The Chloroquine Story in the First Year of the COVID-19 Pandemic

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Abstract
The emergence of COVID-19 has caused worldwide concern due to its high infectivity and mortality. Research groups around the world have prioritised drug development against COVID-19. Repurposing of already approved drugs, including the antimalarial drug chloroquine, has attracted considerable attention. The aim of this article is to (i) provide an overview of the recent chemical methods used to synthesise chloroquine and hydroxychloroquine, and (ii) provide insight into the data collected in 2020 on their efficacy against COVID-19. Unfortunately, the promising early results have not been confirmed and a clear and unambiguous conclusion on their clinical efficacy has not yet been drawn.

Keywords
Chemical synthesis, chloroquine, COVID-19, hydroxychloroquine, SARS-CoV-2

1 Introduction
COVID-19 (Coronavirus Disease of 2019) is an acute respiratory disease, caused by the coronavirus SARS-CoV-2, first reported in Wuhan, China in December 2019. On January 30, 2020, the World Health Organisation (WHO) officially declared the COVID-19 epidemic a public health emergency of international concern. The disease spread extremely rapidly around the world. By the end of October 2021, more than 244 million cases had been confirmed, and more than 4.9 million deaths had been attributed to COVID-19.

Since the beginning of the COVID-19 crisis, both the political and scientific communities have made every effort to provide an effective drug or vaccine. To this end, the U.S. Food and Drug Administration (FDA) created the Coronavirus Treatment Acceleration Program (CTAP), a special emergency program to develop coronavirus therapeutics. According to the CTAP website, as of December 2021, more than 670 drug development programs were in the planning phase, more than 470 were under FDA review, 11 COVID-19 treatments were approved for emergency use, and 1 repurposed drug, remdesivir, was approved for clinical use.

Drug repurposing (repositioning, repurposing, or retasking) is a strategy aimed at finding new therapeutic uses for previously approved or experimental drugs that are outside the scope of the original medical indication. Compared to developing a completely new drug, repurposing has the following advantages, among others: (i) lower risk of failure as the safety profile has already been determined to be acceptable, (ii) shorter time frame for drug development as most preclinical testing has already been completed, (iii) lower cost of preclinical testing and phases I and II, which can reduce the overall cost of bringing the drug to market, and (iv) repurposed drugs open up the possibility of identifying new targets and pathways for further investigation.

Recent drug repurposing studies against SARS-CoV-2 are addressing the cross-reactivity of chemical and biological agents with the activity of viral molecules or immunomodulatory agents. The three main therapeutic targets are determined: (i) inhibition of interactions between viral proteins and surface-cell receptors, (ii) inhibition of transcription, translation, and replication of the viral genome, and (iii) hampering of the viral assembly mechanism by blocking the assembling of the proteins involved. Preclinical and clinical in vitro studies have been conducted with many existing drugs, but only thirteen of them have shown anti-SARS-CoV-2 activity: berberine, CQ, HCQ, cyclosporine A, emetine, homoharringtonine, lopinavir, nafamostat, nitazoxamide, penciclovir, remdesivir, ribavirin, favipiravir, and arbidol. Besides remdesivir, which inhibits viral replication with an EC₅₀ of 0.77–26.9 µM, other drugs tested for their ability to disrupt viral replication (favipiravir, penciclovir, or ribavirin) showed only weak activity against SARS-CoV-2. Of the known viral protease inhibitors, only lopinavir showed antiviral activity (EC₅₀ 5.25–26.62 µM), while its combination with ritonavir showed no clinical effect. Although the compounds with different and partly unknown modes of antiviral action (nitazoxamide, cyclosporine A, emetine, and homoharringtonine) showed strong anti-SARS-CoV-2 activity, they were not readily evaluated in animal models or clinical trials. Veklury (remdesivir), originally developed to treat Ebola virus, is the only known antiviral drug that has demonstrated efficacy against SARS-CoV-2 in preclinical and clinical settings. In addition, remdesivir is the only repurposed drug authorised for use in the EU.

In addition to the known antiviral drugs, small molecules and immunotherapies such as convalescent plasma transfusion, hyperimmune globulin, and monoclonal antibodies have also been tested as treatment against COVID-19. Monoclonal antibodies are proteins that bind to a specific...
target, in the case of SARS-CoV-2 the S-protein, and in this way prevent the virus from entering the cell. The EMA’s Human Medicines Committee (CHMP) has recommended authorisation of four monoclonal antibody medicines for COVID-19 aimed at significantly reducing hospitalisation and mortality in COVID-19 patients: Ronapreve (casirivimab/imdevimab), Regkinona (regnanvimab), RoActemra (tocilizumab) and Xevudy (sotrovimab). One of the four monoclonal antibodies that can be used in the EU to treat COVID-19, RoActemra, is originally used to treat rheumatoid arthritis, giant cell arteritis, and cytokine release syndrome.9

At the very beginning of the pandemic, the repurposing of a well-known antimalarial drug, CQ and its derivative HCQ, was seriously considered as a promising strategy to combat COVID-19. Thus, in this paper, we aim to provide an overview of the chemical synthesis, antimalarial activity, and possible antiviral mechanism of these drugs, as well as the recent findings on their efficacy in the COVID-19 crisis.

2 SARS-CoV-2: Structure and replication

SARS-CoV-2 are enveloped, single-stranded, positive-sense RNA viruses.10 The coronavirus genome contains a number of open reading frames (ORFs). Most of the viral RNA encodes the pp1a and pp1ab polyproteins, from which 16 nonstructural proteins are released by proteolytic cleavage.11 The reminder of the coronavirus genome encodes several accessory proteins that affect the nonspecific (innate) host immune response, as well as four structural proteins, including a glycoprotein spike (S-glycoprotein), a viral envelope protein (E-protein), a membrane protein (M-protein), and a nucleocapsid protein (N-protein).12 The S-glycoprotein, consisting of subunits S1 and S2, plays an important role in the invention and development of drugs against COVID-19. Subunit S1 binds the cell surface receptor ACE2 (angiotensin-converting enzyme 2) to its receptor binding domain, thereby altering the conformation of subunit S2 to allow fusion of the cell membrane and viral envelope.13 This process represents a critical point for viral entry into the host cell, as viral entry ultimately leads to capsid degradation and release of viral RNA into the cytoplasm. The host proteolytic enzyme TMPRSS2 (Transmembrane Serine Protease 2) cleaves the S-glycoprotein at the S1/S2 cleavage site, activating the S-glycoprotein, and allowing SARS-CoV-2 to enter the cell.14,15 Recent studies have confirmed that TMPRSS2-expressing VeroE6 cells are highly susceptible to SARS-CoV-2 infection.8 After entering the cell, the capsid of the viral particle disintegrates and the processes of transcription and translation take place. The first ORF encodes approximately 67 % of the genome and encodes the pp1a and pp1ab polyproteins. Processing of these polyproteins leads to the synthesis of a number of non-structural proteins, including RNA-dependent RNA polymerase and helicase, which exclusively recognise and synthesise viral RNA molecules. RNA polymerase uses the positively stranded (+) RNA as a template for synthesis of the (−) RNA chain. Transcription of the (−) RNA strand results in a new (+) RNA strand and subgenomic mRNA fragments that encode the structural and accessory proteins of the virus.7 The newly synthesised viral RNA, nucleocapsid proteins, and envelope proteins form viral particles that are packaged in vesicles that ultimately emerge from the infected cell.

3 CQ/HCQ: Synthesis, antimalarial and antiviral activity

CQ is on the WHO model list of essential medicines.16 It is mainly used for prevention and treatment of acute forms of malaria caused by the parasite Plasmodium, with the exception of Plasmodium falciparum, which has developed resistance.17 After isosteric modification of CQ with ferrocene, antimalarial activity was fully restored18 and the resulting ferroquine was recently shown to be effective against SARS-CoV-2 at doses commonly administered in malaria treatment.19 Recently, a review of chemical methods for the synthesis of repositioning agents (CQ, HCQ, favipiravir and remdesivir) for the treatment of COVID-19 was provided.20 CQ (3) and HCQ (5) are prepared from the same precursor, 4,7-dichloroquinoline (1), which was condensed with 2-amino-5-(diethylamino)pentane (2) and 5-(N-ethyl-N-2-hydroxy-ethylamino)-2-pentylamine (4), respectively (Fig. 1).11

Since the discovery of CQ, numerous synthetic routes to key components 1, 2, and 4 have been described.21 Recent work on the synthesis of 4,7-dichloroquinoline (1) was mainly based on the procedure reported in 194822 using m-chloroanilin (6) as the starting compound. The

![Fig. 1 – Synthetic route to chloroquine (3) and hydroxychloroquine (5)
Slika 1 – Sintetski put do klorokina (3) i hidroksiklorokina (5)](image-url)
Schiff base 8, obtained by condensation of 6 with ethoxymethyleneenomalonic ester 7, was subjected to thermal cyclisation to give 7-chloro-4(1H)-quinolone-3-carboxylate 9. Subsequent saponification, thermal decarboxylation, and treatment with phosphorus oxychloride gave precursor 1 (Fig. 2).

One of the frequently reported procedures for the preparation of the second key component, 2-amino-5-(diethylamino)pentane (2), uses 2-acetylbutyrolactone 12 as the starting compound. Its acidic hydrolysis followed by decarboxylation gave pentanone 13, which was treated with diethylamine to produce diethylamino pentanone 14. The final step was the catalytic reductive amination of ketone 14 to give the desired precursor 2. The most recent procedure for the synthesis of the third key component, 5-(N-ethyl-N-2-hydroxyethylamino)-2-pentylamine (4), involved the acidic hydrolysis of 12 to iodopentan-2-one 15, which was subjected to nucleophilic substitution with ethanethiol to give 16. The oxidation and subsequent catalytic reduction of oxime 17 gave the precursor 4 (Fig. 3).
The mechanism of the antimalarial activity of CQ is not fully understood. One of the assumptions is based on hydrogen bonding between CQ and a purine base, followed by intercalation of CQ into the DNA helix of the parasite, which prevents the processes of transcription and translation, and consequently limits the synthesis of parasitic nucleic acids. The parasite invades erythrocytes and grows by ingesting haemoglobin from the host cell and depositing it in the digestive vacuole (lysosome) which is enclosed by a lipoprotein membrane and has an acidic pH. As a membrane-soluble weak base, CQ is protonated at acidic pH and subsequently trapped in the vacuole, where it binds to hematin, a toxic product of haemoglobin proteolysis, to prevent its detoxification to hemozoin. The hematin interferes with the detoxification processes of the parasite, resulting in hematin-mediated toxic effects and death of the parasite.

As for the antiviral mechanism, the enveloped viruses require a pH-dependent step for entry (low pH, followed by the action of enzymes, allows the release of the infectious nucleic acid and enzymes necessary for viral replication). The inhibitory effect of CQ/HCQ on pH-dependent endosome-mediated viral entry was reported several decades ago. In addition to inhibiting the viral entry, likely by impairing terminal glycosylation of ACE2, which may lead to decreased binding affinity of the SARS-CoV-5 protein to ACE2, these drugs have been shown to impair viral replication by interfering with terminal glycosylation of envelope glycoproteins. Possible mechanisms of CQ/HCQ anti-SARS-CoV-2 activity include inhibition of attachment and entry of the virus into the host cell, and inhibition of maturation and spread of new viral particles. Infection occurs via sialic acid-mediated recognition and interaction of the viral structural envelope S-protein with ACE2 as a host cell membrane receptor. CQ inhibits N-glycosylation of ACE2 and/or S-protein, thereby reducing the binding affinity for virus-cell recognition, which is required for viral invasion of the host cell. In addition, CQ/HCQ could bind host sialic acids, which could then be responsible for inhibiting the interaction of the S-protein with the host plasma-membrane. It has been speculated that CQ inhibits two pathways through which the SARS-CoV-2-S-protein is activated to promote infection.

One of the pathways is dependent on the endosomal proteases cathepsin L, while the other is dependent on TMPRSS2. Recent work has shown that CQ alone does not block TMPRSS2-mediated viral entry. However, the combination of CQ and clinically tested TMPRSS2 inhibitors was found to effectively inhibit viral entry. Thus, the lack of in vivo efficacy of CQ can be explained by TMPRSS2 expression on SARS-CoV-2 target cells, which likely deactivates the antiviral activity of the drug.

The rapid increase in pH and alkalisation of endosomes in the presence of CQ/HCQ disrupts the endosome-lysosome membrane fusion, which is required for viral receptor membrane recycling, viral uncoating, and viral genome release. CQ/HCQ are also involved in the inhibition of low pH-dependent viral protein maturation processes seen in endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and trans-Golgi network (TGN) vesicles. CQ, which is known to increase endosomal pH, leads to disruption of both glycosylation and proteolytic cleavage of viral proteins, thus inhibiting viral spreading. In addition, formation of mature virions by budding of encapsidated viral genomes into ERGIC membranes is also inhibited by CQ/HCQ. Activation of cells via MAPK (mitogen-activated protein kinase) is required by viruses for their replication. Considering that CQ acts as an inhibitor of p38 MAPK, viral replication could be inhibited by CQ, thus impeding viral infection.

4 How the story on CQ/HCQ efficacy in the treatment of COVID-19 had changed from February to December 2020?

Since no vaccines were available at the beginning of the pandemic, it was extremely important to evaluate the potential preventive and therapeutic effects of available drugs. Among the many established drugs, the antimalarial drug chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) were studied, and initial studies indicated considerable activity and safety of these drugs in COVID-19 treatment.

Right at the beginning of the pandemic, results of the in vitro antiviral activity of CQ/HCQ gave cause for optimism about their efficacy in treating the novel disease, so much so that US FDA granted an Emergency Use Authorisation (EUA) for CQ in late March. However, instead of the expected clinical confirmation of the initial promising results, the overall results of the numerous clinical trials on CQ/HCQ activity in vivo were rather controversial. In addition, serious and growing concerns about reported adverse effects have led the European Medicines Agency (EMA) to revoke the marketing authorisation for CQ.

Data generated during the second year of pandemic show no clinical benefit of CQ/HCQ, and therefore further investigation of CQ/HCQ as pre- and post-exposure prophylaxis and treatment of non-hospitalised and hospitalised patients with COVID-19 is not recommended.

Here, we briefly review the results of the studies on the efficacy of CQ/HCQ against COVID-19 obtained in the first year of pandemic (Fig. 4).

February 2020: The antiviral activity of CQ against 2019-CoV in Vero E6 cells with EC50 value of 6.90 mM was reported. Considering its efficacy and safety record, CQ was expected to be clinically applicable for this novel disease. The use of CQ against SARS-CoV-2 was also described as a spectacular example of potential drug repositioning. If CQ is clinically approved, the novel disease should be among the cheapest and easiest to treat compared to other respiratory diseases.

March 2020: Results obtained from review of PubMed and EMBASE databases, as well as the Chinese Clinical Trials Registry and the International Clinical Trials Registry Platform, indicate the impact of CQ on SARS-CoV-2 replication. It was hypothesised that CQ could improve the
The antiviral activity of CQ: a spectacular example of the drug repositioning.

EMAs: revoking of the approval due to the lack of in vivo activity; the daily dosage recommendation for older patients (2 x 500 mg).

The encouraging in silico inhibitory activity of CQ: a moratorium on prescription of HCQ due to the prolonged QTc intervals; the need to define the proper dose of CQ/HCQ.

Controversial data or viral suppression by CQ/AZ: the findings obtained on animals do not support the broad use of CQ/HCQ.

The initial benefits of CQ/HCQ do not outweigh the known risk of cardiac adverse events; the conducted RCTs do not provide any reason for the repurposing of CQ/HCQ.

The HCQ prophylaxis do not prevent COVID-19: HCQ failure due to inability to reach adequate concentration (higher doses might cause serious safety concerns); NMA: no reduce of mortality rate and no protection by CQ/HCQ.

February
CQ influences the viral replication; HCQ efficiently clear the viral loads; FDA: an emergency use authorization for CQ.

March
CQ/HCQ have beneficial effects; the serious adverse effects of CQ/HCQ, the different dosage regimens for moderate and severe symptoms, the increment of mortality.

April
Different dosage regimens for patients of different age; the numerous side effects and increased mortality.

May
The lack of evidence for CQ/HCQ efficacy and safety; the unsolved questions on optimal dose and risk benefit balance; is treatment with high initial dose followed by low and sparse doses the most beneficial for the patients?

June
The controversies on therapeutic and prophylactic effects of CQ/HCQ: the insignificant difference in the mortality in the high and low dose patient group; many supportive evidences justify the use of HCQ; the potential risk factors: male gender, older age and concurrent intake of NSAIDs.

July
August
September
October
November
December

Fig. 4 – Milestones in the repurposing of CQ/HCQ for treatment of COVID-19 in 2020

Clinical picture in infected patients due to its inhibitory effect on viral replication. It was also hypothesised that CQ affects the interaction of SARS-CoV-2 with ACE2, impairs with proteolytic cleavage of the M-protein, and alters virion assembly and budding. It was found that the viral load of SARS-CoV-2 was cleared from the nasopharynx of COVID-19 patients in less than six days after treatment with HCQ. Moreover, the PCR test of the patients treated with CQ in combination with azithromycin (AZ) was negative, indicating the synergistic effect of CQ/AZ. Therefore, on March 28, the US FDA released an EUA for CQ to treat COVID-19 patients.

April 2020: The potential mechanisms of anti-SARS-CoV-2 action and the results of the clinical trials conducted to date indicate that additional studies are required before these drugs can be widely used for the treatment of COVID-19. Therefore, the EMA denied approval of CQ and limited its use to clinical trials or national emergency programmes. The in vitro and in vivo data on CQ as COVID-19 treatment showed strong differences in efficacy in cell lines and live animals. It was strongly recommended that well-designed clinical trials (randomised and controlled) are urgently needed to establish the safety and efficacy of CQ. Based on the reviewed data from trials of antiviral drugs in the laboratory and in patients with COVID-19, CQ was more efficient in reducing the exacerbation of pneumonia, viral load and symptoms compared to treatment with standard therapy. Therefore, it was suggested that CQ/HCQ should be considered as a weapon to combat COVID-19, and that the dosage of 500 mg (twice daily) should be recommended for elderly patients or patients with severe symptoms.
May 2020: CQ/HCQ have already been shown to have a modulatory effect on activated immune cells during SARS-CoV infection. As for their anti-SARS-CoV-2 activity, HCQ ($EC_{50} = 0.72 \text{ mM}$) exhibited almost 8-fold higher inhibitory potency than CQ. The study, which aimed to explore the safety of CQ and to apply the simulation techniques to determine the relationships between the serious adverse effects and overdose, as well as to propose optimised dosing regimens, was published. Serious adverse effects observed during treatment with CQ included prolongation of the QT interval, cardiac arrhythmias, cardiomyopathy, diffuse parenchymal lung disease, severe hypoglycemia, eye/vision disorders, myasthenia-like symptoms, and liver disorders. CQ overdose results in nausea and vomiting, hypokalemia, metabolic acidosis, neuropsychiatric side effects, headache and visual disturbances, cardiac arrhythmias, and eventually death. The two scenarios based on dose reduction on the second or third day were proposed for COVID-19 patients with mild to moderate symptoms of pneumonia. Clinicians reached mixed conclusions regarding the observed efficacy and safety of CQ/HCQ. A non-randomised open-label clinical trial in France showed the beneficial effect of HCQ on viral negativity at day 6, and HCQ/AZ also had positive effects. The clinical trial in China showed remarkable clinical outcomes and viral clearance. On the other hand, other studies warned of a significant risk of severe QT prolongation and cardiac arrhythmias. The study conducted in the USA showed a higher mortality rate when receiving CQ alone or CQ/AZ.

June 2020: The urgent need for a moratorium on HCQ prescription was highlighted, as the available evidence on safety and toxicity was limited to 8 published studies, and only modest to moderate benefit was found. Considering disease course and patient-related outcomes (need for mechanical ventilation and oxygenation, length of hospital stay, and length of stay in the ICU), the evidence obtained from the studies conducted was insufficient. The major drawback of the studies conducted was that only a very small number of patients were included and there was no control group. The major concern regarding side effects was the prolongation of the QTc interval. The safety profile of HCQ stems from decades of use in autoimmune diseases, which are more common in the population of younger and middle-aged women, whose risk of fatal outcome due to QT prolongation is very low. In contrast, the risks for COVID-19 are much higher in older male patients and in patients with comorbidities. Therefore, the moratorium on the use of CQ/HCQ for the treatment or prevention of COVID-19 has been strongly recommended. To decide whether CQ should be used as standard therapy for COVID-19, the following unresolved questions must be addressed: what is the goal of treatment (shortening hospital stay, reducing the need for mechanical ventilation, etc.), what is the appropriate dose and timing of use, what monitoring is required, and which patients are at greatest risk for adverse effects. The inhibitory activity of CQ and its derivatives against drug targets of SARS-CoV-2 has been studied in silico. The derivatives of CQ were obtained by structural modifications of the amino and trimethylene groups, while the quinoline moiety was retained. The obtained results, based on the molecular docking of the interaction between the tested compounds and the active sites of the virus, showed that CQ and its derivatives could bind to SARS-CoV-2 proteins involved in viral pathogenesis, thus interfering with their normal function. CQ derivatives showed better inhibitory activity against all target proteins of SARS-CoV-2 than HCQ and CQ themselves. These results could be used for further optimisation of CQ derivatives as antiviral drugs.

July 2020: The results of both in vitro and clinical studies on the use of CQ/HCQ in SARS-CoV-2 infections were reviewed and clinical practice guidelines for therapy were given. In the Netherlands, Pakistan, France, and Spain, treatment of patients with CQ was suggested, whereas treatment of Chinese patients aged 18 to 65 years with CQ was recommended only if the basic regimen (lopinavir/ritonavir in combination with arbidol) was not effective. In Ireland and Italy, CQ/HCQ were recommended for all COVID-19 patients over 70 years of age. In the USA, CQ was recommended for the treatment of patients over 60 years of age. At the same time, a retrospective analysis of 671 hospitals in six continents was performed, and found an increased number of deaths associated with CQ/HCQ-induced arrhythmias. Therefore, Italy and France abandoned the recommendation to use CQ/HCQ for the treatment of COVID-19 patients. Systematic and statistical review of current data on controlled COVID-19 treatment trials in humans revealed numerous adverse events and increased mortality. At this time, there was no human study demonstrating the efficacy of HCQ for COVID-19 prophylaxis. The Nature article reports that CQ inhibits SARS-CoV-2 infection of Vero kidney cells, but not infection of human lung cells. This finding underscores the fact that cell lines mimicking respiratory epithelial cells should be used to test antiviral activity.

August 2020: The possible role of HCQ/AZ as a “game-changer” was again investigated in a study conducted in France. After six days, 100 % of patients treated with HCQ/AZ were negative for SARS-CoV-2, while 57.1 % of patients treated with HCQ alone were negative, and only 12.5 % of patients in the control group were negative. Another clinical trial showed a significant shortening of viral shedding in COVID-19 patients treated with HCQ/AZ. At the same time, the other small clinical trials suggest that viral suppression is not improved by treatment with combined drugs. A narrative review discussed the general properties and antiviral history of CQ, HCQ and CQ/AZ, and analysed the available evidence for their anti-COVID activity. Due to the serious cardiac side effects, it was recommended to avoid high doses and to be extremely cautious when using these drugs in the compassionate use.

September 2020: The history of combating life-threatening infections proves the importance of inhibiting the multiplication of the pathogen in the early stages of the disease. To inhibit SARS-CoV-2 infection, it is necessary to achieve high blood concentrations of CQ/HCQ as soon as possible, considering the potential toxicity. Currently, there is no evidence for or against the administration of these drugs for the prevention or early treatment of COVID-19. It has also been emphasised that unwarranted recommendation of CQ/HCQ without evidence of their efficacy and safety could undermine public confidence in the regulatory mechanisms of the pharmaceutical industry.
The results of the study investigating e.g. in vitro, in silico, in vivo and clinical evaluation of drug candidates for COVID-19 has been provided. The various phases of clinical trials on the efficacy of CQ/HQC were ongoing (e.g., phase II of interventional trial in USA; phase III of interventional trial in Spain; phase I of randomised trial in USA; phase III of randomised trial in France, Brazil, China, and India). However, the studies reviewed yielded conflicting results regarding CQ/HQC potential as COVID-19 drugs. The two main therapeutic approaches to COVID-19 treatment (repurposing existing drugs and developing new biologics targeting molecules involved in SARS-CoV-2 viral entry) and the corresponding clinical trials were described. Among the 396 different interventional therapeutics tested, mainly small molecules that were already approved for other indications were presented (73%). HCQ was the most frequently tested interventional therapeutics tested, mainly small molecules and biologics, and the limited treatment duration should be considered. The above doses are much lower compared to the doses used in the reported RCTs (i.e., one third of the doses used in the RECOVERY trial). However, the use of high doses in the RCTs could be explained as an attempt to maximise the direct antiviral activity of the drug. The mortality of patients in the high and low-dose groups was compared, but the difference in mortality was not considered statistically significant. Therefore, further RCT’s should pay attention to drug dose and treatment duration. The review paper on controversies related to repurposing of HCQ for the treatment of COVID-19 was given. There is much evidence to justify the use of HCQ. Data from a non-randomised observational study conducted in China suggest reduced disease severity, improved radiological findings, faster viral clearance, and earlier recovery after treatment with HCQ. Randomised controlled clinical trials in a cohort of 62 COVID-19 patients demonstrated the efficacy of HCQ in terms of shorter time to clinical recovery and faster resolution of pneumonia. In a single-centre, non-randomised clinical trial in France, HCQ was found to be superior to supportive therapy, and contributed to a 70% reduction in viral load. A non-randomised, non-comparative observational study demonstrated the benefits of treatment with HCQ/AZ. The retrospective study examined the effect of the same combination on mortality, recovery, and viral clearance. Viral clearance was seen in 91.7% of patients after less than 10 days of treatment. Adverse clinical outcomes were noted in 4.3% of patients, while 0.75% of patients died due to respiratory failure. The above studies encouraged further investigation. Of the 688 and 2122 COVID-19 studies registered in the Chinese Clinical Trial Registry and the U.S. National Library of Medicine, 11 and 218 studies, respectively, aimed to gain better insight into the therapeutic efficacy of HCQ in curing SARS-CoV-2 infection. However, severe side effects of HCQ were observed. In addition to mild gastrointestinal and cutaneous side effects, this drug can lead to severe cardiotoxic, metabolic, and neuropsychiatric manifestations. Potential risk factors for cardiotoxicity include male gender, older age, and concomitant use of NSAIDs. The incidence of adverse events ranged from 0.06% to 33.67%, depending on the HCQ dose administered and the concomitant presence of cardiac, hepatic, and renal disease in the patients studied. December 2020: The results of the study investigating the preventive effect of HCQ in COVID-19 patients with rheumatic diseases in the Republic of Korea who were prescribed this drug were published. The attack rate of COVID-19 in HCQ users (2.3%) was almost identical to that in non-users (2.2%), suggesting that prophylactic use of HCQ does not prevent COVID-19 in patients with rheumatic diseases. The protective role of CQ and its derivatives as prophylaxis for COVID-19 was studied in Spanish patients conducted in Italy and Belgium in more than 10,000 hospitalised COVID-19 patients reported a significant decrease in mortality, and no safety concerns in patients treated with HCQ compared with those who did not receive HCQ. Despite their limitations (retrospective nature and lack of randomisation of patients), the data obtained from ROS can be taken into account in the planning of new, well-prepared and executed RCT. The low to moderate total HCQ dose used in the Belgian and Italian studies, and the limited treatment duration should be considered. The above initial doses were much lower compared to the doses used in the reported RCTs (i.e., one third of the doses used in the RECOVERY trial). However, the use of high doses in the RCTs could be explained as an attempt to maximise the direct antiviral activity of the drug. The mortality of patients in the high and low-dose groups was compared, but the difference in mortality was not considered statistically significant. Therefore, further RCT’s should pay attention to drug dose and treatment duration. The review paper on controversies related to repurposing of HCQ for the treatment of COVID-19 was given. There is much evidence to justify the use of HCQ. 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with autoimmune diseases. The untreated control group was compared with the treated group in terms of sex, age group, and incidence region. The results obtained showed no differences in the prevalence of COVID-19 between untreated individuals and patients with autoimmune diseases treated with CQ or its derivatives. The inhibitory effect of HCQ on viral entry and transport determined in vitro could not be replicated in in vivo animal models or in clinical observational studies and clinical trials of pre- and post-exposure prophylaxis in healthcare workers. The most likely reason for the HCQ inefficacy is pharmacokinetic failure due to the inability to achieve adequate concentration of the drug at the target site and attenuate its inhibitory effect. However, the use of higher doses of HCQ for prophylaxis could raise serious safety concerns, and other possible interventions should be considered. The first comprehensive network meta-analysis (NMA) of the pharmacological treatment of COVID-19, which included 47 pharmacological agents and treatment regimens used in 49,569 COVID-19 patients, was conducted. HCQ, which attracted considerable interest not only from scientists and professionals but also from the media, failed to reduce mortality or protect against disease progression. This finding is confirmed by an in vitro study which revealed that CQ cannot prevent SARS-CoV-2 from entering human lung cells and subsequently spreading throughout the lungs. In addition, this study found both cardiac and non-cardiac safety risks associated with HCQ.

5 Conclusion

The initial optimism about the efficacy of CQ/HCQ in curing COVID-19 infections did not last long. The various clinical studies indicated that CQ/HCQ helped to clear the viral load of SARS-CoV-2 and improve the imaging findings of the lungs. However, the methodological limitations of the studies conducted (e.g., lack of a control group and small sample size), and the serious adverse effects observed called into question both the efficacy and safety of the drug. As a result, the emergency use authorisation for these drugs issued by the FDA on March 28, 2020 was revoked on June 15, 2020. Due to the spreading pandemic and the rapidly growing demand for effective drugs, large-scale, randomised, double-blind clinical trials of CQ/HCQ were conducted to evaluate their effect on COVID-19 infections. As a result, the role of CQ/HCQ in the treatment of COVID-19 patients not only became the focus of the scientific community, but also attracted the attention of policy makers and industry.

In contrast to the promising early results, the largest randomised controlled trials (SOLIDARITY, RECOVERY) and network meta-analysis conducted in nearly 50,000 COVID-19 patients failed to confirm that CQ/HCQ were able to reduce overall mortality or act as protective agents. In addition, safety issues related to cardiotoxicity remain unresolved.

Based on the available data on the efficacy of CQ/HCQ as pre- and post-exposure prophylaxis and treatment for non-hospitalised and hospitalised patients with COVID-19 collected in 2021, their clinical use is not recommended.

List of abbreviations

**Popis kratica**

<table>
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SAŽETAK

Priča o klorokinu u prvoj godini pandemije COVIDA-19
Matea Hunić, Renata Teparić, Monika Kovačević i Lidija Barišić*

Pojava COVIDA-19 uzrokovala je globalnu zabrinutost zbog visoke zaraznosti i mortaliteta. Stoga je razvoj lijekova protiv COVIDA-19 postao prioritet istraživačkim skupinama diljem svijeta. Pri tom je posebnu pozornost privukla moguća prenamjena prethodno odobrenih lijekova, uključujući i antimalarijski lijek klorokin. Cilj ovog rada je (i) prikazati pregled recentnih kemijskih metoda primijenjenih za sintezu klorokina i hidroksiklorokina te (ii) dati uvid u podatke o njihovoj učinkovitosti protiv COVIDA-19 prikupljene tijekom 2020. Nažalost, početni obećavajući rezultati nisu potvrđeni, a jasni i nedvosmisleni zaključci o kliničkoj učinkovitosti klorokina i hidroksiklorokina još nisu postignuti.

Ključne riječi
Kemijska sinteza, klorokin, COVID-19, hidroksiklorokin, SARS-CoV-2

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