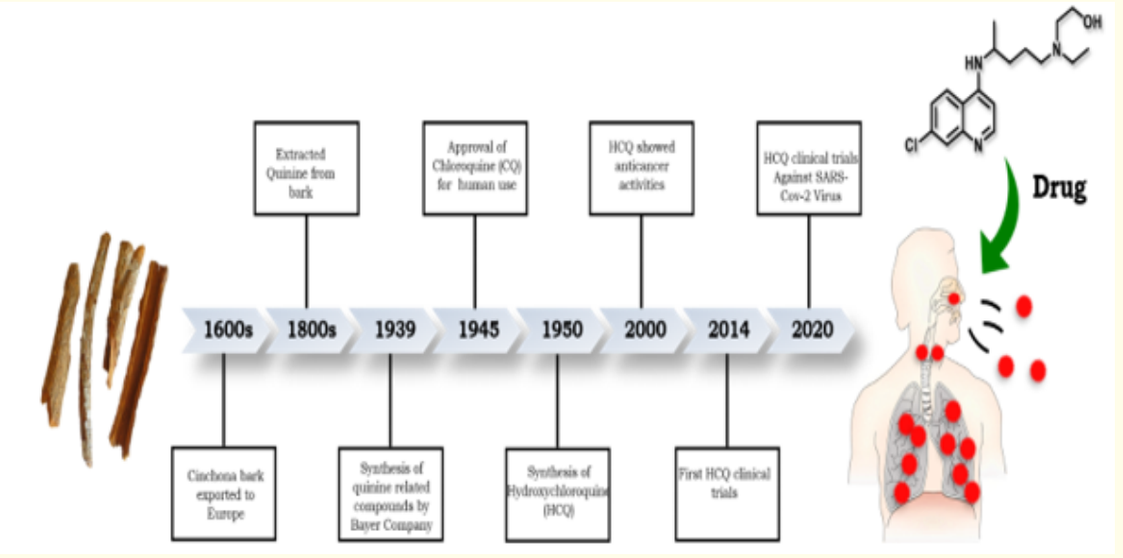


Figure 1: *Schematic showing the milestones in the history of hydroxychloroquine (HCQ).*

Chemical composition

Hydroxychloroquine (HCQ) sulfate is a more polar, less lipophilic quinoline antimalarial drug (Figure 2) which belongs to the 4-Amioquinoline family that can diffuse across cell membranes as discussed earlier [4]. Moreover, HCQ is often used as a slow-acting antirheumatic drug in the treatment of disorders of connective tissue. It is chemically 2-[[4-[(7-Chloro-4-quinolyl) amino] pentyl] ethylamino] ethanol sulfate with two fused aromatic rings having conjugated double bonds containing a chiral centre, as shown in figure 2. In 2008, Semeniuk and coworkers studied the structural properties and intermolecular interactions of monocrystal HCQs using crystallography and X-ray diffraction techniques [5]. The studies revealed that the nitrogen atoms of the free base are forming intermolecular hydrogen bonds with oxygen atoms of the sulfate anion, where the nitrogen is acting as a proton donor. Another aspect of the study explained the addition of one more hydrogen bond-forming hydroxyl group (-OH group) increased the interaction of the drug with the transmembrane receptor of the pathogen by changing the conformation of the molecule significantly.

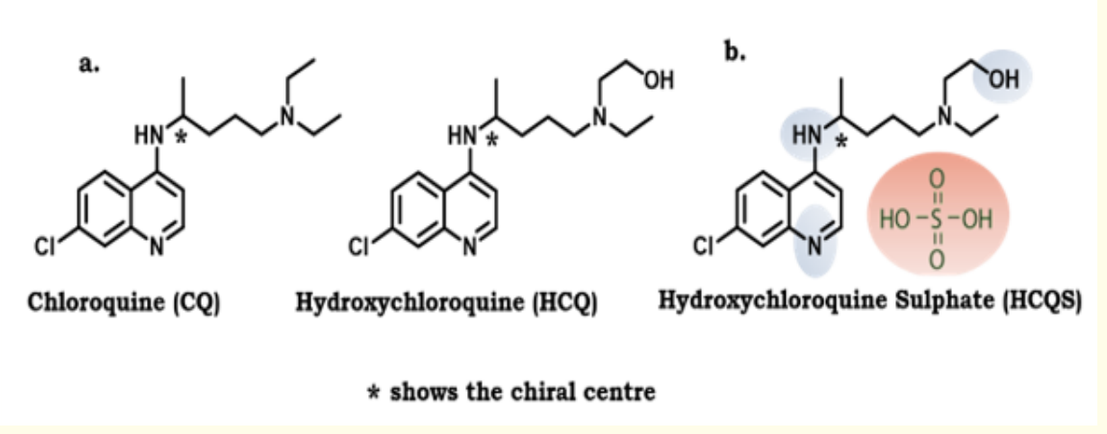


Figure 2: *a. Showing the chemical structure of Chloroquine (CQ) and Hydroxychloroquine (HCQ). b. Chemical Structure of Hydroxychloroquine sulphate (HCQS) showing hydrogen bonding sites.*

The HCQ drug with different brand names available in the global market contains dibasic calcium phosphate, hypromellose, magnesium stearate, polyethylene glycol, polysorbate, corn starch, titanium dioxide, carnauba wax, shellac and black iron oxide as inactive ingredients other than the Active pharmaceutical Ingredient (API). The drug in racemic form is marketed as a sulphate salt for minimizing the solubility issues. According to the reports, the HCQ sulphate has a solubility of 0.0261 mg/mL in water [6]. The administration of HCQ sulphates to patients requires more attention due to the commercialization of HCQ in its racemic form, i.e. equimolar mixture of S- (+)-HCQ and R- (-)-HCQ enantiomers. Racemic mixtures can be dangerous or can lead to toxicity based on the less active isomer. However, there are no conclusive reports regarding the stereoselective properties as well as the pharmacological impact of the R and S isomers of HCQs [7]. One report demonstrated the high concentration of R- (-)-HCQ enantiomers in blood and plasma over S- (+)-HCQ enantiomer [8]. The authors postulated that the cellular blood components preferably utilize R- (-)-HCQ enantiomers over the other, keeping it available for metabolic activities. Despite a few studies, there is no evident information regarding the toxicity of both the isomers as well as the stability of HCQs.

Effect on Covid-19

HCQ has a broad-spectrum activity against viruses. HCQ increases the endosomal pH, inhibits the quinone reductase 2, and interferes with the glycosylation of angiotensin-converting enzyme 2 (ACE2). The anti-inflammatory and anti-viral properties of hydroxychloroquine offer it as a suitable candidate for Covid-19. The experimental studies suggest that the hydroxychloroquine interferes with the glycosylation of angiotensin-converting enzyme 2 (ACE2), the cellular receptor of SARS-CoV, which is expressed in lungs, kidney, heart, and intestine. Since the novel coronavirus, SARS-CoV-2, also utilizes a similar surface receptor, the scientific community believes in the use of HCQ for the treatment of COVID-19. HCQ may interfere with the glycosylation of ACE 2 receptors, thereby preventing the attachment of SARS-CoV 2 to the target cells. The in vitro studies of chloroquine against SARS-Cov 2 using the Vero E6 cell have shown its ability to reduce viral replication. Clinical data demonstrated a high concentration of cytokines in the plasma of COVID-19 positive patients. By virtue of the successful anti-inflammatory activity, HCQs may help in the decline of cytokine production and thereby reduce the effect of the inflammatory response [9].

The treatment of COVID 19 by using hydroxychloroquine has demonstrated a reduction in the viral load. Additionally, the combination of hydroxychloroquine with other drugs such as azithromycin also found to decrease the viral load [10]. The binding of SARS-CoV-2 by ACE2 receptor cause in the uncontrolled hyperglycemia induced by the glycosylation of ACE2 as well as the viral spike protein [11]. The hydroxychloroquine considered a hypoglycemic agent [12]. HCQ intake can offer a more significant reduction in haemoglobin A1c compared to methotrexate for rheumatologic patients with diabetics [13], which implies the use of HCQ in the treatment of COVID since hyperglycemia is a possible risk factor of COVID-19.

Mode of action

One recent report by Fantini., *et al.* suggested a new mechanism of action of HCQ against the novel coronavirus by identifying a new primary attachment factor of the virus [14]. The inhibition of MERS-CoV entry into human airways by the depletion of cell surface sialic acids by neuraminidase treatment showcased the importance of further studies on sialic acids as molecular targets of HCQs [15]. Besides using their protein membrane receptor called angiotensin-converting enzyme-2 (ACE-2) to bind to the cell membrane for cell entry, these novel viruses also utilize sialic-acid-containing glycoproteins and gangliosides binding domain for primary attachment along the respiratory tract for cell entry [16]. They identified a new ganglioside-binding site in the N-terminal domain of the spike glycoprotein of SARS-CoV-2 virus. The authors investigated the interaction between HCQ and sialic acids with the aid of molecular modelling approaches. The results explained the potential of HCQs to bind to the sialic-acid-containing gangliosides of the cell membrane and thereby inhibiting spike glycoprotein ganglioside interaction (Figure 3). The promising results of this study suggest the use of HCQs as prime drug candidates for treating patients with SARS-CoV-2 virus.

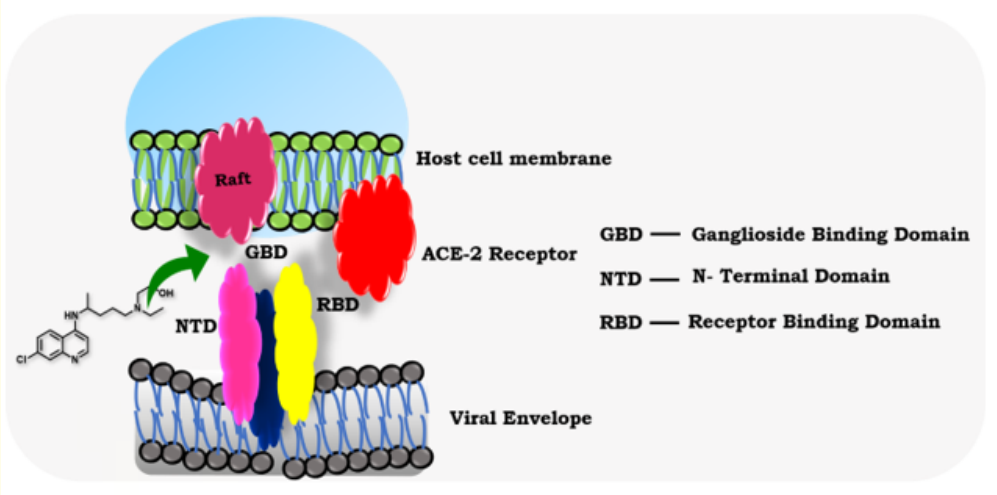


Figure 3: *Schematic showing the mode of inhibition of HCQs by binding to the GBD.*

Chloroquine is involved in the alkalisation of endosomes leading to the prevention of endocytosis along with the rapid increase in endosomal pH. In the case of SARS-CoV-1, pH-dependent entry is prevented after the binding of the DC-SIGN receptor [17]. The fusion of endosomal membrane and virus leads to the release of viral nucleic acid to the cytosol, which is favoured by the low pH of the endosome (Figure 4). Thus, the chloroquine can inhibit and prevent the pH-based entry and replication of viruses. The elevation in the pH also inhibits the cathepsins, which is required for the formation of autophagosomes to cleave the viral spike proteins. Additionally, the chloroquine interferes with the biosynthesis of sialic acid, a receptor moiety of SARS-CoV-2, through constraining the essential agent quinone reductase. Furthermore, chloroquine can inhibit the MAP-kinase pathway (Figure 4) and alter the viral assembly through the virus molecular crosstalk.

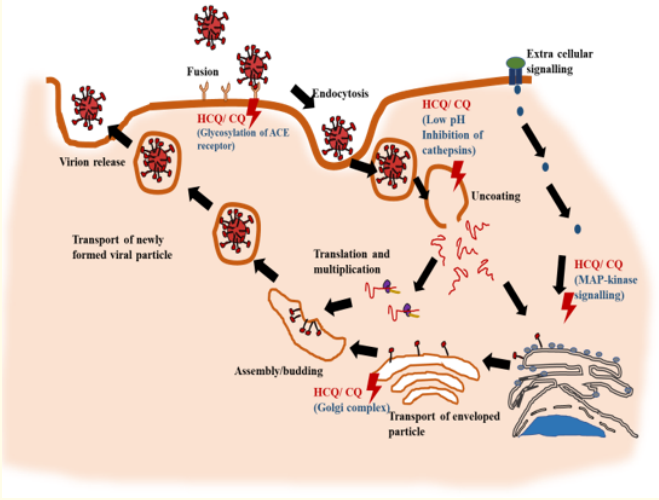


Figure 4: *Schematic representation of the possible mode of action of hydroxychloroquine/chloroquine on the SARS-CoV-2 replication cycle.*

The localization of membrane M protein of non-human coronaviruses determines the site of intracellular budding of viruses and which accumulates in the Golgi complex [18]. Since chloroquine proved to affect the proteolytic processing of the flavivirus PrM protein to M protein, thereby produce impaired viral infectivity [19], suggesting a similar scenario in the case of SARS-CoV-2 at this stage of replication [20].

Clinical trials

In early March 2020, a pilot clinical trial was conducted by Chen and co-workers in 30 COVID-19 positive patients to evaluate the efficacy and safety of hydroxychloroquine [21]. Out of 30, 15 patients were administered with 400 mg HCQ per day, and the rest were given conventional treatments for five days. The study revealed that the HCQ group showed 86.7% (13 cases) recovery, whereas 93.3% (14 cases) recovered in the control group. In other words, there was no significant difference in the viral load with and without hydroxychloroquine. The data from the study was inappropriate for concluding due to the smaller sample size.

Following this, another group evaluated the effect of HCQ through a randomized clinical trial in 62 COVID positive patients in Wuhan consisting of 29 males and 32 females [22]. They administered 200 mg of HCQ every 12h to 31 patients for five days. The results showed the recovery of around 80% (25/31) patients from pneumonia in the HCQ treated group compared to the 55% (17/31) of the control group. Despite a few adverse cases (6/31), the study demonstrated the effect of HCQ in shortening the time to clinical recovery and the reduction of pneumonia. In contrast with *in vitro* tests, a recent time-to-event analysis of HCQ administration in patients with COVID-19 along with a primary endpoint of intubation or death conducted by Joshua Geleris and co-workers observed that there is no significant relationship between the HCQ administration and the endpoint (intubation or death) [23].

Combination with azithromycin

Due to less drug-drug interactions along with a higher daily dose of HCQ, other drugs can also be administered for the reduction of virus load. Azithromycin has been efficiently used as a combination drug with HCQ for the treatment of the SARS-CoV-2 virus. Raoult's research group from France demonstrated an open-label non-randomized clinical trial of 42 COVID-19 infected patients with a combination of HCQ and Azithromycin [24]. They grouped the patients into three categories of symptoms: asymptomatic, upper respiratory tract infection (URTI) symptoms and lower respiratory tract infection (LRTI) symptoms and administered them with 200 mg of HCQ every 8h with 500 mg of azithromycin on Day 1 followed by 250 mg on Day 2 - 5. When compared to the controls, 20 cases showed a significant reduction of the viral load at D6-post inclusion. Moreover, the viral carrying duration was lower than the previous reports.

The same group conducted one more non-comparative study of 80 mildly infected patients with HCQ and Azithromycin treatment for three days [10]. They observed improved health conditions in all cases except the death of one patient aged above 86. The combination of azithromycin with HCQ is critical in the early reduction of the disease. Contrasting to this, Molina and co-workers reported that there is no crucial evidence in terms of antiviral activity for the combination of HCQ with azithromycin in severe COVID-19 infected patients [25]. After giving the combo to 11 patients in the same dose as previously reported, resulted in the death of one patient and no reduction of viral load even after six days of treatment. It is challenging to conclude from these studies regarding the pros and cons of the treatment of hydroxychloroquine as a potential COVID-19 drug. The *in-vitro* study done by Julien Andreani and co-workers also pointed out the synergistic effect of hydroxychloroquine and azithromycin on SARS-CoV-2. The optimal combination of 5 μM of hydroxychloroquine with 10 μM and 5 μM of azithromycin demonstrated a relative viral inhibition of 97.5% and 99.1%, respectively [26].

Anti-viral properties

By interacting with the endosome mediated cell entry or the late stage of replication of the enveloped virus, the chloroquine/hydroxychloroquine can inhibit the viral replication. The chloroquine/hydroxychloroquine found to inhibit the pH-dependent entry of viruses

and it increases the lysosomal and trans-Golgi network (TGN) vesicle pH results in the disruption of many enzymes which may include the acid hydrolase, which is required for the post-translational modification of newly prepared proteins. Chloroquine influenced rise of endosomal pH can result in the decreased concentration of intracellular iron through the impaired release of iron from ferrated transferrin. The reduction of iron concentration can affect the DNA replication and expression of many genes since iron acts as the cofactor of many enzymes [27,28].

Most of the endocytosis mediated viral entries targeting the virus to the lysosome, where the low pH and variety of enzymes breaks the particle and releases the infectious nucleic acid. Whereas, the chloroquine/hydroxychloroquine increases the lysosomal pH. The hepatitis A virus, chloroquine, exhibit the inhibition of the uncoating of the virus particle and thereby impairing the replication cycle [29]. The chloroquine/hydroxychloroquine can also inhibit the viral replication by producing non-infectious retroviral particles through the inhibition of glycosylation of envelope glycoproteins. The increase in the pH of TGN leads to the impaired function of glycosyltransferase, which is required for the post-translational modification of HIV glycoproteins [30,31]. In the case of SARS, the endosomal transport is necessary for the HCoV-229E infection [32]. Surprisingly, cells treated with chloroquine showed reduced quantities of HCoV-229E antigens.

Anti-malarial properties

Chloroquine and hydroxychloroquine widely used for the treatment of malaria. It can affect the functions of plasmodia as well as the lysosome in humans. HCQ interferes with the proteolytic ability of plasmodia and inhibit the haemoglobin lysis, which is necessary for their energy requirement. Thus, HCQ prevents the normal growth and multiplication of parasites. HCQ is also causing the accumulation of toxic beta-hematin by interfering with the parasitic heme polymerase [33]. The antiparasitic properties of chloroquine can describe by the increase in the pH of intravesical. The unprotonated chloroquine diffused across the cell membrane and protonated inside the acidic environment of the vesicle, which prevents the diffusion of a more polar molecule from the vesicle. Interestingly, due to the difference in pH between the parasite vesicle and extracellular medium, parasitic vesicle contains more than 1000 fold chloroquine [34].

Anticancer properties

Many research groups have studied the potential use of CQ/HCQ in anticancer therapy. Surprisingly, the anti-malarial drug CQ/HCQ can act as an anticancer agent in different ways such as by delaying [35] and decreasing the tumour growth [36], increasing tumour cell apoptosis [37], through inhibiting the autophagy, TLR9/nuclear factor-kappa B signalling pathway and CXCL12/CXCR4 signalling pathway along with interfering with the p53 pathway.

Autophagy can suppress the tumour at the early stage of carcinogenesis by eradicating the defective cell components. However, in the more advanced stage of cancer or the late carcinogenesis, autophagy can enhance tumour growth [38,39]. The HCQ/CQ uptake of the cell can elevate the lysosomal pH by the accumulation of protonated form drug in the lysosome. The increased pH in the lysosome leads to the inhibition of degradative enzymes present in lysosomes which further inhibit the autophagy process [40]. Toll-like receptor 9 (TLR9), situated in the endosomal compartment, has found to over express in many different cancerous cells compared to the neighbouring normal cells [41]. The activation of the NF-κB signalling pathway mediated by TLR9 can lead to tumour progression and migration of cancer. It was believed that the upraised level of pH in endosome could inhibit TLR9 activity. However, the study conducted by Alenka Kuznik and co-workers noted that CQ intercalates and alter the structure of nucleic acid thereby modifying the TLR-binding epitope and preventing it from binding to the TLR9 receptor [42]. The CXCL12/CXCR4 signalling plays a vital role in the chemotaxis and adhesion of cells [43]. Independent studies conducted by Kim [44] and Baliac [45] proved that the CQ/HCQ reduced the proliferation of pancreatic cancer cells through the internalisation of CXCR4, decreased phosphorylation of signal transducer and activator of transcription 3 (STAT 3) and extracellular signal-regulated kinase (ERK). CQ can prevent the tumour development through the stabilisation of the tumour suppressor gene, p53 and subsequent activation of p53-dependent transcription of pro-apoptotic genes [37]. CQ also has found to inhibit the tumour growth and metastasis through paracrine apoptosis induced by Par-4 secretion, a tumour suppressor protein [46].

The promising results of these preclinical studies of HCQ as efficient autophagy inhibitor ended up in the clinical trials of HCQ/CQ in cancer patients to appraise its therapeutic activity. Wolpin and co-workers evaluated the therapeutic effect of HCQ in patients diagnosed with pancreatic cancer (Metastatic Pancreatic Adenocarcinoma). Out of 20 patients, 10 were administered with 400mg HCQ whereas another half with 600mg HCQ twice a day [47]. Unfortunately, the results of this seminal work showcased the less competency of the administration of HCQ alone in the anticancer treatment.

Other applications

A vital step in the autoimmune response is antigen processing and the antigen-MHC complex presentation to CD4+ cells by macrophages. The antimalarial drug can alter the auto antigenic protein presentation by reducing the formation of peptide-class-II MHC complexes. Digestion of antigenic protein and further assembling of peptides with the α and β chains of class-II MHC require acidic cytoplasmic compartments. HCQ reduces the antigenic protein digestion and thereby peptide-MHC complex, which is essential to stimulate CD4+ T cell formation by increasing the lysosome pH. Hence, down regulating the immune response towards the auto antigenic peptides along with the reduced release of cytokines [48].

There are other methods in which the chloroquine interferes with autoimmune response. Either by directly stabilizing and reducing the dissociation of the α and β non polymorphic invariant complexes or through the impaired digestion pattern of antigenic peptides, which reduces the degradation of non polymorphic invariant, the antimalarial drug can reduce the autoimmune response. The elevation of pH influences the formation of MHC complexes containing the auto antigens. Specifically, the increase in the pH will stabilize the non polymorphic invariant complexes, and they would not displace by low-affinity self-antigens. The treatment with HCQ/CQ also showed a reduced amount of fibronectin and fibrinogen in *in-vivo* experiments, which can be linked to the decreased cytokine production. HCQ also inhibits phospholipids A2 [49], TNF-α [50,51], toll-like receptor signals [52] and T and B cells calcium signaling [53]. Most importantly, reducing interleukin-1 and interleukin-6 production by macrophage [54].

Toxicity profile

The mode of action of hydroxychloroquine and chloroquine is almost similar [55]. Hydroxychloroquine and chloroquine have been widely used for the treatment of malaria, rheumatoid arthritis, and lupus, and it is comparatively safe for consumption. Due to the presence of the hydroxyl group in the hydroxychloroquine, it is less permeable in the blood-retinal barrier, and it will get cleared from retinal pigment cells. Hence, hydroxychloroquine offers low risk than chloroquine.

The *in-vitro* studies of chloroquine with Vero E6 cells upon novel corona virus (2019-n CoV) infection (multiplicity of infection (MOI) of 0.05) demonstrated the half-cytotoxic concentration (CC_{50}) is more than 100 μM with a half-maximal effective concentration (EC_{50}) of 1.13 μM and 90% of maximal effective concentration (EC_{90}) is 6.90 μM along with a selectivity index (SI) higher than 88.50 [9]. In a different study, Yao *et al.,* used viral infected (MOI = 0.01) Vero cells to study the anti-viral activity of both HCQ and chloroquine and found that the EC_{50} values at 24h for chloroquine was 23.90 μM while the HCQ was 6.14 μM. Furthermore, the EC_{50} at 48h was 5.47 μM and 0.72 μM for chloroquine and HCQ, respectively [55]. The margin between the safer therapeutic, adverse and lethal doses of chloroquine is very close compare to hydroxychloroquine. Compared to Chloroquine, HCQ is less toxic [56]. Physiochemical properties including the plasma half-life and the solubility of chloroquine and HCQ is different. Hydroxychloroquine (1300h) is having more plasma half-life than chloroquine (900h) [57]. Various studies using HCQ against COVID-19 has resulted in prolonged QTc [58-60]. The prolonged exposure of HCQ for several months has found to cause lethal heart failure [61].

Overdose and Side effects

Overdose of hydroxychloroquine and chloroquine can be severe and fatal and it may cause arrhythmia [62]. Nausea, hypokalaemia, shock, drowsiness, and even death can happen after the intake of an overdose of the drug, and the side effects can observe between 1 to

3 hours after the consumption of the drug. Retinal and psychiatric symptoms have also found when the chloroquine dose higher than the required for malarial treatment [63]. The macular retinopathy can occur due to the cumulative dose rather than the daily dose, and it can prevent by the regular ophthalmologic evaluation along with the treatment. Cardiac and respiratory arrest can also occur with an overdose of chloroquine [64]. The continuous use of hydroxychloroquine may result in corneal vortex keratopathy, which is mostly dose-dependent [65]. This occurring due to the accumulation of lipid bearing intra-lysosomal inclusion bodies in the corneal epithelial basal layers [66]. The chloroquine can also result in neuromyotoxicity [67], ototoxicity [68] and hyperpigmentation [69]. The potential adverse effect due to the consumption of chloroquine/hydroxychloroquine is outlined in figure 5.

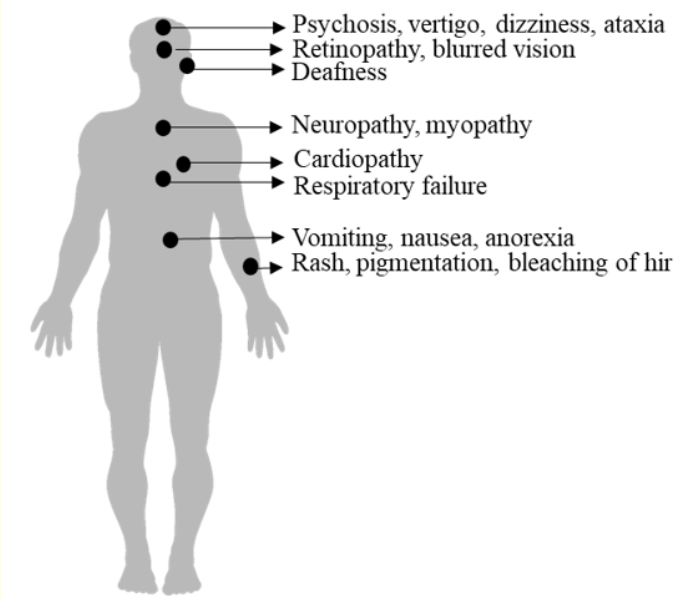


Figure 5: *Adverse effect of chloroquine/hydroxychloroquine consumption.*

Pharmacokinetics

Yao., *et al.* have developed the physiologically-based pharmacokinetic model (PBPK) of HCQ by combining the drug deposition and physiological data to forecast the drug concentration in human tissue *in silico* methods [55]. The mathematical modelling and simulation are necessary for predicting the drug dosage. They simulated the drug in lungs, blood and plasma under different dosing regime via a permeability rate limiting model with a high lung to plasma partition coefficient (Kp) ratio. The oral administration of chloroquine can cause the distribution of the drug in the whole body, including the lungs. The simulation data predicted an optimal dosage of hydroxychloroquine sulfate of 400 mg twice a day, followed by 200mg twice a day for four days as a maintenance dose [55].

The population pharmacokinetics (PopPK) is also used to predict the dosing regime of hydroxychloroquine. Mahmoud Al-Kofahi and colleagues suggest a dosing regime of 800 mg loading followed by 400 mg twice or thrice a week under pre-exposure prophylaxis settings required for maintaining EC_{50} > 50%. Whereas, in exposure driven post-exposure settings, 800 mg loading and 600 mg after six hours followed by 600mg for four days to keep the EC_{50} > 50% [70].

The pharmacokinetics studies of HCQ in malaria patients and healthy individuals (n = 91) conducted by Lim., *et al.* have analysed the plasma concentration through non compartmental, mixed effect and two-compartment model [71]. The experiment has demonstrated the volume distribution of clearance, central and peripheral was 15.5 L/h, 733 L/h and 1630 L/h respectively.

Conclusion

Hydroxychloroquine (HCQ) is an aminoquinoline molecule which is having a wide spectrum of therapeutic application. HCQ can be used for antimalarial treatment, anticancer treatment, antiviral treatment and autoimmune disease treatments. Owing to its unique properties, WHO urged the use of HCQ and its combination with other drugs against COVID-19. Many *invitro* studies demonstrated its ability to reduce the viral load. HCQ/CQ is a broad-spectrum antiviral agent which can interfere with the fusion of viral particle to the receptors, the viral assembly, MAP kinase pathway and the proteolysis of viral peptides. The studies also proved that the combination of HCQ with other drugs like azithromycin is more efficient than monotherapy by HCQ. The exposure of HCQ can also result in the cardiovascular complications. Although HCQ has shown promising results in the *in vitro* studies against COVID-19, the large observational studies and clinical trials have resulted in a lack of efficacy.

Conflicts of Interest

The authors declare that they have no conflict of interests.

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Data Availability

The authors declared that the research data referred correctly cited in the manuscript's reference section.

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