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Hydroxychloroquine in SARS−CoV−2 infection: Understanding the misadventure

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ABSTRACT

The widespread outbreak of SARS-CoV-2 was declared a public health emergency by the World Health Organization and various governments worldwide. This prompted the implementation of stringent infection control measures to curb the spread of the virus. Amidst this, the medical community faced the challenge of treating the virus without specific therapies or a vaccine, leading to reliance on empirical treatment approaches. In this context, hydroxychloroquine, an antimalarial and antirheumatic drug, gained attention as a potential treatment option. Despite its theoretical benefits, such as inhibiting viral entry, reducing inflammation, and modulating immune responses, empirical studies yielded inconsistent results. Some indicated a potential for symptom relief, while others showed no significant improvement in patient outcomes. The initial enthusiasm waned as the lack of substantial evidence led to revoking its Emergency Use Authorization, and several clinical trials were prematurely halted. The review in question critically examines the factors contributing to the ineffectiveness of hydroxychloroquine in treating SARS-CoV-2 infection, highlighting the complexities of drug repurposing during a rapidly evolving pandemic.

KEYWORDS: SARS-CoV-2; Hydroxychloroquine; Pharmacogenomics; TLR signaling; Cardiotoxicity

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) poses a serious risk to human health. As of the latest data, the pandemic has resulted in 774 631 444 reported cases and has tragically led to several fatalities worldwide[1]. Characterized by its ability to spread swiftly among people, the average time between successive cases (serial interval) was approximately 7.5 days. Additionally, the timeframe from the onset of symptoms to death can be as brief as 14 days, with this duration often being even shorter in elderly individuals. This highlights the rapid progression of the disease, particularly in older populations[2]. Moreover, SARS-CoV-2 commonly leads to acute respiratory distress and pneumonia, and many patients have also reported experiencing gastrointestinal symptoms[2].

Coronaviruses are characterized by their enveloped structure, positive-sense single-strand RNA, and distinctive glycoprotein spikes on their surface. The genetic composition of SARS-CoV-2, spanning 29 881 base pairs, shares similarities with bat-derived SARS-like coronaviruses, specifically bat-SL-CoVZC45 and SL-CoVZXC21. In patients infected with SARS-CoV-2, there is an observed increase in interleukin (IL) levels, particularly IL-1B, as well as heightened levels of interferon-γ (IFN-γ), IFN-γ-inducible protein 10, monocyte chemoattractant protein, granulocyte colony-stimulating factor, macrophage inflammatory protein, and tumor necrosis factor (TNF)-α[2,3].

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In the early phase of the pandemic, the evaluation of evidence-based data resulted in the recognition of aminoquinolines for their efficacy against various viral infections. This led to the exploration of hydroxychloroquine as a potential treatment option for managing SARS-CoV-2.

Hydroxychloroquine (HCQ), belongs to the 4-aminoquinoline class and has a long history of being prescribed for preventing and treating uncomplicated malaria, typically administered with an initial loading dose of 800 mg, followed by doses of 400 mg at 6, 24, and 48 hours[4]. Its ability to inhibit plasmacytoid dendritic cells and reduce the release of proinflammatory cytokines has led to its application in treating rheumatoid arthritis and systemic lupus erythematosus[5]. Moreover, HCQ is effective against Whipple disease[6] and is used in combination with doxycycline to treat Q fever endocarditis[7].

Following early indications of potential effectiveness, the Food and Drug Administration granted an Emergency Use Authorization for the use of HCQ in treating SARS-CoV-2 infection on March 28, 2020[8]. Nevertheless, various research studies have challenged the assertions regarding the effectiveness and safety of HCQ, leading to the suspension, withdrawal, or termination of numerous clinical trials. The World Health Organization, which had started the Solidarity trial to investigate HCQ, halted it promptly due to interim findings indicating no reduction in mortality rates among patients[9]. The Medicines and Healthcare Products Regulatory Agency has concluded clinical trials investigating the use of HCQ in the treatment of SARS-CoV-2[10]. The University of Oxford launched a Randomised Evaluation of COVID-19 Therapy trial focusing on hospitalized patients. This trial was subsequently discontinued when it was determined that HCQ did not effectively reduce mortality rates in these patients[11]. The Oregon Health and Science University had started a randomized, double-blind study to evaluate the effects of HCQ on patients with SARS-CoV-2 but this study was ultimately discontinued[12]. New York University Langone Health conducted a study to investigate the efficacy of HCQ in treating COVID-19 patients, but this study was subsequently suspended[13]. Furthermore, after considering the risk-benefit analysis and recognizing the lack of effectiveness, the Food and Drug Administration has rescinded the Emergency Use Authorization of HCQ and ceased its application in the treatment of SARS-CoV-2 infection. Therefore, this review focuses on the disadvantages of using HCQ and explores potential reasons for its ineffectiveness.

2. HCQ–how did the use begin?

The decision to use HCQ for treating SARS-CoV-2 infection was likely influenced by initial *in vitro* and *in silico* studies. These studies demonstrated that HCQ exhibited antiviral properties against SARS-CoV-2 in Vero E6 cells, observed after incubation periods of 24 and 48 hours. Specifically, in the therapeutic context, HCQ showed a median effective concentration (EC_{50}) of 6.14 μM after a 24-hour incubation and 0.72 μM following a 48-hour incubation. For prophylactic use, the EC_{50} values were 6.25 μM and 5.85 μM at 24 and 48 hours of incubation, respectively[14]. Consequently, the proposed dosage of HCQ for use was an estimation derived from its effectiveness observed in *in vitro* studies. Additionally, the potential of HCQ as an anti-inflammatory and immunomodulatory agent might have been a key factor in considering its application for SARS-CoV-2 infection. Furthermore, the capacity of HCQ to accumulate in the lungs at concentrations up to ten times higher than in the bloodstream also played a role in its proposed use[15] and led to the belief that it could be suitable for respiratory tract infections. Moreover, its long elimination half-life, suggested that it could be administered in fewer doses[16]. Furthermore, the longstanding use of HCQ in treating rheumatological conditions with minimal notable side effects has been well-documented[17]. Its affordability also made it a viable option for patients who bear the cost of treatment themselves. Additionally, the compatibility with antiviral agents and immunoglobulins ensures no adverse impact on its therapeutic effectiveness when used concurrently, further supporting its potential use[18]. These factors likely contributed to the initial consideration of HCQ in the treatment of SARS-CoV-2 infection.

3. Mechanistic role of HCQ

The S glycoprotein on the surface of the SARS-CoV-2 virus interacts with the angiotensin-converting enzyme-2 (ACE2) receptors, which are abundant in human epithelial airway cells. HCQ acts by inhibiting the terminal glycosylation of ACE2, leading to molecular alterations that hinder the virus's ability to attach and fuse with the host cell membrane[19]. HCQ, a lysosomotropic amine[20], interferes with the action of hydrolases contained within lysosomes. HCQ raises the pH, alters lysosomal enzyme activity, inhibits proteases, and affects viral maturation[21]. HCQ is a late-phase inhibitor of autophagy and interferes with the fusion of lysosomes with autophagosomes. Also, HCQ attenuates antigen processing by inhibiting the T-cell receptor-dependent calcium signaling leading to apoptosis of a subset of effector T cells[22]. HCQ restrains T cell activation by repressing the expression of IL-1, IL-6, and TNF-α[23]. Besides, the post-translational modification of viral proteins within the endoplasmic reticulum and the trans-Golgi network organelle are curtailed.

4. Plausible reasons for poor response

4.1. Lack of dose optimization

In various studies, a non-standardized dose of HCQ was

recommended for treating SARS-CoV-2, as there was no dose optimization through clinical trials. The chosen dose was based on the lower half of the maximum effective concentration observed in *in vitro* studies. In March 2020, the Italian National Institute for Infectious Disease advised using 400 mg of HCQ in combination with an antiviral drug for this purpose[24]. In a multicenter, randomized, controlled trial involving hospitalized patients with suspected COVID-19, participants were given 400 mg of HCQ twice daily for 7 days. However, it was found that the risks associated with this treatment outweighed the potential benefits[25]. Following the recommendations of a task force, a study in Belgium implemented a low-dosage regimen of HCQ. The protocol started with administering 400 mg twice on the first day, followed by a maintenance dose of 200 mg twice daily for the next four days, culminating in a total dosage of 2 400 mg over five days[17]. In the HCQ-treated population, the case fatality rate was lower (804/454; 17.7%) relative to the group that did not receive HCQ (957/3 533; 27.1%)[17]. In a retrospective study involving 368 male patients, the group was divided into three categories: 97 patients treated with HCQ, 113 treated with HCQ in combination with azithromycin, and 158 who received no medication. The study found that the mortality rates were notably higher, at 27.8%, in the group treated with HCQ alone[26]. Mahevas *et al*. found no significant beneficial effects in hospitalized COVID-19 patients who were treated with 600 mg of HCQ per day during the first 48 hours, compared to patients who did not receive any HCQ treatment[27]. During a randomized, placebo-controlled trial, participants were given an initial loading dose of 800 mg of HCQ followed by a subsequent dose of 600 mg after 6-8 hours on the first day. This was then continued for four more days with a daily dose of 600 mg[28]. HCQ was also found to be ineffective as a postexposure prophylactic. A contributing factor to these poor outcomes could be the recommended dosage, which may result in either a sub-therapeutic concentration, insufficient for effective treatment, or a higher concentration that could lead to toxicity.

4.2. Pharmacodynamic factors

4.2.1. Impact on ACE2

The ACE2 is key in converting angiotensin Ⅱ into angiotensin (1-7), a process that reduces the risks associated with vasoconstriction and water retention, and offers cardioprotective benefits. ACE2 receptors, which are highly present in human epithelial airway cells, act as the essential mechanistic site of attachment for HCQ. This enzyme is a critical target for HCQ, as it disrupts ACE2 glycosylation, thereby hindering the attachment of the virus. In patients with COVID-19, there is an observed correlation between heightened angiotensin Ⅱ activity and an increase in viral load[29]. Elevated levels of angiotensin Ⅱ indicate a reduction in the production of angiotensin (1-7), which is believed to result from the blockade of ACE2 receptors[30]. Furthermore, angiotensin Ⅱ is known to facilitate the accumulation of neutrophils and contribute to the occurrence of microvascular thrombosis[31,32]. Hence, ACE2 receptors represent a significant focal point for HCQ, preventing viral entry and potentially reducing their numbers, which could contribute to a weakened response. Additionally, variations in the *ACE2* gene, known as single nucleotide polymorphisms, may also play a role in diminishing the effectiveness of HCQ[33].

4.2.2. Effects on TLR signaling

HCQ exhibits a reduced response toward specific Toll-like receptors (TLRs), particularly TLRs 7 and 8. These receptors bind to single-stranded viral RNA that has been endocytosed, initiating the MyD88 signaling pathway. This, in turn, triggers signaling pathways involving MAPK and NF-κB. The activation of NF-κB is believed to play a role in regulating autophagy through the expression of genes that control this cellular process[34]. Additionally, the activation of IRF-5 and IRF-7 occurs through IL-1 receptor-associated kinases 1/2/4 and TNF receptor-associated factor-3/6[35], resulting in the production of type Ⅰ IFN. Interferons exert a protective role in viral infections and contain their replication[36].

Consequently, HCQ may not significantly interfere with the generation of IFN-α, which is induced through the stimulation of TLR7 or TLR7-activated plasmacytoid dendritic cells (pDCs). While TLR7 is constitutively expressed in pDCs and B cells, its expression in macrophages can be induced in the presence of antigenic components. TLR7 activation is mediated by proteases like furin-like proprotein convertases, which do not require the acidification of endosomes and function at a neutral pH[37].

As a result, HCQ may have a limited impact on TLR7 signaling, potentially allowing for the release of type Ⅰ IFN and heightened immune activation. Furthermore, TLR7 activation can trigger the MyD88 signaling pathway, promoting the production of proinflammatory cytokines. Interestingly, proinflammatory cytokines can enhance the expression of furin-like proprotein convertases in immune cells, further supporting TLR7 processing. Consequently, HCQ may not be effective in suppressing TLR7-mediated IFN-α production by pDCs[37].

4.2.3. Vacuolar type H^+ ATPases (V type H^+ ATPases)

Vacuolar ATPase (V-ATPase) plays a crucial role in facilitating the ATP-mediated transport of molecules across intracellular membranes. It consists of V1 and V0 domains, with the V-ATPase G subunit 1 belonging to the V1 subunit, responsible for ATP hydrolysis. These complexes are central in acidifying organelles and maintaining autophagy flux. Organelles like lysosomes have macromolecular protein binding sites on their membranes and contain hydrolases such as glucosidases, phosphatases, lipases, and nucleases. These hydrolases require an optimal pH of around five to exhibit catalytic activity, and this acidic environment is maintained

within lysosomes by proton pumps like V-ATPases, which transport H^+ ions from the cytosol into the lysosome interior.

During the process of lysosomal ATPases pumping H^+ ions, unprotonated HCQ from the extracellular environment is transported along with H^+ ions, leading to its protonation and accumulation within the lysosome. Consequently, it is plausible to hypothesize that an inadequate clinical response to HCQ could be linked to dysregulation in V-type H^+ ATPases, potentially resulting in drug resistance due to alterations in the pH gradient between the intracellular and extracellular environments[38]. Furthermore, it is worth noting that SARS-CoV-3 CLpro has been reported to cleave V-ATPase G1, potentially enhancing viral pathogenicity[39]. This suggests that the administered dose of HCQ might not be sufficient to counteract the increased virulence of the virus.

4.2.4. Effect on trained immunity

In the context of the immune system, the adaptive immune system cells develop a memory of pathogens upon exposure, and it is now understood that innate immune system cells undergo a process akin to training or education during their initial encounter with antigens. This training involves the reprogramming of monocytes and macrophages through various mechanisms, including changes in mitochondrial function, metabolic processes, and epigenetic modifications. These changes ultimately lead to the activation of specific gene transcription, which is essential for the body's host defense mechanisms against future infections[40]. The reprogramming of bone marrow myeloid progenitor cells leads to the generation of monocytes that are readily equipped to combat the invading pathogen[41]. As a result of epigenetic reprogramming, the chromatin structure undergoes conformational changes that modify the accessibility of DNA to transcription factors. Yao *et al*. have documented the presence of a trained immunity phenotype in alveolar macrophages[42]. When trained cells encounter a secondary stimulus, they demonstrate an enhanced immune response, which can effectively combat the viral load and reduce the likelihood of excessive inflammation. Metabolomic and epigenetic investigations have indicated that HCQ hinders the crucial host-directed mechanism through which innate immune system cells eliminate the pathogen[40].

The impact of HCQ on trained immunity has been substantiated through various experiments. In the context of human peripheral blood mononuclear cells repeatedly stimulated with bacterial antigens, a typical cytokine response was observed. However, when HCQ was administered concurrently, this response was notably inhibited. Furthermore, upon restimulation with IFN-γ, monocytes typically express IL-6 and TNF-α, but this effect was significantly reduced in the presence of HCQ.

Investigation into changes in lysosomal function and their role in trained immunity revealed that the inhibition of vacuolar ATPase (V-ATPase) led to a decrease in trained immunity. Notably, cells exposed to HCQ exhibited reduced transcription in trained monocytes, resulting in a substantial decrease in the expression of interferon-stimulated genes and other inflammatory genes. This modulation of metabolic processes plays a pivotal role in enhancing the effector functions of innate immune system cells[43]. HCQ as part of its suppressive impact on trained immunity, has been found to influence the metabolism of cellular lipids. When monocytes are activated by bacterial antigens in the presence of HCQ, their lipid composition and physiology are significantly altered[44].

4.2.5. Impact on drug transporters

P-glycoprotein (P-gp) is a membrane-bound efflux pump that falls under the category of ATP-binding cassette transporters[45]. P-gp plays a crucial role in expelling intracellularly available drugs, which in turn can modify their pharmacodynamics and pharmacokinetics, ultimately diminishing their effectiveness. Reduced expression of P-gp can lead to a decreased drug efflux, potentially resulting in drug toxicity. Conversely, overexpression of P-gp can make cells resistant to drugs. HCQ inhibits the activity of P-gp[46] and as a result, it can potentially lead to pharmacokinetic drug interactions. This means that patients who are treated with HCQ and are taking medications that are substrates for P-gp, such as digoxin and cyclosporine, may experience an accumulation of these drugs in their system, potentially leading to toxicity.

4.2.6. Genomic changes

The levels of drug-metabolizing enzymes play a significant role in the metabolism of drugs and xenobiotics. Among these enzymes, the cytochrome (CYP) P450 enzymes are responsible for catalyzing the biotransformation of drugs. The expression of these enzymes can be influenced by various factors, including heritable genetic factors, disease states, cytokine levels, age, and sex. CYP P450 enzymes can exist in multiple allelic forms, and these genetic variations can either slow down or enhance the metabolism of drugs, affecting their efficacy and safety[47]. Allelic variants of drug-metabolizing enzymes can have significant implications for drug concentration in the blood. Variants that lack function may lead to higher blood concentrations of the drug, while overactive variants may result in rapid drug clearance, potentially affecting the drug's effectiveness. In the case of HCQ, it undergoes a metabolic transformation to desethylhydroxychloroquine (DHCQ) and desethylchloroquine, with further conversion to bisdesethylchloroquine. The metabolism of HCQ is facilitated by the enzyme CYP2D6. However, it is important to note that individuals with rheumatoid arthritis may exhibit interindividual variations in blood concentrations of HCQ, which can be attributed to these allelic variations in drug-metabolizing enzymes[16]. Single nucleotide polymorphisms occurring in genes that encode CYP enzymes have the potential to modify the blood concentrations of HCQ and its metabolite DHCQ. A study conducted on systemic lupus patients in Korea investigated the

relationship between the clinical effects of HCQ and the presence of polymorphisms in the genes encoding CYP2C8 and CYP2D6 enzymes. This research aimed to discern how these genetic variations might influence the response to HCQ treatment in these patients[48]. Functional polymorphisms in CYP enzymes have the potential to disrupt the balance between HCQ and DHCQ concentrations in the body. Specifically, patients with the G/G type of CYP2D610 (rs1065852) polymorphism and the C/C type of CYP2D610 (rs1135840) polymorphism tend to exhibit a high ratio of [DHCQ]/[HCQ], indicating a greater conversion of HCQ to DHCQ. In contrast, individuals with the A/A type of CYP2D610 (rs1065852) and the G/G type of CYP2D610 (rs1135840) polymorphism have the lowest [DHCQ]/[HCQ] ratio, suggesting a reduced conversion of HCQ to DHCQ in these cases. Functional CYP polymorphism can disturb the equilibrium between HCQ and DHCQ concentration[48].

An alternative study proposed that the ratio of DHCQ to HCQ represented as [DHCQ]:[HCQ], could serve as a valuable predictor of a patient's response to HCQ treatment. This ratio has been suggested as a potential indicator of how individuals may react to HCQ therapy[49]. Phase Ⅰ enzymes encoded by the *CYP2D6* gene exhibit over 70 allelic variants, making them highly polymorphic among human subjects, and differ in different populations[50]. These variations in *CYP2D6* gene alleles can significantly impact the metabolism and response to drugs like HCQ. Patients who possess the A/G rs1065852 (CYP2D610) genotype tend to exhibit elevated blood concentrations of DHCQ compared to those with the A/A genotype. This genetic variation in the CYP2D610 genotype can influence the metabolism and levels of DHCQ in the bloodstream[48]. Hence, the variability in treatment response can also be influenced by the specific genotype that a patient carries[48]. The simultaneous use of CYP2D6 substrates like haloperidol and ondansetron in conjunction with HCQ can lead to a notable prolongation of the QT interval. This prolonged QT interval is a cardiac parameter that can have implications on heart rhythm and potentially lead to arrhythmias[51]. Approximately 20% of patients with European ancestry possess the nonfunctional CYP2D6*4 allele, which increases the risk of experiencing toxicity when administered HCQ. This genetic variant can impair the metabolism of HCQ, leading to the accumulation of the drug in the body and an elevated risk of adverse effects[51].

Single nucleotide polymorphisms within the *ACE2* gene, including variants like rs4646156, rs879922, rs4240157, and rs233575, may give rise to allelic variations that are more prone to causing left ventricular hypertrophy[33,52]. Therefore, in individuals with these genetic variations, the utilization of HCQ may potentially trigger a synergistic response, amplifying the risk of cardiotoxicity.

4.2.7. Proinflammatory cytokines impact drug-metabolizing enzymes

During an infection, the levels of proinflammatory cytokines are elevated, and this can have a notable impact on the downregulation of drug metabolism, occurring at either the transcriptional or post-transcriptional level[53,54]. Elevated cytokine levels can potentially have adverse effects on hepatic gene expression and the functionality of enzymes. As an example, IL-6 has been observed to inhibit the activity of CYP3A, a crucial enzyme involved in drug metabolism, particularly in patients with rheumatoid arthritis[55]. Cytokines like IL-2 and TNF-α can hinder CYP3A activity and reduce the expression of CYP enzymes, respectively. These actions can disrupt drug metabolism processes in the body[56]. Changes in drug metabolism can potentially lead to negative clinical outcomes, particularly when drugs have a narrow margin of safety. Additionally, the down-regulation of CYP enzymes can be facilitated by the activation of transcription factors such as NF-κB and mitogen-activated protein kinase. These molecular mechanisms can further impact the processing of drugs within the body[56]. Hence, in individuals with COVID-19, the surge in cytokine production may exert an influence on the metabolism of HCQ, resulting in its accumulation and potentially contributing to its toxicity.

4.3. Toxicities

4.3.1. Cardiotoxicity

Cardiac complications have been documented in individuals receiving HCQ as a treatment for coronavirus infections. Patients infected with SARS-CoV-2 who are administered HCQ may face an elevated risk of corrected QT (QTc) interval prolongation and the potential development of Torsades de pointes, a serious heart rhythm disorder. This response can partly be attributed to the larger volume of distribution observed in some cases. Hooks *et al*. have reported a moderate prolongation of QTc interval, with approximately 1.5% of patients experiencing QTc prolongation exceeding 500 milliseconds[57]. Mercuro *et al*. have also found an association between the use of HCQ and QTc prolongation[58]. Hence, patients who display QT prolongation exceeding 50 milliseconds from their baseline or reaching above 500 milliseconds are prone to develop adverse outcomes with HCQ. Patients who are on multiple medications (polypharmacy) and those with inflammatory medical conditions may be at a heightened risk of developing QTc prolongation[59].

The QT-interval triggering Torsades de pointes is prolonged by HCQ[60]. This safety concern arises from the slowing of outward potassium ion movement and an increase in the influx of sodium or calcium ions, causing a delay in ventricular repolarization immediately after depolarization. Aminoquinolines like HCQ can inhibit the rectifier potassium currents (IKr) required for the repolarization process, potentially causing morphological or structural changes in ion channels and proteins, further contributing to the risk of polymorphic VT which is a significant safety concern with HCQ[61]. Additionally, HCQ influences repolarization by

targeting the *KCNH2* gene, which results in the inhibition of protein expression in the potassium channel[62].

4.3.2. Drug interactions

HCQ has the potential to synergize with other drugs, especially antiarrhythmic class IA drugs, when used concurrently, leading to further QT interval prolongation. This interaction can pose an increased risk of arrhythmias and cardiac complications[63]. Potassium-wasting diuretics[63], antipsychotic drugs[64], antidepressants[64], and macrolides prolong the QT interval[65,66]. Azithromycin, a macrolide, prolongs the QT/QTc interval with mortality risk[67,68]. Macrolides can influence the hERG (human ether-a-go-go-related gene) and the repolarization process, particularly by targeting the His-Purkinje tissue and the M cells in the heart. This effect on cardiac repolarization can be significant and may contribute to the risk of arrhythmias when macrolides are used in combination with other drugs, such as HCQ[69]. Long-term use of proton pump inhibitors can cause hypokalemia or hypomagnesemia[63,70], leading to metabolic abnormalities. The significant decrease in potassium ion levels can be a contributing factor to the development of torsades de pointes, especially when combined with HCQ. Additionally, drugs that inhibit CYP2D6, such as amiodarone, quinidine, cimetidine, diphenhydramine, paroxetine, ritonavir, and terbinafine may interfere with the metabolism of HCQ, leading to its accumulation and potential toxicity.

5. Conclusion

The use of HCQ in the management of SARS-CoV-2 infection was initially empirical, driven by early reports suggesting potential benefits. However, as more extensive population-based studies were conducted, the lack of efficacy and increased risks, including mortality and cardiovascular toxicity, have become apparent. In addition to concerns about cardiotoxicity, therapeutic failure with HCQ may be attributed to various factors, including patient-related factors, genetic mutations, and a suboptimal response to HCQ. Hence, the risk-benefit profile of the medication should be assessed in the context of COVID-19 treatment.

Conflict of interest statement

The authors report no conflict of interest.

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Authors' contributions

DB: Conceptualization, supervision, methodology, original draft preparation and editing. OJFB: Literature review, methodology, original draft preparation and editing.

References

[1] WHO. COVID-19 dashboard. [Online] Available from: https://data.who.int/dashboards/covid19/cases?n=c [Accessed on April 3, 2024].

[2] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J Antimicrob Agents* 2020; **55**(3): 105924.

[3] Wang Z, Chen X, Lu Y, Chen F, Zhang W. Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. *Biosci Trends* 2020; **14**(1): 64-68.

[4] Pastick KA, Okafor EC, Wang F, Lofgren SM, Skipper CP, Nicol MR, et al. Hydroxychloroquine and chloroquine for treatment of SARS-CoV-2 (COVID-19). *Open Forum Infect Dis* 2020; **7**(4): ofaa130.

[5] Morawski PA, Bolland S. Expanding the B cell centric view of systemic lupus erythematosus. *Trends Immunol* 2017; **38**(5): 373-382.

[6] Boulos A, Rolain JM, Raoult D. Antibiotic susceptibility of *Tropheryma whipplei* in MRC5 cells. *Antimicrob Agents Chemother* 2004; **48**(3): 747.

[7] Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. *Int J Antimicrob Agents* 2007; **30**(4): 297-308.

[8] FDA U. Coronavirus (COVID-19) update: FDA revokes emergency use authorization for chloroquine and hydroxychloroquine. [Online] Available from: https://fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-revokes-emergency-use-authorization-chloroquine-and [Accessed on November 15, 2020].

[9] WHO. WHO discontinues hydroxychloroquine and lopinavir/ritonavir treatment arms for COVID-19. [Online] Available from: www.who.int/news/item/04-07-2020-who-discontinues-hydroxychloroquine-and-lopinavir-ritonavir-treatment-arms-for-covid-19 [Accessed on November 15, 2020].

[10] Gov.UK. MHRA suspends recruitment to COVID-19 hydroxychloroquine trials. [Online] Available from: www.gov.uk/government/news/mhra-suspends-recruitment-to-covid-19-hydroxychloroquine-trials [Accessed on November 15, 2020].

[11] National Institute of Health Research N. Case study: The UK's flagship COVID-19 treatment trial - the RECOVERY trial. [Online] Available from: www.nihr.ac.uk/case-studies/the-uks-flagship-covid-19-treatment-trial-the-recovery-trial/25358 [Accessed on November 15, 2020].

[12] ClinicalTrials.gov. Assessing hydroxychloroquine in patients with SARS-CoV-2 (COVID-19). [Online] Available from: www.clinicaltrials.gov/ct2/show/NCT04363866?cond=hydroxychloroquine+for+covid-19&draw=5 [Accessed on November 13, 2020].

[13]ClinicalTrials.gov. Chloroquine/Hydroxychloroquine prevention of coronavirus disease (COVID-19) in the healthcare setting (COPCOV). [Online] Available from: https://clinicaltrials.gov/ct2/show/NCT04303507 [Accessed on November 13, 2020].

[14]Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. *In vitro* antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis* 2020; **71**(15): 732-739.

[15]Braun S, Ferner M, Kronfeld K, Griese M. Hydroxychloroquine in children with interstitial (diffuse parenchymal) lung diseases. *Pediatr Pulmonol* 2015; **50**(4): 410-419.

[16]Tett SE, Cutler DJ, Beck C, Day RO. Concentration-effect relationship of hydroxychloroquine in patients with rheumatoid arthritis--A prospective, dose ranging study. *J Rheumatol* 2000; **27**(7): 1656-1660.

[17]Catteau L, Dauby N, Montourcy M, Bottieau E, Hautekiet J, Goetghebeur E, et al. Low-dose hydroxychloroquine therapy and mortality in hospitalised patients with COVID-19: A nationwide observational study of 8 075 participants. *Int J Antimicrob Agents* 2020; **56**(4): 106144.

[18]Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. *Int J Antimicrob Agents* 2020: 105932.

[19]Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection *in vitro*. *Cell Discov* 2020; **6**: 16.

[20]De Clercq E. Potential antivirals and antiviral strategies against SARS coronavirus infections. *Expert Rev Anti Infect Ther* 2006; **4**(2): 291-302.

[21]Meyerowitz EA, Vannier AG, Friesen MG, Schoenfeld S, Gelfand JA, Callahan MV, et al. Rethinking the role of hydroxychloroquine in the treatment of COVID-19. *FASEB J* 2020; **34**(5): 6027-6037.

[22]Goldman FD, Gilman AL, Hollenback C, Kato RM, Premack BA, Rawlings DJ. Hydroxychloroquine inhibits calcium signals in T cells: A new mechanism to explain its immunomodulatory properties. *Blood* 2000; **95**(11): 3460-3466.

[23]Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet* 2020; **395**(10229): 1054-1062.

[24]Nicastri E, Petrosillo N, Ascoli Bartoli T, Lepore L, Mondi A, Palmieri F, et al. National institute for the infectious diseases "*L. spallanzani*", IRCCS. Recommendations for COVID-19 clinical management. *Infect Dis Rep* 2020; **12**: 8543.

[25]Cavalcanti AB, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A, et al. Hydroxychloroquine with or without azithromycin in mild-to-moderate COVID-19. *New Engl J Med* 2020. PMID: 32706953.

[26]Molina JM, Delaugerre C, Le Goff J, Mela-Lima B, Ponscarme D, Goldwirt L, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. *Med Mal Infect* 2020; **50**(4): 384.

[27]Mahévas M, Tran VT, Roumier M, Chabrol A, Paule R, Guillaud C, et al. Clinical efficacy of hydroxychloroquine in patients with COVID-19 pneumonia who require oxygen: Observational comparative study using routine care data. *BMJ* 2020; **369**: m1844.

[28]Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for COVID-19. *New Engl J Med* 2020; **383**(6): 517-525.

[29]Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020; **63**(3): 364-374.

[30]Shete A, Urgent need for evaluating agonists of angiotensin-(1-7)/Mas receptor axis for treating patients with COVID-19. *Int J Infect Dis* 2020; **96**: 348-351.

[31]Nabah YNA, Mateo T, Estellés R, Mata M, Zagorski J, Sarau H, et al. Angiotensin Ⅱ induces neutrophil accumulation *in vivo* through generation and release of CXC chemokines. *Circulation* 2004; **110**(23): 3581-3586.

[32]Senchenkova EY, Russell J, Almeida-Paula LD, Harding JW, Granger DN. Angiotensin Ⅱ- mediated microvascular thrombosis. *Hypertension* 2010; **56**(6): 1089-1095.

[33]Sieńko JK, Bogacz A, Lechowicz K, Drożdżal S, Rosik J, Sietnicki M, et al. COVID-19: The influence of ACE genotype and ACE-Ⅰ and ARBs on the course of SARS-CoV-2 infection in elderly patients. *Clin Interv Aging* 2020; **15**: 1231-1240.

[34]Copetti T, Bertoli C, Dalla E, Demarchi F, Schneider C. p65/RelA modulates BECN1 transcription and autophagy. *Mol Cell Biol* 2009; **29**(10): 2594-2608.

[35]Trocoli A, Djavaheri-Mergny M. The complex interplay between autophagy and NF-kappaB signaling pathways in cancer cells. *Am J Cancer Res* 2011; **1**(5): 629-649.

[36]Teijaro JR. Type Ⅰ interferons in viral control and immune regulation. *Curr Opin Virol* 2016; **16**: 31-40.

[37]Hipp M, Shepherd D, Gileadi U, Aichinger M, Kessler B, Edelmann M, et al. Processing of human toll-like receptor 7 by furin-like proprotein convertases is required for its accumulation and activity in endosomes. *Immunity* 2013; **39**: 711-721.

[38]Simon S, Roy D, Schindler M. Intracellular pH and the control of multidrug resistance. *Proc Natl Acad Sci U S A* 1994; **91**(3): 1128-1132.

[39]Lin CW, Tsai FJ, Wan L, Lai CC, Lin KH, Hsieh TH, et al. Binding interaction of SARS coronavirus 3CL(pro) protease with vacuolar-H^+ ATPase G1 subunit. *FEBS Lett* 2005; **579**(27): 6089-6094.

[40]Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. *Science* 2016; **352**(6284): aaf1098.

[41]Mitroulis I, Ruppova K, Wang B, Chen LS, Grzybek M, Grinenko T, et al. Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell* 2018; **172**(1-2): 147-161.e12.

[42]Yao Y, Jeyanathan M, Haddadi S, Barra NG, Vaseghi-Shanjani M, Damjanovic D, et al. Induction of autonomous memory alveolar macrophages requires T cell help and is critical to trained immunity. *Cell* 2018; **175**(6): 1634-1650.e17.

[43]O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK

and pseudo-starvation. *Nature* 2013; **493**(7432): 346-355.

[44]Rother N, Yanginlar C, Lindeboom RGH, Bekkering S, van Leent MMT, Buijsers B, et al. Hydroxychloroquine inhibits trained immunity - implications for COVID-19. *medRxiv* 2020: 2020.06.08.20122143.

[45]Borst P, Elferink RO. Mammalian ABC transporters in health and disease. *Annu Rev Biochem* 2002; **71**: 537-592.

[46]Zamora JM, Pearce HL, Beck WT. Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. *Mol Pharmacol* 1988; **33**(4): 454-462.

[47]Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* 2013; **138**(1): 103-141.

[48]Lee JY, Vinayagamoorthy N, Han K, Kwok SK, Ju JH, Park KS, et al. Association of polymorphisms of cytochrome P450 2D6 with blood hydroxychloroquine levels in patients With systemic lupus erythematosus. *Arthritis Rheumatol* 2016; **68**(1): 184-190.

[49]Hersberger M, Marti-Jaun J, Rentsch K, Hanseler E. Rapid detection of the CYP2D6*3, CYP2D6*4, and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. *Clin Chem* 2000; **46**(8 Pt 1): 1072-1077.

[50]Bertilsson L, Dahl ML, Dalén P, Al-Shurbaji A. Molecular genetics of CYP2D6: Clinical relevance with focus on psychotropic drugs. *Br J Clin Pharmacol* 2002; **53**(2): 111-122.

[51]Takahashi T, Luzum JA, Nicol MR, Jacobson PA, Pharmacogenomics of COVID-19 therapies. *NPJ Genomic Med* 2020; **5**(1): 35.

[52]Lieb W, Graf J, Götz A, König IR, Mayer B, Fischer M, et al. Association of angiotensin-converting enzyme 2 (ACE2) gene polymorphisms with parameters of left ventricular hypertrophy in men. Results of the MONICA Augsburg echocardiographic substudy. *J Mol Med (Berl)* 2006; **84**(1): 88-96.

[53]Slaviero KA, Clarke SJ, Rivory LP. Inflammatory response: An unrecognised source of variability in the pharmacokinetics and pharmacodynamics of cancer chemotherapy. *Lancet Oncol* 2003; **4**(4): 224-232.

[54]Jover R, Bort R, Gómez-Lechón MJ, Castell JV. Down-regulation of human CYP3A4 by the inflammatory signal interleukin-6: Molecular mechanism and transcription factors involved. *FASEB J* 2002; **16**(13): 1799-1801.

[55]Zhuang Y, de Vries DE, Xu Z, Marciniak SJ Jr, Chen D, Leon F, et al. Evaluation of disease-mediated therapeutic protein-drug interactions between an anti-interleukin-6 monoclonal antibody (sirukumab) and cytochrome P450 activities in a phase 1 study in patients with rheumatoid arthritis using a cocktail approach. *J Clin Pharmacol* 2015; **55**(12): 1386-1394.

[56]Mallick P, Taneja G, Moorthy B, Ghose R. Regulation of drug-metabolizing enzymes in infectious and inflammatory disease: Implications for biologics-small molecule drug interactions. *Expert Opin Drug Metab Toxicol* 2017; **13**(6): 605-616.

[57]Hooks M, Bart B, Vardeny O, Westanmo A, Adabag S. Effects of hydroxychloroquine treatment on QT interval. *Heart Rhythm* 2020; **17**(11): 1930-1935.

[58]Mercuro NJ, Yen CF, Shim DJ, Maher TR, McCoy CM, Zimetbaum PJ, et al. Risk of QT interval prolongation associated with use of hydroxychloroquine with or without concomitant azithromycin among hospitalized patients testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol* 2020; **5**(9): 1036-1041.

[59]Haugaa KH, Bos JM, Tarrell RF, Morlan BW, Caraballo PJ, Ackerman MJ. Institution-wide QT alert system identifies patients with a high risk of mortality. *Mayo Clin Proc* 2013; **88**(4): 315-325.

[60]Jankelson L, Karam G, Becker ML, Chinitz LA, Tsai MC. QT prolongation, torsades de pointes and sudden death with short courses of chloroquine or hydroxychloroquine as used in COVID-19: A systematic review. *Heart Rhythm* 2020; **17**(9): 1472-1479.

[61]Porta AD, Bornstein K, Coye A, Montrief T, Long B, Parris MA. Acute chloroquine and hydroxychloroquine toxicity: A review for emergency clinicians. *Am J Emerg Med* 2020; **38**(10): 2209-2217.

[62]Smulyan H. The computerized ECG: Friend and foe. *Am J Med* 2019; **132**(2): 153-160.

[63]Schwartz PJ, Woosley RL. Predicting the unpredictable: Drug-induced QT prolongation and Torsades de Pointes. *J Am Coll Cardiol* 2016; **67**(13): 1639-1650.

[64]Glassman AH, Bigger JT Jr. Antipsychotic drugs: prolonged QTc interval, torsade de pointes, and sudden death. *Am J Psychiatry* 2001; **158**(11): 1774-1782.

[65]Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral erythromycin and the risk of sudden death from cardiac causes. *N Engl J Med* 2004; **351**(11): 1089-1096.

[66]Goldstein EJ, Owens RC, Jr, Nolin TD. Antimicrobial-associated QT interval prolongation: Pointes of interest. *Clin Infect Dis* 2006; **43**(12): 1603-1611.

[67]Huang BH, Wu CH, Hsia CP, Yin Chen C. Azithromycin-induced torsade de pointes. *Pacing Clin Electrophysiol* 2007; **30**(12): 1579-1582.

[68]Lu ZK, Yuan J, Li M, Sutton SS, Rao GA, Jacob S, et al. Cardiac risks associated with antibiotics: Azithromycin and levofloxacin. *Expert Opin Drug Saf* 2015; **14**(2): 295-303.

[69]Albert RK, Schuller JL, Network CC. Macrolide antibiotics and the risk of cardiac arrhythmias. *Am J Respir Crit Care Med* 2014; **189**(10): 1173-1180.

[70]Makunts T, Cohen IV, Awdishu L, Abagyan R. Analysis of postmarketing safety data for proton-pump inhibitors reveals increased propensity for renal injury, electrolyte abnormalities, and nephrolithiasis. *Sci Rep* 2019; **9**(1): 2282.

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