European Review for Medical and Pharmacological Sciences 2020; 24: 7164-7172

The antiviral mechanisms, effects, safety and adverse effects of chloroquine

Z.-X. WEI, T.-T. TANG, S.-P. JIANG

[1]Department of Pulmonary and Critical Care Medicine, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China
[2]Institute of Pulmonary Diseases, Sun Yat-sen University, Guangzhou, Guangdong, China

Abstract. – Chloroquine, a 4-aminoquinoline derivative, was initially used to treat malaria. It was later found to have immunomodulating, anti-infective, anti-thrombotic, anti-tumor, and metabolic effects. Recently, many studies have focused on the application of chloroquine in viral infections. Most *in vitro* studies suggested that chloroquine exerted some benefit in infections from viruses. However, animal experiment and clinical trials that attempted to use chloroquine in prevention or treatment of viral infections have reported disappointing results. It might be attributable to inadequate steady-state whole blood chloroquine concentration necessary for exerting its antiviral effects. A 16 μM/L steady-state whole blood concentration of chloroquine should suffice in antiviral treatment with minimal toxicity. Furthermore, chloroquine has both acute and cumulative toxicity. Hence, not only the appropriate treatment dose is crucial, the occurrence of adverse reactions should also be closely monitored and treated in time. Herein, we report the antiviral mechanisms, effects, safety and adverse effects of chloroquine.

Key Words:
Chloroquine, Antiviral mechanisms, Antiviral effects, Safety, Adverse effects, COVID-19.

Introduction

At the end of 2019, a Novel Coronavirus (SARS-CoV-2) broke out in Wuhan of China. This brand-new virus can cause pneumonia and other complications, including acute respiratory distress syndrome, acute kidney injury, cardiac injury, liver dysfunction, hyperglycemia, gastrointestinal hemorrhage, pneumothorax, urinary tract infection, etc[1]. Still no specific vaccine or drugs have been found for its prevention or treatment. In the context of this pandemic, the multicenter collaboration group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province of China announced an expert consensus on chloroquine phosphate for the treatment of Novel Coronavirus pneumonia. The expert consensus recommended that chloroquine should be used in the treatment of Coronavirus disease 2019 (COVID-19)[2].

Chloroquine (CQ) is a veteran drug, which was first synthesized by Hans Andersag through modification of quinine structure, and was originally used for the treatment of malaria[3]. During World War II, malaria-infected soldiers who were treated with CQ had significant improvement of rashes and arthritis. Hence the immunomodulating activity of CQ was found and it was gradually used in autoimmune diseases[4]. However, the exact mechanism for the action of CQ is not fully understood.

Widely accepted mechanisms at present include interfering with DNA functions, inhibiting prostaglandin formation, inhibiting the chemotaxis and phagocytic effects of polymorphonuclear cells, and regulating the release of cytokines[5]. Hydroxychloroquine (HCQ) is an analogue of CQ and has a similar mechanism of action to CQ. With continuous studies on CQ and its analogue HCQ, more therapeutic properties have been discovered, which can be summarized as immune regulation, anti-infection (including anti-parasite, anti-bacterial, anti-fungal and anti-viral), anti-thrombotic, anti-tumor and metabolic effects[6]. The outbreak of novel coronavirus disease has raised concerns about the antiviral effect of CQ. This paper will focus on the antiviral mechanisms, effects, safety and adverse effects of CQ.

Antiviral Mechanisms

Inhibition of Viral Infection

When a virus enters the body, viral attachment protein binds to the specific receptor on the plas-

Corresponding Author: Shanping Jiang, MD; e-mail: jiangshp@mail.sysu.edu.cn

ma membrane of susceptible cells, such as tissue macrophages, monocytes, dendritic cells, endothelial cells, and hepatocytes. Then, virions can be internalized into vesicles which traffic through the way of pinocytosis, fusion, and penetration. After the acidification and cleavage of viral glycoproteins in acidic lysosomes (pH ~5.5), the virus can start to replicate under several enzymes. Without acidification and cleavage, the infection is abrogated[7,8]. CQ is a synthetic 4-aminoquinoline derivative and a weak base. On oral administration, CQ is readily absorbed and concentrated in tissues, such as the liver, spleen, kidney, and lung[9]. Once in the cells, CQ becomes protonated and concentrated in acidic organelles, such as the endosomes and Golgi vesicles. As a weak base, CQ, then, destroys the structure and function of these acidic organelles by increasing PH, resulting in inhibition of virus infection and replication. The known mechanisms include: (1) interfering with endocytosis and blocking viral entry in cells; (2) inhibiting the activity of several proteases and interfering with the proteolytic process; (3) inhibiting the activity of protein-modifying enzymes, thus changing the process of viral protein modification to reduce or inactivate the ability of viral infection; (4) disfunction the late endosomes and inhibiting viral assembling and budding from the infected cells[10]. Furthermore, CQ can also interfere with DNA replication and gene expression to exert viral inhibition by inhibiting transferrin from releasing iron ions and reducing the iron content in cells[11].

Reducing the Damage of Inflammatory Response

Mostly in the final stage of the viral infections, a cytokine storm may be induced by the overactivated immune system. The cytokine storm might then cause acute respiratory distress syndrome and multiple organ failure in such extreme immune attack[12]. CQ and HCQ can inhibit immune overactivation in different ways: (1) increasing the pH value of intracellular compartments of antigen presentation cells and interfering with the function of phagocytosis and antigen presenting; (2) preventing the T cells activation, proliferation and inhibiting the expression of co-stimulation molecules on account of less antigen presenting, thus decreasing the release of several pro-inflammation factors, including interferon-γ, tumor necrosis factor (TNF), interleukin (IL)-1, IL-6 and IL-2; (3) blocking the interaction of Toll-like receptors (TLRs) with ligands, and inhibiting its mediated cellular activation and cytokine production[6]. Therefore, CQ and HCQ may delay or contain the deterioration of diseases by blocking the generation of cytokine storms.

Anticoagulation and Antithrombosis

Respiratory virus infection can cause acute lung injury by inducing abnormalities in the clotting system[13,14]. Infection of severe influenza A virus can induce abnormal activation of the pulmonary epithelial cells. The activated pulmonary epithelial cells can release tissue factors into the blood to activate the endogenous coagulation pathway and lead to platelet aggregation and thrombosis[14]. Highly pathogenic coronaviruses, i.e. severe acute respiratory syndrome coronavirus (SARS-CoV), the Middle East respiratory syndrome coronavirus (MERS-CoV) and the novel coronavirus (SARS-CoV 2), induce blood coagulation dysfunction and high level of inflammatory factors[15-17]. Increasing expression of inflammatory cytokines may enhance coagulation cascade and break the physiological balance of the blood coagulation and fibrinolysis, which may induce hypercoagulable state and promote thrombosis. Post-mortem examination of 20 patients confirmed with SARS in 2003 in Toronto, Canada showed that in addition to predominant diffuse alveolar damage and acute fibrinous organizing pneumonia, the pathological features of SARS-lung still included vascular endothelial damage of both small-sized and mid-sized pulmonary vessels and vascular fibrin thrombi, which were often associated with pulmonary infarcts[18].

CQ and HCQ can prevent thrombosis by inhibiting the release of inflammatory factors, reducing red blood cell aggregation, inhibiting platelet aggregation and adhesion, reducing blood viscosity, and enhancing antiplatelet activity. HCQ has been shown to prevent significant thromboembolic events after total hip replacement or during pregnancy[9]. Whether CQ and HCQ can play a positive role in preventing hypercoagulable state and thromboembolic risk in coronavirus infection remains to be confirmed.

Antiviral Effects of Chloroquine

HIV

As early as 1993, HCQ was found to inhibit the replication of human immunodeficiency virus (HIV) in primary T cells and monocytes[19]. CQ can change the glycosylation pattern of the

HIV-1 gp120 envelope, inhibit HIV replication in CD4+ T cells, inhibit transactivation of the HIV-1 long terminal repeat and induce the selective apoptosis of the memory T cell compartment (CD45RA–CD45RO+) *in vitro*[20-22]. Two clinical trials conducted by Sperber et al[23,24] in 1995 and 1997 demonstrated the antiviral effect of HCQ on HIV-1. Subsequent clinical trials suggested that HCQ showed no effect when given alone in HIV patients but synergistically acted when combined with other antiretroviral drugs[25,26]. Furthermore, using CQ in HIV positive pregnancy women is associated with a decreased rate of vertical transmission of the virus to their infants[27]. However the same dosage of CQ did not show the similar anti-HIV effects in other clinical trials[28]. Some researchers[29] have emphasized on the starting time and dosage selection in order to maximize efficacy of CQ.

Influenza Virus

CQ was able to inhibit the replication of influenza virus A *in vitro* at low concentrations[30]. *In vitr*o, the inhibitory effect was maximal when the drug was given at the time of infection and was lost after 2 h post-infection. This timing approximately corresponded to that of virus/cell fusion and it suggested that CQ may be effective in the prevention of influenza[31]. However, CQ was not as effective as preventive therapy in animal models *in vivo*[32]. In a randomized, double-blind clinical trial, chloroquine phosphate (500 mg/day for one week, followed by once a week for 12 weeks) had no protection against influenza viruses compared to placebo[33].

Dengue Virus

Farias et al[34,35] found that CQ inhibited the replication of Dengue virus-2 by increasing the pH value of endosome in Vero cells and U937 cells. CQ could also inhibit the cytotoxic and immune-inducing effects of dengue virus-2[36]. Later, they also confirmed that CQ could interfere with the replication of dengue virus-2 in macaques[37]. A randomized, double-blind clinical trial showed that CQ improved the clinical symptoms of dengue fever patients[38].

Ebola Virus

Ebola virus belongs to filoviruses family, and it is one of the most virulent pathogens, with a mortality rate ranging from 59 to 88%[39]. It has been reported that CQ could interfere with the process of the virus entrance and replication *in vitro* to inhibit Ebola virus in mice. In 2015, Long et al[40] claimed that CQ could be a candidate for the treatment of Ebola according to their *in vitro* experimental result. Although CQ has been reported to be ineffective in Ebola patients, the survival rate of infected mice was 80% in a mouse model. Mice were delivered CQ with a dosage of 90 mg/kg body-weight twice daily for 14 days to maintain the stable whole blood concentration of CQ at 2.5 μg/ml[39]. Therefore Akpovwa[39], considered that negative results of the clinical trials might attribute to the failure of maintaining therapeutic concentration of CQ in plasma.

Coronary Virus

In 1984, Krzystyniak et al[41] found that CQ could change the pH value of endosomes to inhibit the entry of mouse hepatitis viruses (Coronaviruses) into cells. In 2003, after the SARS outbreak, Savarino et al[42] proposed that CQ might have an effect on the treatment of SARS. Subsequently, Keyaerts et al[43] of the University of Leuven, Belgium showed that CQ phosphate could inhibit the replication of SARS-CoV in Vero E6 cell line with 50% inhibitory concentration [IC_{50} = (8.8±1.2) μM], close to the serum CQ concentration during the treatment of acute malaria but significantly lower than its 50% cell inhibitory concentration (cytostatic activity) [CC_{50}=(261.3±14.5) μM], suggesting the safety of CQ in this cell line. In addition, chloroquine was significantly effective even when the drug was administered 3-5 h after infection, suggesting an antiviral effect even after the establishment of infection. Vincent et al[44] from the US Centers for Disease Control and Prevention (CDC). further elucidated that CQ inhibited viral replication by reducing the terminal glycosylation of the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of Vero E6 cells and interfering with the binding of SARS-CoV to ACE2 receptor. Mice experiments proved that CQ could not reduce the viral load in mice infected with SARS-CoV, but it had a certain inhibitory effect on the inflammatory response caused by viral infection[45]. I*n vitro,* it was found that CQ also had a certain inhibitory effect on another Coronavirus, MERS-CoV[46].

A paper published by *Science China Life Sciences*[47] on March 2020 found that the SARS-CoV-2 spikes (S) protein was similar to the S protein structure of SARS-CoV, and could also infect host epithelial cells by binding to ACE2 receptor on the surface of host cells *via* S protein. Moreover, a joint study[48] conducted by the Wuhan In-

stitute of Virology, Chinese Academy of Sciences and the Institute of Toxicology and Pharmacology, Chinese Academy of Military Medical Sciences, showed that Remdesivir (GS-5734) and CQ (Sigma-c6628) could effectively inhibit SARS-CoV-2 at the cellular level.

According to the Diagnosis and Treatment Protocol for COVID-19 (Trial version 7) by National Health Commission of the People's Republic of China and National Administration of Traditional Chinese Medicine, the clinical sub-types are as follows. (1) Mild type: patients with mild clinical symptoms and no imaging evidence of pneumonia. (2) Moderate type: patients with fever and respiratory symptoms as well as imaging evidence of pneumonia. (3) Severe type: for adults meeting any of the following: i. polypnea, respiratory rates (RR) >30 times/min; ii. pulse oxygen saturation (SpO_2) ≤93% in a resting state; iii. arterial partial pressure of oxygen (PaO_2)/fraction of inspiration O_2 (FiO_2) ≤ 300 mmHg and for patients in the region of high altitude (>1000m), the value of PaO_2/FiO_2 should be corrected as following formula: $PaO_2/FiO_2 \times$ [Atmosphere(mmHg)/760]. Besides, adult patients with rapidly progress of pulmonary lesions >50% in imaging should be managed as severe type; for children meeting any of the following: i. polypnea (<2 months, respiratory rates (RR) ≥60 times/min; 2-12 months, RR ≥50 times/min; 1-5 years old, RR ≥40 times/min; >5 years old, RR ≥30 times/min) excluding the impact of fever and crying; ii. pulse oxygen saturation (SpO_2) ≤93% in a resting state; iii. signs of accessory respiration (groan, nasal flaring or three concave sign); iv. drowsiness, convulsions; v. antifeedant or feeding difficulty, with dehydration. (4) Critical type: patients meeting any of the following: i. respiratory failure and requiring mechanical ventilation; ii. shock; iii. combining with any other organ dysfunction and requiring ICU management[49].

Expert consensus on chloroquine phosphate for the treatment of Novel Coronavirus pneumonia of China recommends that mild, moderate or severe patients aged between 18 to 65 can be treated with chloroquine phosphate after exclusion of contraindications[2]. A dosage of 500 mg twice a day for 7 days is recommended for patients weighing over 50 kg, while a dosage of 500 mg twice a day for the first 2 days and once a day for the rest of 5 days is recommended for those weighing less than or equal to 50 kg[50]. At present there are 23 clinical trials of CQ for the treatment of COVID-19 in China according to the register information on Chinese Clinical Trial Registry[51]. The contraindications of using chloroquine listed in the consensus included: (1) age <18 years old or age >65 years old; (2) women in pregnancy; (3) documented history of allergy to 4-aminoquinoline compounds; (4) documented history of hematological system diseases; (5) documented history of chronic liver and kidney diseases, especially at the end stage; (6) documented history of cardiac arrhythmia or chronic heart diseases; (7) documented history of retina or hearing dysfunction; (8) documented history of mental illnesses; (9) documented history of skin disorders (including rashes, dermatitis, psoriasis); (10) documented history of deficiency of glucose-6-phosphate dehydrogenase (G6PD); (11) use of any of the medications below due to the previous diseases including primaquine, digitalis, benzedrine, thyroxine, streptomycin, metronidazole, monoamine oxidase inhibitor, chlorpromazine, triamcinolone, proguanil, mefloquine, phenylbutazone, heparin, penicillamine, aminoglycoside antibiotics. The consensus especially emphasized that to avoid the potential risk of prolonged QT intervals and even torsade de pointes, antibiotics, including respiratory quinolones and macrolides should not be prescribed when patients are undergoing the treatment of CQ. Besides, the serum electrolyte levels (potassium, sodium, chlorine), blood glucose, liver and kidney function are ensured to be normal[2].

A clinical study to evaluate the efficacy and safety of CQ in hospitalized patients with COVID-19 was initiated in China from January 27, 2020 to February 15, 2020. In this study, patients were randomized into two groups. For experimental group, 10 patients including 3 severe and 7 moderate cases were treated with chloroquine phosphate 500 mg orally twice daily for 10 day. For control group, 12 patients including 5 severe and 7 moderate cases were treated with Lopinavir/Ritonavir 400/100 mg orally twice daily for 10 days. Comparing to the Lopinavir/Ritonavir group, the percentages of patients who became SARS-CoV-2 negative in the CQ group were slightly higher at day 7, day 10, and day 14. By day 14, the incidence rate of lung improvement based on CT imaging from the CQ group was more than doubled to that of the Lopinavir/Ritonavir group (rate ratio 2.21, 95% CI 0.81-6.62). By day 14, all 10 patients (100%) from the CQ group were discharged compared to 6 patients (50%) from the Lopinavir/Ritonavir group. These results demonstrated that CQ is superior to Lopinavir/Ritonavir in promoting a virus negative conversion, inhibiting the exacerbation of

pneumonia, improving lung imaging findings, and shortening the duration of hospital stays[52]. It suggests that CQ may be an effective and inexpensive drug to fight COVID-19.

Safety Issues

As an old drug used in clinical practice for decades, both CQ and HCQ show high tolerance even during pregnancy[6]. For a long time, CQ has been controversial for its narrow therapeutic window concentration to the toxic concentration, while HCQ, which is similar to CQ in a therapeutic effect, has been more favored for its less toxicity. Still, the toxicity of CQ or HCQ is considered to be dose-related and the probability of severe adverse effects at a therapeutic dose is rather low[53]. Therefore, it is critical to maintain a stable blood concentration during administering CQ, not only given to exert its antiviral effect but also in order to avoid the toxicity by excessive accumulation in circulation.

According to Akpovwa[39] we found that CQ did exert a significant antiviral effect *in vitro* experiments but the results from animal models and clinical trials were not satisfactory. Hence some scholars speculated that the CQ dosage was insufficient to reach the steady blood concentration, which we now understand is necessary for the treatment of diseases caused by acidic pH-dependent virus. Some *in vitro* studies have shown that CQ at a range of 10-20 μM/L in blood might be optimal for cell uptake, while a lesser uptake might occur at a concentration above 30 μM/L because higher concentrations of chloroquine existed in the form of the free base[50,54]. *In vivo,* the toxicity threshold was found to be of 17.7 μM/L[55]. A concentration of 16 μM/L in whole blood is considered as the steady state, and is sufficient to alkalize acidic organelles and inhibit viral replication and excessive release of cytokines, without significant cardiovascular events[55]. From the above studies, it can be concluded that the steady-state CQ concentration in the whole blood of 16 μm/L may be an effective and safe concentration for antiviral treatment[39] Nevertheless, due to the individual differences in the pharmacokinetics of CQ, the dosage to maintain the above effective concentration still needs to be further studied.

Adverse Effects

The adverse effects of CQ in short-term treatment are rare and reversible. Common adverse effects, include headache, dizziness, tinnitus, gastrointestinal reaction, irritability, and skin itchiness[53]. During long-term treatment, such as in the treatment of rheumatic diseases, the most apparent side effect is visual impairment[56]. Large dosage or rapid intravenous administration can lead to hypotension, cardiac dysfunction, abnormal electrocardiogram, cardiac arrest; while single dosage higher than 5 g can be an accurate predictor of a fatal outcome[57]. The other occasional adverse effects, include hypoglycemia, myopathy, hemolysis, acute liver injury, hair loss, gray hair, etc[6].

Acute toxicity

Riou et al[57] reported that the lethal dose of CQ in a single dose of acute toxicity was 3-5 g, and therefore this dosage was chosen as the criterion for severe CQ poisoning. Nevertheless, it may be more accurate to determine the dose of CQ ingested per kilogram of body weight. The National Institutes of Health webpage reported that the lowest toxic dose for acute CQ poisoning in adults was 138 mg/kg body weight, and the lowest lethal dose was 179 mg/kg body weight (Table I). However, the blood concentration may be of higher value in the determination of acute CQ poisoning due to the interindividual differences in the pharmacokinetics of CQ absorption.

Acute CQ poisoning can be manifested as follows: nausea, vomiting, alalia, visual impairment, dyspnea due to pulmonary edema, and apnea, cardiac arrhythmia, seizures, coma, and even death. For patients with severe CQ poisoning, the criteria for predicting fatal risk include single ingested dose $\geq$5 g, a systolic pressure $\leq$80 mm Hg, and QRS duration $\geq$0.120 s on an electrocardiogram, some presented hypokalemia in severe cases. Severe CQ poisoning is usually fatal. The cardiovascular toxicity of CQ is related to the transiently high blood concentration present early in the distribution phase and toxic effects usually do not persist for more than 24 h after CQ ingestion. Marks[58] indicated that early mechanical ventilation combined with diazepam and epinephrine might be effective in the treatment of severe CQ poisoning.

Cumulative Toxicity

The most common side effect of long-term use of CQ is visual impairment. CQ has a strong binding affinity to with melanin cells in the body, which makes it easier to deposit on the cornea and retina, resulting in corneal diseases and retinopathy[59]. Following termination of treatment, corneal changes are usually entirely reversible. However, the changes in the retina, for instance retinitis pigmentosa, vision loss, optic atrophy, visual field defects, and blindness are irreversible.

Table I. Acute effects of chloroquine phosphate as reported in NIH webpage.

No.	Gender	Test Type	Route of administration	Dosage	Adverse effects	Reference
1	Woman	LDLo	oral	298 mg/kg	Behavioral: coma; cardiac: other changes; lungs, thorax, or respiration: other changes	Muhm M, Stimpfl T, Malzer R, Mortinger H, Binder R, Vycudilik W, Berzlanovich A, Bauer G, Laggner AN. Suicidal chloroquine poisoning: clinical course, autopsy findings, and chemical analysis. J Forensic Sci 1996; 41: 1077-1079.
2	Woman	TDLo	oral	138 mg/kg	Behavioral: coma; vascular: blood pressure lowering not characterized in autonomic section; lungs, thorax, or respiration: other changes	National Center for Biotechnology Information. PubChem Database. Chloroquine, CID=2719, https://pubchem.ncbi.nlm.nih.gov/compound/Chloroquine (accessed on May 29, 2020)
3	Woman	TDLo	oral	600 mg/kg/17W	Peripheral nerve and sensation: spastic paralysis with or without sensory change; sense organs and special senses: other: eye; behavioral: changes in motor activity (specific assay)	Marks JS. Motor polyneuroapthy and nystagmus associated with chloroquine phosphate. Postgrad Med J 1979; 55: 569.
4	Man	TDLo	oral	8.571 mg/kg	Gastrointestinal: nausea or vomiting; gastrointestinal: other changes	Bhasin DK, Chhina RS. Chloroquine phosphate induced haematemesis. Hum Toxicol 1989; 8: 387-388.
5	Man	TDLo	oral	8.571 mg/kg	Gastrointestinal: ulceration or bleeding from duodenum; gastrointestinal: nausea or vomiting; gastrointestinal: other changes	Bhasin DK, Chhina RS. Chloroquine phosphate induced haematemesis. Hum Toxicol 1989; 8: 387-388.
6	Child	LDLo	oral	250 mg/kg	Behavioral: coma	Ifftsits-Simon C. Fatal, suicidal chloroquine poisonings. Arch Toxikol 1968; 23: 204-208.
7	Woman	TDLo	oral	167 mg/kg	Cardiac: EKG changes not diagnostic of above; vascular: blood pressure lowering not characterized in autonomic section; lungs, thorax, or respiration: respiratory depression	Heath A, Ahlmén J, Mellstrand T, Wickström I. Resin hemoperfusion in chloroquine poisoning. J Toxicol Clin Toxicol 1982; 19: 1067-1071.
8	Man	LDLo	oral	179 mg/kg	Null	Ifftsits-Simon C. Fatal, suicidal chloroquine poisonings. Arch Toxikol 1968; 23: 204-208.

LDLo: lowest published lethal dose; TDLo: lowest published toxic dose. Data source: https://pubchem.ncbi.nlm.nih.gov/compound/Chloroquine Phosphate.

An accumulative dosage above 100 g increases the risk of retinopathy. Retinopathy can also occur even at doses not exceeding 250 mg per day[60]. The American Academy of Ophthalmology (AAO) summarized the risk factors assumed to play key roles in the development of retinopathy as (1) high daily dosage per kilogram body weight (>6.5 mg/ kg of HCQ or >3 mg/ kg of CQ); (2) long duration of consistent intake (>5 years); (3) high body fat level (High body fat level was defined as a BMI >25 kg/m^2); (4) inherent liver or kidney disease; (5) concomitant retinal disease; (6) age >60 years. It is also suggested that patients should have regular ophthalmologic screening, of which intervals should be based on the risk profile[60].

It is worth noting that chronic CQ poisoning can also cause cardiomyopathy and skeletal myopathy[61]. In animal models, chronic CQ poisoning can lead to vacuolation and necrosis of cardiomyocytes and skeletal muscle cells, with more severe myocardial injury. They are generally more severe in the region of the ventricular septum than in the rest of the ventricles[61]. Ladipo et al[62] reported a complete cardiac conduction block caused by chronic CQ poisoning, in which the blood concentration of CQ was more than twice higher than that in the other patients receiving therapeutic doses of CQ; and the ratio of CQ to its metabolites in the heart block patients was significantly lower than that in the other patients receiving therapeutic doses of CQ.

Conclusions

Although the antiviral mechanism of CQ is not fully understood, a large number of studies have shown the antiviral effect of CQ, a potential candidate in emergency viral contagions. Because of the narrow therapeutic window of CQ, it is of utmost challenge for us to formulate an appropriate treatment scheme, which can not only achieve effective antiviral concentration but also avoid the occurrence of severe adverse reactions.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

We sincerely acknowledge Nanshan Zhong (Academician, Chinese Academy of Engineering; Guangzhou Institute of Respiratory Disease), Tao Xu (Academician, Chinese Academy of Science; Guangzhou Regenerative Medicine and Health Guangdong Laboratory), Erwei Song (Academician, Chinese Academy of Science; Sun Yat-sen Memorial Hospital of Sun Yat-sen University) for guidance, and Professor Phei Er Saw for valuable revision of this manuscript

Funding Information

This study was funded by the Emergency Program for Major Public Health Event of Ministry of Science and Technology, Department of Science and Technology of Guangdong Province of China (2020B111113001), the Emergency Program for Guangzhou Regenerative Medicine and Health Guangdong Laboratory of China (2020GZR110106003) and Tencent Charity Foundation of China.

References

1) Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020. pii: S2213-2600(20)30079-5. doi: 10.1016/S2213-2600(20)30079-5. [Epub ahead of print].
2) Multicenter Collaboration Group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for chloroquine in the treatment of novel coronavirus pneumonia [Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia]. Zhonghua Jie He He Hu Xi Za Zhi 2020; 43: E019.
3) Krafts K, Hempelmann E, Skórska-Stania A. From methylene blue to chloroquine: a brief review of the development of an antimalarial therapy. Parasitol Res 2012; 111: 1-6.
4) Ben-Zvi I, Kivity S, Langevitz P, Shoenfeld Y. Hydroxychloroquine: from malaria to autoimmunity. Clin Rev Allergy Immunol 2012; 42: 145-153.
5) Fox RI, Kang HI. Mechanism of action of antimalarial drugs: inhibition of antigen processing and presentation. Lupus 1993; 2 Suppl 1: S9-12.
6) Plantone D, Koudriavtseva T. Current and future use of chloroquine and hydroxychloroquine in infectious, immune, neoplastic, and neurological diseases: a mini-review. Clin Drug Invest 2018; 38: 653-671.
7) Martinson JA, Montoya CJ, Usuga X, Ronquillo R, Landay AL, Desai SN. Chloroquine modulates HIV-1-induced plasmacytoid dendritic cell alpha interferon: implication for T-cell activation. Antimicrob Agents Chemother 2010; 54: 871-881.
8) Shivanna V, Kim Y, Chang KO. Endosomal acidification and cathepsin L activity is required for calicivirus replication. Virology 2014; 464-465: 287-295.
9) Al-Bari MAA. Chloroquine analogues in drug discovery: new directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. J Antimicrob Chemother 2015; 70: 1608-1621.
10) Al-Bari MAA. Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. Pharmacol Res Perspect 2017; 5: e00293.
11) Legssyer R, Josse C, Piette J, Ward RJ, Crichton RR. Changes in function of iron-loaded alveolar macrophages after in vivo administration of desferrioxamine and/or chloroquine. J Inorg Biochem 2003; 94: 36-42.
12) Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol 2017; 39: 517-528.
13) Yang M, Ng MHL, Li CK. Thrombocytopenia in patients with severe acute respiratory syndrome (review). Hematology (Amsterdam, Netherlands) 2005; 10: 101-105.
14) Yang Y, Tang H. Aberrant coagulation causes a hyper-inflammatory response in severe influ-

enza pneumonia. Cell Mol Immunol 2016; 13: 432-442.
15) Franks TJ, Chong PY, Chui P, Galvin JR, Lourens RM, Reid AH, Selbs E, McEvoy CPL, Hayden CDL, Fukuoka J, Taubenberger JK, Travis WD. Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore. Human Pathol 2003; 34: 743-748.
16) Singh SK. Middle East Respiratory Syndrome virus pathogenesis. Semin Respir Crit Care Med 2016; 37: 572-577.
17) Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet (London, England) 2020; 395: 497-506.
18) Hwang DM, Chamberlain DW, Poutanen SM, Low DE, Asa SL, Butany J. Pulmonary pathology of severe acute respiratory syndrome in Toronto. Mol Pathol 2005; 18: 1-10.
19) Sperber K, Kalb TH, Stecher VJ, Banerjee R, Mayer L. Inhibition of human immunodeficiency virus type 1 replication by hydroxychloroquine in T cells and monocytes. AIDS Res Hum Retroviruses 1993; 9: 91-98.
20) Naarding MA, Baan E, Pollakis G, Paxton WA. Effect of chloroquine on reducing HIV-1 replication in vitro and the DC-SIGN mediated transfer of virus to CD4+ T-lymphocytes. Retrovirology 2007; 4: 6.
21) Jiang MC, Lin JK, Chen SS. Inhibition of HIV-1 Tat-mediated transactivation by quinacrine and chloroquine. Biochem Biophys Res Commun 1996; 226: 1-7.
22) van Loosdregt J, Spreafico R, Rossetti M, Prakken BJ, Lotz M, Albani S. Hydroxychloroquine preferentially induces apoptosis of CD45RO+ effector T cells by inhibiting autophagy: a possible mechanism for therapeutic modulation of T cells. J Allergy Clin Immunol 2013; 131: 1443-1446.e1441.
23) Sperber K, Louie M, Kraus T, Proner J, Sapira E, Lin S, Stecher V, Mayer L. Hydroxychloroquine treatment of patients with human immunodeficiency virus type 1. Clin Ther 1995; 17: 622-636.
24) Sperber K, Chiang G, Chen H, Ross W, Chusid E, Gonchar M, Chow R, Liriano O. Comparison of hydroxychloroquine with zidovudine in asymptomatic patients infected with human immunodeficiency virus type 1. Clin Ther 1997; 19: 913-923.
25) Paton NI, Goodall RL, Dunn DT, Franzen S, Collaco-Moraes Y, Gazzard BG, Williams IG, Fisher MJ, Winston A, Fox J, Orkin C, Herieka EA, Ainsworth JG, Post FA, Wansbrough-Jones M, Kelleher P. Effects of hydroxychloroquine on immune activation and disease progression among HIV-infected patients not receiving antiretroviral therapy: a randomized controlled trial. JAMA 2012; 308: 353-361.
26) Piconi S, Parisotto S, Rizzardini G, Passerini S, Terzi R, Argenteri B, Meraviglia P, Capetti A, Biasin M, Trabattoni D, Clerici M. Hydroxychloroquine drastically reduces immune activation in HIV-infected, antiretroviral therapy-treated immunologic nonresponders. Blood 2011; 118: 3263-3272.
27) Neely M, Kalyesubula I, Bagenda D, Myers C, Olness K. Effect of chloroquine on human immunodeficiency virus (HIV) vertical transmission. Afr Health Sci 2003; 3: 61-67.
28) Murray SM, Down CM, Boulware DR, Stauffer WM, Cavert WP, Schacker TW, Brenchley JM, Douek DC. Reduction of immune activation with chloroquine therapy during chronic HIV infection. J Virol 2010; 84: 12082-12086.
29) Savarino A, Shytaj IL. Chloroquine and beyond: exploring anti-rheumatic drugs to reduce immune hyperactivation in HIV/AIDS. Retrovirology 2015; 12: 51.
30) Ooi EE, Chew JSW, Loh JP, Chua RCS. In vitro inhibition of human influenza A virus replication by chloroquine. Virol J 2006; 3: 39.
31) Di Trani L, Savarino A, Campitelli L, Norelli S, Puzelli S, D'Ostilio D, Vignolo E, Donatelli I, Cassone A. Different pH requirements are associated with divergent inhibitory effects of chloroquine on human and avian influenza A viruses. Virol J 2007; 4: 39.
32) Ooi EE, Chew JS, Loh JP, Chua RC. In vitro inhibition of human influenza A virus replication by chloroquine. Virol J 2006; 3: 39.
33) Paton NI, Lee L, Xu Y, Ooi EE, Cheung YB, Archuleta S, Wong G, Wilder-Smith A. Chloroquine for influenza prevention: a randomised, double-blind, placebo controlled trial. Lancet Infect Dis 2011; 11: 677-683.
34) Farias KJ, Machado PR, da Fonseca BA. Chloroquine inhibits dengue virus type 2 replication in Vero cells but not in C6/36 cells. ScientificWorldJournal 2013; 2013: 282734.
35) Farias KJ, Machado PR, de Almeida Junior RF, de Aquino AA, da Fonseca BA. Chloroquine interferes with dengue-2 virus replication in U937 cells. Microbiol Immunol 2014; 58: 318-326.
36) Gandini M, Gras C, Azeredo EL, Pinto LM, Smith N, Despres P, da Cunha RV, de Souza LJ, Kubelka CF, Herbeuval JP. Dengue virus activates membrane TRAIL relocalization and IFN-α production by human plasmacytoid dendritic cells *in vitro a*nd *in vivo.* PLoS Negl Trop Dis 2013; 7: e2257.
37) Farias KJ, Machado PR, Muniz JA, Imbeloni AA, da Fonseca BA. Antiviral activity of chloroquine against dengue virus type 2 replication in Aotus monkeys. Viral Immunol 2015; 28: 161-169.
38) Borges MC, Castro LA, Fonseca BA. Chloroquine use improves dengue-related symptoms. Mem Inst Oswaldo Cruz 2013; 108: 596-599.
39) Akpovwa H. Chloroquine could be used for the treatment of filoviral infections and other viral infections that emerge or emerged from viruses requiring an acidic pH for infectivity. Cell Biochem Funct 2016; 34: 191-196.
40) Long J, Wright E, Molesti E, Temperton N, Barclay W. Antiviral therapies against Ebola and other emerging viral diseases using existing medicines that block virus entry. F1000Res 2015; 4: 30.
41) Krzystyniak K, Dupuy JM. Entry of mouse hepatitis virus 3 into cells. J Gen Virol 1984; 65 (Pt 1): 227-231.
42) Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old

drug against today's diseases? Lancet Infect Dis 2003; 3: 722-727.
43) Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun 2004; 323: 264-268.
44) Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, Nichol ST. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J 2005; 2: 69.
45) Barnard DL, Day CW, Bailey K, Heiner M, Montgomery R, Lauridsen L, Chan PK, Sidwell RW. Evaluation of immunomodulators, interferons and known in vitro SARS-coV inhibitors for inhibition of SARS-coV replication in BALB/c mice. Antivir Chem Chemother 2006; 17: 275-284.
46) Dyall J, Coleman CM, Hart BJ, Venkataraman T, Holbrook MR, Kindrachuk J, Johnson RF, Olinger GG, Jr., Jahrling PB, Laidlaw M, Johansen LM, Lear-Rooney CM, Glass PJ, Hensley LE, Frieman MB. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob Agents Chemother 2014; 58: 4885-4893.
47) Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, Zhong W, Hao P. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 2020; 63: 457-460.
48) Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020; 30: 269-271.
49) The General Office of the National Health Commission of the People's Republic of China, The Office of the National Administration of Traditional Chinese Medicine, "Notice on publishing Diagnosis and Treatment Protocol for COVID-19 (Trial version 7)"(National Administration of Traditional Chinese Medicine Office Medical Letter 2020 No. 184) http://www.nhc.gov.cn/xcs/zhengcwj/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml
50) The General Office of the National Health Commission of the people's Republic of China, The Office of the National Administration of Traditional Chinese Medicine, "Notice on adjusting the usage and dosage of chloroquine phosphate for the COVID-19 Infection" (National Administration of Traditional Chinese Medicine Office Medical Letter 2020 No. 165). http://www.nhc.gov.cn/yzygj/s7653p/202002/0293d017621941f6b2a4890035243730. shtml
51) The register information of Chinese Clinical Trial Registry access on March 22th, 2020. http://www.chictr.org.cn/index.aspx
52) Huang M, Tang T, Pang P, Li M, Ma R, Lu J, Shu J, You Y, Chen B, Liang J, Hong Z, Chen H, Kong L, Qin D, Pei D, Xia J, Jiang S, Shan H. Treating COVID-19 with chloroquine. J Mol Cell Biol 2020. pii: mjaa014. doi: 10.1093/jmcb/mjaa014. [Epub ahead of print].
53) Frisk-Holmberg M, Bergkvist Y, Domeij-Nyberg B, Hellström L, Jansson F. Chloroquine serum concentration and side effects: evidence for dose-dependent kinetics. Clin Pharmacol Ther 1979; 25: 345-350.
54) MacIntyre AC, Cutler DJ. Role of lysosomes in hepatic accumulation of chloroquine. J Pharm Sci 1988; 77: 196-199.
55) Mégarbane B, Bloch V, Hirt D, Debray M, Résiére D, Deye N, Baud FJ. Blood concentrations are better predictors of chioroquine poisoning severity than plasma concentrations: a prospective study with modeling of the concentration/effect relationships. Clin Toxicol (Phila) 2010; 48: 904-915.
56) Bergholz R, Schroeter J, Rüther K. Evaluation of risk factors for retinal damage due to chloroquine and hydroxychloroquine. Br J Ophthalmol 2010; 94: 1637-1642.
57) Riou B, Barriot P, Rimailho A, Baud FJ. Treatment of severe chloroquine poisoning. N Engl J Med 1988; 318: 1-6.
58) Marks JS. Chloroquine retinopathy: is there a safe daily dose? Ann Rheum Dis 1982; 41: 52-58.
59) Al-Bari MAA. Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. Pharmacol Res Perspect 2017; 5: e00293.
60) Marmor MF, Carr RE, Easterbrook M, Farjo AA, Mieler WF. Recommendations on screening for chloroquine and hydroxychloroquine retinopathy: a report by the American Academy of Ophthalmology. Ophthalmology 2002; 109: 1377-1382.
61) Fischer VW. Evolution of a chloroquine-induced cardiomyopathy in the chicken. Exp Mol Pathol 1976; 25: 242-252.
62) Ladipo GO, Essien EE, Andy JJ. Complete heart block in chronic chloroquine poisoning. Int J Cardiol 1983; 4: 198-200.