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Efficacy and safety of chloroquine and hydroxychloroquine for COVID-19: A comprehensive evidence synthesis of clinical, animal, and in vitro studies

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Abstract

Background: The world is facing a pandemic of COVID-19, a respiratory disease caused by a novel coronavirus which is now called SARS-CoV-2. Current treatment recommendations for the infection are mainly repurposed drugs based on experience with other clinically similar conditions and are not backed by direct evidence. Chloroquine (CQ) and its derivative Hydroxychloroquine (HCQ) are among the candidates. We aimed to synthesize current evidence systematically for in vitro, animal, and human studies on the efficacy and safety of chloroquine in patients with COVID-19.

Methods: The Cochrane Library, Google Scholar, PubMed (via Medline), Embase, Scopus, and Web of Science, MedRxiv, clinical trial registries including clinicaltrials.gov, ChiCTR (Chinese Clinical Trial Registry), IRCT (Iranian Registry of Clinical Trials), and the EU Clinical Trials Register. We used the Cochrane tool for risk of bias assessment in randomized studies, the ROBINS tool for non-randomized studies, and the GRADE methodology to summarize the evidence and certainty in effect estimates.

Results: The initial database searching retrieved 24,752 studies. Of these, 15,435 abstracts were screened and 115 were selected for full-text review. Finally, 20 human studies, 3 animal studies, and 4 in vitro studies were included in this systematic review. The risk of bias within studies was unclear to high and the overall certainty in evidence-based on GRADES- was very low. HCQ may be effective in clinical improvement in a subset of patients with COVID-19. However, the frequency of adverse events was higher in patients taking HCQ compared to standard of care alone. In contrast, animal studies, did not report any adverse effects. Furthermore, clear benefit of the drug in the survival of the animals has been reported. Most in vitro studies indicated a high selectivity index for the drug and one study that used a human coronavirus reported blockage of virus replication.

Conclusion: Current evidence background is limited to six poorly conducted clinical studies with inconsistent findings which fail to show significant efficacy for HCQ. Safety data is also limited but the drug may increase adverse outcomes. Routine use of the drug is not recommended based on limited efficacy and concerns about the drug safety especially in high-risk populations.

Keywords: Efficacy, Safety, Hydroxychloroquine, COVID-19, Systematic review

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↑What is "already known" in this topic:
The world is facing a pandemic of COVID-19, so it is essential to survey in vitro, animal, and clinical evidence background for the effectiveness and safety of Hydroxychloroquine for treating patients with COVID-19 in around the world.

→What this article adds:
There is insufficient evidence to draw conclusions on the efficacy and safety of chloroquine and Hydroxychloroquine based on current evidence.

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Introduction

From the black plague in the 14th century Europe to the cholera pandemics of the 19th century and the 1918 Spanish flu, epidemics have always been a subject of concern and a cause of mass mortality as well as considerable economic, social, and psycho logic harm to populations throughout history. The most recent one, to date, has been the COVID-19 pandemic, caused by SARS-CoV-2, a member of the family Coronaviridae, that is mainly characterized by severe acute respiratory syndrome. This virus appeared in China in December 2019 and rapidly spread worldwide, creating a public health emergency around the world in 6 months.

Since SARS-CoV2 is a novel pathogen, no standardized treatment is currently available. One proposed agent is chloroquine (CQ), classically known as an antimalarial and immunomodulatory agent. Preclinical evidence suggests that clinical research on CQ in COVID-19 patients is justified (1). In a previous SARS-CoV epidemic, it was proposed that CQ could be considered as a treatment (2, 3). During the first months of the COVID epidemic, a letter-to-the-editor claimed that CQ has shown efficacy and safety in an ongoing multicenter study in China for pneumonia caused by SARS-CoV-2, and it was recommended to be included in treatment regimens for COVID-19 pneumonia (4). Therefore, CQ and Hydroxychloroquine (HCQ) have been considered as potential treatments for COVID-19.

Whether systematic reviews of preclinical studies can accurately predict clinical outcomes is controversial. However, preclinical research can provide useful information about the biological plausibility of human drug trials (5). Its special settings that enable direct study of the mechanisms of disease as well as drug pharmacodynamics and pharmacokinetics can aid clinical decision-making (6).

In this systematic review, we aimed to comprehensively synthesize in vitro, animal, and clinical evidence background for the effectiveness and safety of CQ, including its sulfate and phosphate salts, and Hydroxychloroquine for treating patients with COVID-19.

Methods

Protocol and Registration

This systematic review was conducted under emergency conditions of the global coronavirus pandemic. The protocol was developed by a team of clinical epidemiologists (H.R.B. and Y.M.), physicians (H.R.B., MAK, and F.B.), and a librarian (R.V.A.).

Eligibility Criteria

Human controlled studies (including interventional and observational studies), animal studies, and in vitro studies evaluating the effect of CQ, Hydroxychloroquine, or other quinine derivatives on coronavirus infections, including SARS, MERS, and COVID-19, up to June 30, 2020 were included. No limitation was used based on language, publication status, or length of follow-up.

Information Sources

The Cochrane Library, Google Scholar, PubMed (via Medline), Embase, Scopus, and Web of Science, MedRxiv; and clinical trial registries, including clinicaltrials.gov, ChiCTR (Chinese Clinical Trial Registry), IRCT (Iranian Registry of Clinical Trials), and the EU Clinical Trials Register.

Search

The search strategy was developed based on study questions and relevant key words by a medical librarian (R.V.A.) and a physician (F.B.) with systematic review experience for all information sources. An update search was done on July, 30, 2020 for further human studies.

Study Selection

All retrieved records were comprehensively screened based on title by 2 authors independently (F.B. and M.A.K.). Relevant studies were imported into a citation manager (Endnote X7) for screening the abstract after removal of duplicated sources. The abstracts matching the eligibility criteria were selected and categorized into groups based on study type and participants by one reviewer (M.A.K. or F.B.) and the full-texts of the studies were retrieved. We contacted the authors when we could not access the full-texts. The full-texts of the in vitro studies as well as the animal models were reviewed by a virologist (B.S.). The full-texts of clinical studies were evaluated for eligibility by 2 physicians (F.B. and MAK). The references of included articles were hand-searched for further relevant studies.

Data Collection Process

To ensure uniform and comprehensive data extraction among different data extractors, the reviewers developed Microsoft Access forms and tables, including information recommended in the Cochrane Handbook of Systematic Reviews of Interventions (7). These items included the name of the first author, publication year, region, descriptions of study design, participants, interventions, comparisons, outcomes and results, as the sources of funding and key conclusions by the original study authors. Data were extracted by the same review authors who screened the article full-texts.

Risk of Bias in Individual Studies

Risk of bias within the clinical studies was assessed using the Cochrane risk of bias tool, which evaluates studies in 5 domains and rates the study for each domain of bias as having low, unclear, or high risk of bias (8). The ROBINS tool was used to assess risk of bias within nonrandomized studies. The SYRCLE's tool was used to assess the risk of bias in animal studies (9). OHAT risk-of-bias tool was employed for in vitro studies (10, 11). The risk of bias for each study was assessed by 2 reviewers independently (F.B., B.S., Y.M.) and in case an agreement could not be reached between the first 2 reviewers, a third reviewer would intervene (H.R.B).

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Summary Measures and Synthesis of Results

Relative risk and relative risk reduction were used to summarize data for dichotomous outcomes, and mean difference was used for continuous variables. Because of the significant variation in study methodology and outcome measurements, a meta-analysis was not possible for most of the outcomes. Qualitative synthesis was done using the GRADE approach per study outcome**.** We followed the SWIM guidelines for reporting synthesis without meta-analysis (12).Viral clearance was summarized as the odds ratio of the proportion of patients who tested negative on PCR testing within 10 days of medication use.

Results

Study Selection

A total of 24,752 studies were retrieved by searching the mentioned databases, that were screened by title. Of these, 15 435 abstracts were screened and 115 were selected for full-text review. The eligible studies were categorized at this stage based on their subject (in vitro, animal, and human studies) and were assigned to expert authors for full-text review and final inclusion. Seven human studies were excluded based on full-text, as they were reviews or perspective articles.

The update search retrieved 1628 studies that were screened by title by 1 author. A total of 248 abstracts were screened and 82 full-text articles were screened by 2 authors independently. Finally, we included 18 non-randomized studies and 6 randomized human studies.

The PRISMA flow diagram is presented in Figure 1.

Study Characteristics

Human Studies: We included 6 RCTs and 14 nonrandomized controlled human studies, all of which were conducted among hospitalized patients. None of the studies were placebo-controlled. Also, pregnant and breastfeeding women as well as patients with underlying conditions were excluded. Most studies compared the recommended dose of HCQ daily (ranging from 400mgs/day to 800mgs/day) with drug regimens without HCQ/CQ. Also, 3 studies used a high dose of HCQ, 6 had AZI, and 2 had Lopinavir/ritonavir as part of their control regimen. The follow-up duration varied among the studies and ranged from 7 to 30 days (Tables 1 and 2).

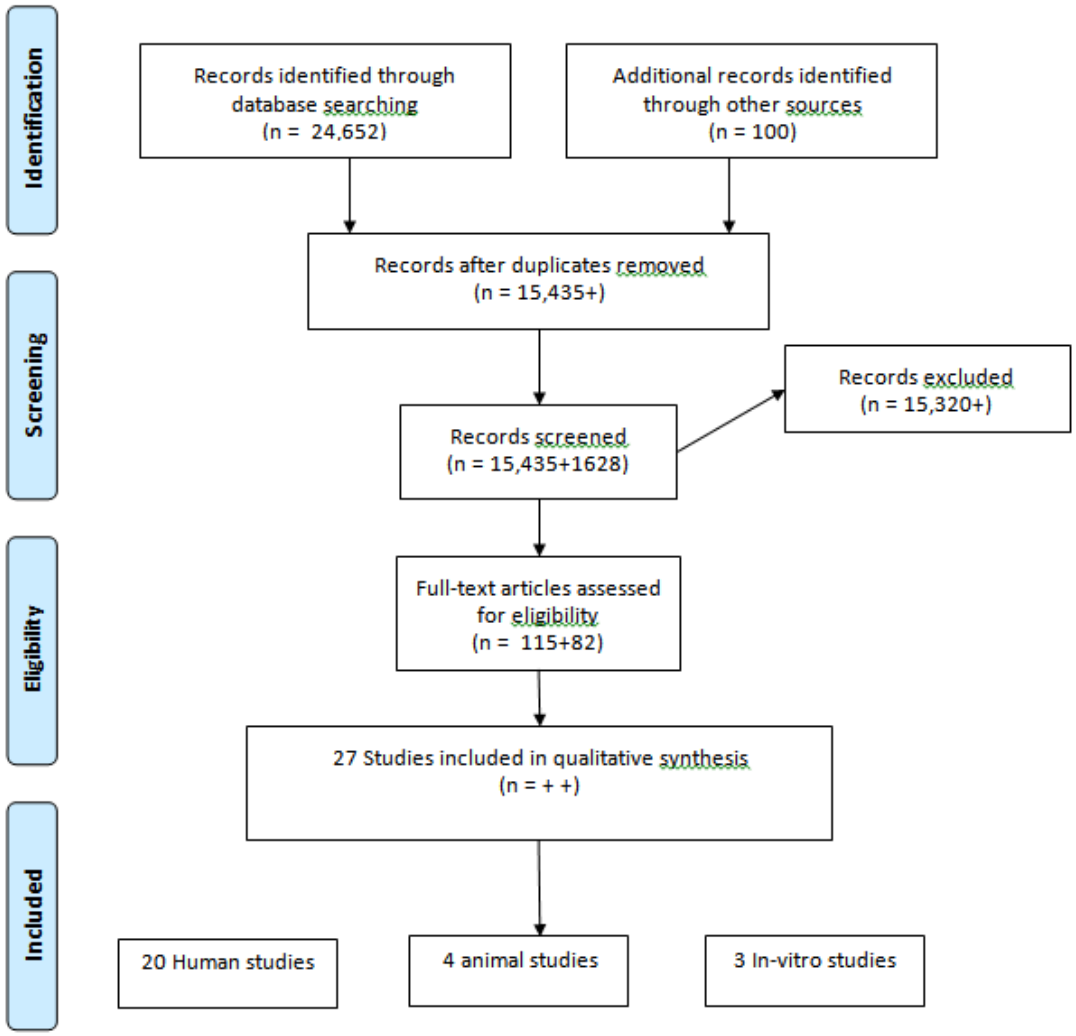

Fig. 1. RISMA Flow Diagram

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Table 1. The Risk of Bias within Randomized Controlled Trials

ID	First Author	Random Sequence Generation	Allocation Concealment	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Overall
1	Chen, Zh[1]	L	U	U	U	L	H	L
2	Chen, J[2]	U	H	H	H	U	U	H
3	Tang, W[3]	L	L	H	H	U	L	H
4	Borba, MGS[4]	L	L	U	L	L	U	L
5	Cavalcanti, AB[5]	L	U	H	H	L	L	L
6	Huang, M[6]	U	L	H	H	H	L	H

Guide to the table: Unclear RoB Low RoB High RoB

1. Allocation concealment and blinding procedures were neither explained in the article nor in the protocol. The outcomes reported were completely different from the ones initially planned in the protocol. In the protocol, the researchers planned to evaluate viral clearance and immunologic response. However, they merely reported clinical outcomes in the article.
2. Unclear description of randomization is given. Antiviral regimens were not the same between groups.
3. Open-label randomized trial. With control group receiving standard care. Reporting of outcomes was complete and dropout frequency was 6 out of 75. Use of intervention varied among participants especially regarding the timing in relation to symptom onset.
4. Randomized controlled trial. The pharmacist distributing the drugs was not blinded, which might have been the source of uncertainty in blinding of participants and personnel.
5. Open-label randomized study with 6-item randomization blocks. Both the patients and the personnel were aware of the randomization group. Selective reporting was unlikely because the protocol was available, and all the predetermined outcomes have been reported. Appropriate use of intention-to-treat analysis makes it unlikely for incomplete reporting to affect study results.
6. Open-label randomized study with 4-item randomization blocks (according to the protocol but not mentioned in the published report). There was a baseline difference between the groups in "days from onset to treatment." Concealment of randomization was done using sealed envelopes. The study protocol was available and the risk of selective reporting was low. However, the published results are preliminary and there is serious risk of data incompleteness.

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Table 2. The Risk of Bias within Non-Randomized Controlled Human Studies

No	First Author	Bias due to Confounding	Selection Bias	Classification Bias	Deviation from Exposure	Missing Data	Measurement of Outcomes	Selective Reporting	Overal
1	Matthieu Mahévas	L	L	L	M	L	S	NI	M
2	Gautret, Philippe	M	NI	L	L	S	M	NI	S
3	Singh, Sh	M	M	L	S	M	NI	NI	S
4	Sbidian	M	M	L	S	S	M	NI	S
5	Rosenberg	M	L	L	S	M	S	NI	M
6	Mehra, MR	M	L	L	M	NI	S	NI	M
7	Mallat, J	S	S	L	S	NI	NI	NI	C
8	Magagnoli	L	S	M	S	L	M	L	S
9	Lagier, JC	S	S	L	NI	S	M	NI	C
10	Geleris,J	M	M	L	S	M	L	NI	M
11	Yu, B	M	M	M	S	L	L	NI	S
12	Arshad, S	M	L	L	M	L	M	NI	M
13	IP, A	M	M	L	M	L	M	L	M
14	Mazzanti,	L	L	L	M	L	M	L	M

Guide to the table: L: Low RoB; NI: No Information; M: Moderate RoB; S:Serious RoB; C: Critical RoB

1. Observational study with well-matched between-group baseline characteristics, and the same dosage of chloroquine for all patients. Inconsistency in measurement and recording of the outcomes is suspected.
2. Children who usually have milder course of disease were not included to the treatment group, while they were included in the comparison group. No placebo was used in comparison group. Participants and personnel were not blinded. There was a high dropout rate (6/26) in the treatment group with reasons potentially relevant to the side effects of hydroxychloroquine, including admission to ICU and treatment cessation. An intention-to-treat analysis should have been used.
3. Retrospective cohort analysis of hospital data. Patient selection based on international criteria. Confounding variables addressed by propensity scores in analysis. No information was given on how the exposure use was confirmed. No information was given on how outcomes were assessed. The study protocol was not available to assess the selective reporting. No information was given on missing data.
4. Retrospective study of hospital data on PCR confirmed COVID19 patients. Augmented inverse probability of treatment weighted (AIPTW) estimates of the average treatment effect (ATE) were used to account for confounding. Data were extracted using artificial intelligence from data systems and manually from medical text reports. Exposures measured according to hospital-registered prescriptions only.
5. Retrospective multicenter cohort. Random sample of patients admitted to 25 hospitals. The exposure dosage and regimens differed across the study sample. A low proportion of patients used HCQ alone. There was inadequate description of how outcome measurement and data extraction were done and there is a high risk of bias due to the variability in exposures and outcome measurements across hospitals.
6. Multinational registry analysis (retrospective observational) on patients with PCR-confirmed COVID-19. Confounding was adjusted for in statistical analysis. No protocol was available; therefore, risk of bias due to selective reporting cannot be estimated. Outcome assessment is suspected to differ significantly across the study centers and due to the observational nature of the study.
7. Retrospective observational study on patient data from 1 hospital. The study methodology was poorly reported. All patients had PCR-confirmed COVID-19. There was significant difference in the frequency of comorbidities between HCQ and control groups, and consequently, a serious risk of bias due to confounding. No information was given on missing data. No study protocol was available to confidently assess selective reporting.
8. Retrospective observational study in 1 veterans' hospital. All patients had PCR-confirmed COVID19. Propensity score analysis was used to address confounding. Exposure was defined based on the information from hospital registry of drug dispensing for each patient. No study protocol was available to assess selective reporting. Outcome assessment may cause moderate risk of bias due to the observational nature of the study.
9. Retrospective cohort study. All patients had PCR and culture-confirmed COVID-19. Cardiovascular disease was more common among the control group. The treatment was initiated at an earlier stage of the infection in this study. It is not clear how exposure to the treatments was assessed. No study protocol was available to assess the risk of selective reporting.
10. Retrospective observational study at a quaternary, acute care hospital. Patients had PCR confirmed COVID-19. Confounding was adjusted using propensity score matching/analysis. The exposure was evaluated by patient exposure to the drug before or during the admission and thus may vary across participants. No study protocol was available and risk of selective reporting could not be assessed.
11. Retrospective observational study on critically-ill (selection bias) inpatients with CT and PCR-confirmed COVID19. There was no significant baseline difference in confounding variables between groups. Patient classification (based on dug exposure) may have been subject to error because mere prescription may not show drug use by the patient. No information is given on how the outcome measurements were standardized.
12. A multicenter retrospective observational study on inpatients with PCR-confirmed COVID-19. Treatment regimens were uniform across hospitals. There was moderate risk of bias due to confounding because of the nonrandomized nature of the study and that HCQ was used among patients with more severe disease. This may underestimate the effects of chloroquine. This confounding was partially adjusted for statistically.
13. Retrospective multicenter cohort based on HER data. The study design made it susceptible to bias due to confounding and misclassification. Drug administration was well documented. There was moderate risk of bias due to missing outcome data.
14. Initial results of a prospective observational study with an available protocol. Patients had PCR-confirmed COVID-19. Confounding variables have been well adjusted for. Although the study was observational, enough documentation was performed for the degree of exposure to drugs. Outcome measurement was at moderate risk of bias due to the nature of nonblinded and observational nature of the study.

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Animal Studies: One of the animal studies used SARS-CoV-1 in BALB/c female mice and tested the efficiency of Amodiaquine and CQ, with a dose response design detecting the virus replication in lung homogenates. The other 2 animal studies used HCoV-OC43 virus as the model. One of these 2 studies aimed at identifying the effect of CQ-sulphate in adult C57BL/6 mice followed by studying the protective effect of the drug transferred to litters by placenta or milk of the drug treated mothers. The other study optimized the detection of a luciferase labelled virus in adult BALB/c mice but used the drug CQ as a control to block the virus replication and control the background luciferase detection in treated mice. The study characteristics of the 3 animal studies are summarized in Table 3 in Appendix.

Interestingly, 2 of the animal studies included in vitro examinations as part of their design, which were added to the in vitro studies.

Risk of Bias within Studies

Among the 6 randomized human studies included to the systematic review, the risk of bias due to random sequence generation was unclear among 2 studies. Allocation concealment introduced a high risk of bias in 1 study and was unclear in 2 other studies. Only 1 study was blinded. Two studies were at high risk of bias due to incomplete outcome data, and 1 study was at high risk of bias due to selective reporting. The details are presented in Table 4.

Among the 14 nonrandomized human studies, 7 were at moderate overall risk of bias, 5 were at serious risk, and 2 had a critical risk of bias. The details are presented in Table 5.

Risk of bias was also assessed in animal studies, and they were found to be generally reliable (Table 6).

Synthesis of Results

Human Studies: Because of the heterogeneity in the type of included studies, different methods of outcomes assessment, and the statistical methods for summarizing the results, we could not combine the results in a meta-analysis. Therefore, we synthesized the results qualitatively.

Clinical Response: One study showed a reduced mean of days of having fever for patients in the HCQ group compared to the control (2.2 vs 32) and another study showed fewer number of days with fever for the HCQ group compared to the control group. Taking HCQ was also associated with shortened duration of cough in 1 study (an average of 2 vs 3.1 days). In 1 study, administering HCQ was related to a higher rate of clinical improvement within a course of 28 days, whereas in 2 other studies, HCQ treatment was associated with a higher rate of progression to severe illness.

Adverse Events: The most notable adverse events were GI events, death due to unclear cause, and cardiac adverse events, all of which were more prevalent among the HCQ groups compared to controls (SOC). One study that considered the incidence of "any" adverse event showed a higher incidence among the HCQ group compared to that of the control group. The incidence of other symptoms, such as headache, rashes, nausea, and weakness, were low in both groups, without a significant difference between the HCQ and the control groups.

Table 3. The Risk of Bias within Animal Studies

First Author Study Year	Randomization	Allocation concealment	Experimental conditions	Exposure characteristics	Reliability of outcome assessment methods	Blinding of outcome assessment	Incomplete outcome data	Selective Reporting
Dale L Barnard[1] 2006	5	5	1	3	1	1	4	3
Els Keyaerts[2] 2009	2	3	2	3	1	2	3	3
Junwei Niu[3] 2020	2	2	2	1	1	1	4	5
Scoring system:	Definitely High =1	Probably High = 2		Probably Low = 3	Definitely Low = 4		Unclear = 5	

1. The reporting of the outcome and the statistical methods were appropriate.
2. The presentation on the design of the study has some mistakes and the outcome based on the published design is suboptimal.
3. The number of animals used in the experiment was not clear.

Table 4. Virological Response

Outcome	Study	Outcome description	Study design	HCQ	Control	Effect Estimates	p
Viral clearance after 14 days	Mingxing Huang	Proportion of PCR negative on day 14	Randomized Clinical study	10/10	11/12	-	-
	Chen, J	Proportion of PCR negative on day 7	RCT	13/14	2/16	91.00 (7.34–112.94)	0.004
Viral clearance After 6-10 days	Gautret, P	Proportion of PCR negative on day 6	Non-randomized trial	8/16	14/16	0.142 (0.024–0.844)	0.03
	Mingxing Huang	Proportion of PCR negative on day 10	Randomized Clinical study	9/10	9/10	1.00 (0.053–18.57)	0.99
	Tang, W	Proportion of PCR negative on day 6	Randomized trial	34/75	41/75	0.687 (0.36–1.30)	0.25
	Lagier	Proportion of PCR negative after 10 days	Retrospective cohort	643/3119	151/618	0.803 (0.655–0.983)	0.03

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Tables 4 to 6 present the synthesis of the effect estimates per outcome and Table 7 is the summary of findings in GRADE format.

Animal Studies: Two animal studies showed the effectiveness of CQ in mice models using a wild type HCoV-OC43 and another recombinant rOC43-ns2DelRluc from

Table 5. Clinical improvement

Outcome	Study		Hydroxychloroquine	Control	Effect Estimate (Odds Ratio) Or Mean Difference (SD) with Standardized Mean Difference 95 % CI	p
Fever	Chen, Zh	Mean days (SD)	2.2 (0.4)	3.2 (1.3)	1.00 (0.51-1.48)	0.0001
	Chen, J	Median (range)	1 (0-2)	1 (0-3)	0.00 (-0.50-0.50)	0.98
Cough	Chen, Zh	Mean Days (SD)	2 (0.2)	3.1 (1.5)	1.1 (0.55-1.64)	0.002
Clinical improvement	Tang,	The improvement rate of clinical symptoms within 28-day	47/70	48/80	1.36 (0.69-2.66)	0.36
Discharge home or to a rehab center	Sbidian	Number of patients	351/623	1507/3792	1.95 (1.64-2.32)	0.001
	Magagnoli	Number of patients	70/97	140/158	2.92 (1.77-4.81)	0.001
	Mingxing Huang	Proportion of hospital discharge on day 14	10/10	6/12	-	-
	Mahevas	Proportion of patients discharged by day 21	67/84	71/89	1(0.9-1.2)	
Clinical progression to severe illness	Chen, Zh	Rate of Progression to severe illness	4/31	0/31	-	-
	Lagier	Combined death/ICU admission/long hospitalization	8/101	13/162	0.98 (0.39-2.46)	0.97
Mean length of hospital stay	Cavalcanti	Duration of hospital stay	9.6(6.5)	9.5(7.2)	-0.1(-1.58-1.38)	0.89
Radiological response	Mingxing Huang	Proportion of CT-scan improvement (day 10)	7/10	5/12	3.26 (0.55-19.25)	0.19
		Proportion of CT-scan improvement (day 14)	10/10	9/12	-	-

Table 6. Adverse events

Outcome category	Outcome	Study	Hydroxychloroquine	Control	Effect Estimate (Odds Ratio) Or Mean Difference (SD) with Standardized Mean Difference 95 % CI	p
Fatal outcomes	Death (any cause)	Sbidian	111/623	865/3792	0.781 (0.630–0.968)	0.02
		Gautret, P	1/26	0/16	-	
		Rosenberg	54/271	28/221	1.715 (1.044–2.816)	0.03
		Singh	104/910	109/910	0.948 (0.71–1.26)	0.14
		Lagier	2/101	4/162	0.798 (0.14–4.43)	0.79
		Magagnoli	27/97	18/158	3.00 (1.54–5.81)	0.001
		Yu, B	9/48	238/502	0.25 (0.12–0.53)	0.001
		Cavalcanti, AB	7/159	6/173	1.28 (0.42–3.89)	0.6
		Andrew, Ip	110/441	119/598	1.33 (1.00–1.79)	0.050
	Combined Intubation or death	Mahevas	9/84	8/89	1.2 (0.5-3)	
		Geleris	262/811	84/565	2.73 (2.07–3.59)	0.000
		Mahévas, M	3/84	4/97	0.86 (0.18–3.96)	0.84
		Borba,	16/41	6/41	3.73 (1.28-10.57)	0.01
Cardiac adverse outcomes	Cardiac arrest	Rosenberg	37/271	15/221	2.19 (1.69-4.11)	0.01
	Arrhythmia	Rosenberg	44/271	23/221	1.668 (0.972–2.860)	0.06
	QT prolongation	Rosenberg	39/271	13/221	2.689 (1.397–5.17)	0.003
		Nicholas J Mercuro	3/37	7/53	0.579 (0.14–2.40)	0.45
		Mahévas, M	8/84			
Severity-related outcomes	Need for mechanical ventilation	Rosenberg	51/271	18/221	2.61 (1.47-4.62)	0.001
	ICU transfer	Singh	46/910	57/910	0.796 (0.53-1.18)	0.26
		Cavalcanti, AB	12/159	12/173	1.09 (0.47-2.51)	0.83
		Rosenberg	55/271	27/221	2.19 (1.16–4.11)	0.01
		Sbidian	206/623	739/3792	2.04 (1.69-2.45)	
		Mahevas	24/84	23/95	1.25 (0.64-2.44)	0.50

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Table 6. Ctd

Outcome category	Outcome	Study	Hydroxychloroquine	Control	Effect Estimate (Odds Ratio) Or Mean Difference (SD) with Standardized Mean Difference 95 % CI	p
Gastrointestinal adverse outcomes	Gastrointestinal	Chen, J	4/13 (26.7%)	3/14 (20%)	1.62 (0.28–9.25)	0.58
	Nausea	Gautret, P	1/26	0/16	-	
		Mingxing Huang	4/10	0/12	-	
	Diarrhea	Rosenberg	22/271	16/221	1.13 (0.58–2.21)	0.71
Other adverse outcomes	Any Adverse effect	Tang	21/70	7/80	4.49 (1.75-11.31)	0.001
		Mingxing Huang	5/10	0/12	-	
	Rash	Chen, Zh	1/31	0/31	-	
	Hypoglycemia	Rosenberg	9/271	6/221	1.230 (0.44–3.51)	0.38
	Paresthesia	Tang	9/80	2/80	4.943 (1.03–23.65)	0.04
	Paresthesia Weakness	Chen, J	1/13	0/14	-	

Table 7. GRADE summary of findings table

Outcomes	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
Virological Response assessed with: negative throat swabs on day 6-7 post-enrollment	232 (9 RCTs)	⊕◯◯◯ VERY LOW [a,b,c,d]	The evidence is very uncertain about the effect of hydroxychloroquine on the rate of viral clearance after one week of treatment. There are two RCTs, two non-randomized trials, and two retrospective cohort studies with significantly inconsistent results.
Symptom Improvement	286 (4 RCTs)	⊕◯◯◯ VERY LOW [a,b,e,f,g]	The evidence is very uncertain about the effect of hydroxychloroquine on symptom Improvement. The study results are inconsistent and inconclusive.
Radiological Progression	89 (2 RCTs)	⊕◯◯◯ VERY LOW [d,l]	Hydroxychloroquine may have little positive to no effect on radiological progression but the evidence is very uncertain and more research is needed.
ICU Admission	5433 (5 observational studies)	⊕◯◯◯ VERY LOW [b,h,i]	The evidence is very uncertain whether chloroquine/hydroxychloroquine could affect the rate of ICU admission.
Cardiac Adverse Events	501 (5 RCTs)	⊕◯◯◯ VERY LOW [b,j,k]	Hydroxychloroquine may significantly increase the rate of adverse events especially QT prolongation and cardiovascular death but the evidence is very uncertain.
Death	10668 (10 observational studies)	⊕◯◯◯ VERY LOW [a,b,d,e,g]	Current available evidence is very uncertain whether and in what direction chloroquine/hydroxychloroquine could affect mortality due to COVID-19. The evidence is inconclusive and inconsistent.
Gastrointestinal Adverse Effects follow up: range 5 days to 30 days	693 (6 observational studies)	⊕◯◯◯ VERY LOW	The evidence is very uncertain and variable regarding the effect of HCQ on the rate of gastrointestinal adverse events (nausea/vomiting/diarrhea) in patients with COVID19.

Explanations

a. Three studies reported this outcome. None was blinded and one was not randomized.
b. There is significant heterogeneity in trial results. This might be due to differences in outcome measurements and also study populations.
c. Total number of patients in the control group=110. confidence intervals
d. There is special concern for publication bias due to the recent emergence of the disease and the timing of this review. Trials that are not published yet may show different results.
e. None of the studies were placebo-controlled and the standard-of-care which was the control intervention in all of the studies, differed within and across studies. The methods of outcome measurement also differed across studies. Another concern is about time from symptom-onset to treatment initiation which is different and not described in adequate detail.
f. Total number of patients in the control group for this outcome =111
g. Precision is downgraded due to the wide confidence intervals of effect estimates as presented in the results table.
h. Please refer to risk of bias table.
i. Total number of events in the control group was 21.
j. The quality is downgraded due to risk of bias concerns. None of the studies measured the adverse effects systematically. There is a special concern for under detection of side effects in the included studies.
k. Total number of events in control group was 12.
l. total number of events in the control group was 9.

which the ns-2 gene was replaced with a reporter gene. In both models, intracerebral inoculation of the virus was treated with CQ and improved the survival of the infected mice compared with the animals in the control group. The models were not similar: one study examined survival in suckling mice but the other assessed survival of adult mice. Also, the viruses were not identical in the 2 models. CQ in another mice model did not block infection by SARS-CoV.

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In vitro Studies: Recent studies of COVID-19 showed promising results with CQ and HCQ in Vero cell models reported in 3 studies published in 2020 (13). Also, all studies, except 1 which used human primary cells, reported the efficiency of the drug in cell models. Although the selectivity index of the studies varied, they generally show that the drug is safe. Additionally, the amount of virus used to assess the blocking effect of the drugs on virus replication varied between studies, and higher viral inoculates correlated with a higher dose of drug needed to block the virus infection. All in vitro studies supported the antiviral effect of CQ and HCQ in human coronaviruses, including SARS, MERS, and COVID-19. Factors that may limit the applicability of these results include the use of established cell lines, mostly Vero cells, and only 1 or 2 strains of each virus.

Discussion

Summary of the Evidence

Figure 2 presents a concept map of the interaction between HCQ and different mechanisms involved in pathogenesis of COVID19.

Antiviral Effects of CQ/HCQ

HCQ has been proposed to have antiviral activity and has been used against many viral infections, including HIV (14-26), influenza (27-32), chikungunya virus (33-39), and many other viruses (36, 37, 40-44), albeit with variable levels of clinical effectiveness. Our systematic review reveals a consistently positive antiviral effect for HCQ in vitro and animal studies; however, these effects varied significantly in human studies. Most of human studies used the drug at later stages of the illness compared with animal studies, which may be the reason for the inconsistency in findings across human and animal studies. Moreover, previous studies have shown that animal studies cannot accurately predict clinical outcomes in humans (6). This concept has been reproduced in our systematic review. Thus, based on these limited findings, we cannot conclude whether HCQ is a safe and effective antiviral agent for the treatment of COVID19.

Immunomodulatory Effects of CQ/HCQ

COVID-19 is asymptomatic to mildly symptomatic in most patients. However, in a smaller number of patients with more severe disease, severe inflammatory response, cytokine storm, and microphage activation syndrome, it may lead to ARDS and multiorgan failure, which may potentially lead to death (45, 46). Cytokine release syndrome (CRS) is associated with higher adverse outcomes among patients with COVID19 (47-49) and may indirectly decrease viral clearance via decreasing T-cell number and function (50, 51). Immune modulating drugs such as steroids have been shown to improve outcomes (52, 53). Hydroxychloroquine is one of these potential agents that modulates immune reaction by decreasing pro-inflammatory cytokine release and decreasing the risk of macrophage activation syndrome in patients with systemic lupus erythematous without significant immunosuppression (54-60). In our systematic review, 2 included clinical studies evaluated immunologic markers in COVID-19 patients under treatment with HCQ and found significant reduction in inflammatory cytokines in these patients, although the overall quality of these studies was very low (61, 62). Three other studies compared cytokines (IL-6 and TNF-alpha) between HCQ and SOC patients and did not find any significant difference, but our certainty in these results is also very low (63-67).

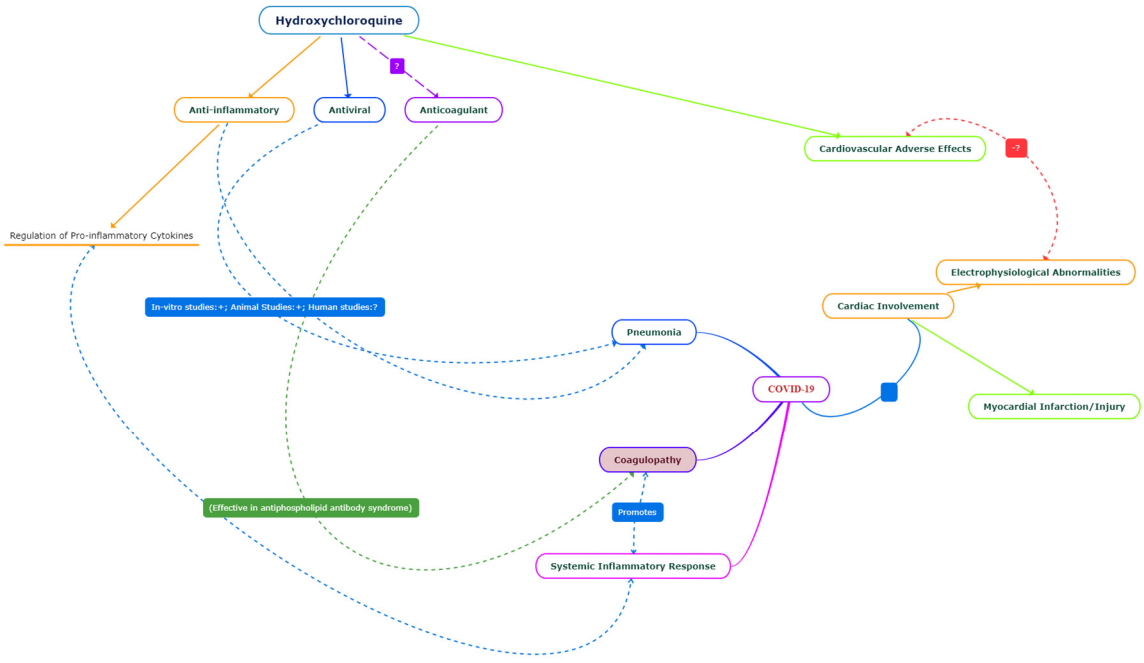

Fig. 2. Concept Note

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Anticoagulant Effects of CQ/HCQ

In light of the evidence suggesting hypercoagulable state and its possible role in the pathogenesis of severe COVID-19, this particular pathway may be an important target for treatment (68, 69). Anticoagulants have been shown to decrease mortality in COVID-19 patients (70). In 1 study, patients with COVID19-associated pneumonia had a markedly higher level of D-dimer and higher platelet count than patients with non-COVID-19-associated pneumonia (71). A multicenter study that included 150 patients with COVID-associated ARDS found that >95% of the patients had an elevated D-dimer level and 50 of 57 of the tested patients were positive for lupus anticoagulant antibodies (72). Nevertheless, these results are inconsistent with another study which found no association between antiphospholipid antibodies and COVID-19 prothrombotic state (73). In another cohort of hospitalized patients with COVID-19, the hypercoagulable state in these patients is more consistent with a severe inflammatory state than DIC (74). HCQ has been shown to decrease thrombotic events in patients with antiphospholipid syndrome by interfering with assembly of endosomal NADPH oxidase-2, which is involved in thrombotic events and affects inflammatory state in antiphospholipid syndrome (58, 75).

Drug and Disease-related Adverse Outcomes

Cardiovascular events, including acute cardiac injury, shock, and arrhythmias, were present in 7.2%, 8.7%, and 16.7% of patients with COVID-19, according to a cohort study of hospitalized patients (76). These patients are at an increased risk of in-hospital events, including ICU admission and death (77-79). In a recent study, a combination of azithromycin and HCQ was associated with an increase in the QTc interval to more than 500 milli seconds in 11% of the 84 patients with COVID-19. This effect was significantly more common among patients with renal failure (80). In a retrospective cohort study on multinational databases collectively including millions of patients, it was found that new users of HCQ alone were not at a significantly increased risk of adverse effects; nonetheless, its combination with azithromycin was associated with an increased risk of death associated with cardiovascular events among patients with rheumatoid arthritis (81). These findings present indirect evidence on cardiovascular outcomes among chloroquine users. Moreover, the doses and disease-drug interaction and effect on the heart may differ considerably in COVID-19. Therefore, clinicians should be especially cautious about the cardiac adverse effects of chloroquine, especially its combination with azithromycin or Oseltamiovir, and their possible synergistic activity with COVID-19 effects on the heart (82).

Toxicity with quinine agents, including chloroquine and HCQ, has also been reported to cause pulmonary side effects, including an ARDS-like syndrome (83-85), bronchiolitis obliterans organizing pneumonia (BOOP) (86), and pulmonary edema (87-89). A retrospective study of Wuhan patients with COVID-19 reported nervous system involvement among 78 of 214 (36.4%) patients as a part of the clinical presentation in COVID-19. It involves both the peripheral and the central nervous system, with symptoms ranging from dizziness and headache to impairments in taste and smell and even to stroke, seizures, and encephalitis. The proposed pathophysiologic mechanism is a direct brain invasion as in SARS and MERS (90). Seizure has also been reported with chloroquine use, although this was not confirmed in trials as an adverse effect, clinicians should be cautious when prescribing this drug in patients with epilepsy (91).

Relevance to Researchers and Care Providers

The available studies have significant methodological limitations, many of which are due to the difficulties associated with the special circumstances during a pandemic. Whether chloroquine can be clinically effective remains a question. One potential reason for the inconsistency between preclinical and clinical studies can be the interval between infection and treatment initiation, which may affect the potential antiviral effects of chloroquine. We recommend researchers to design trials of chloroquine at earlier phases of the infection and among outpatients. We also recommend systematic and globally homogenous monitoring and reporting of the side effects and clinical outcomes.

Relevance to Care providers

Based on current evidence, we recommend clinicians against the routine use of chloroquine/HCQ in patients with COVID-19, as the drug has not shown clinical efficacy and may be associated with life-threatening side effects, especially when prescribed with other routinely prescribed medications, such as azithromycin and Oseltamiovir. Thus, special care must be taken for patients at risk for QT prolongation.

Limitations of the Evidence and the Review

The quality of the included studies was low to very low. No placebo-controlled studies were available and only one of the studies was blinded. There was significant variation in outcomes and methodology across the studies, thus, a meta-analysis could not be conducted. All of the studies included hospitalized patients only and the results cannot be generalized to all patients with COVID-19.

Novelty

We present a holistic viewpoint by synthesizing the in vitro, animal, and human (observational and interventional) evidence on the benefits and risks associated with the use of chloroquine/HCQ in patients with COVID-19 with a rigorous methodology. A few systematic reviews have already been published that focused on the same review question. However, our study is unique in presenting a holistic approach combining the results from preclinical studies with nonrandomized and randomized human studies and presenting a basic science based clinically oriented viewpoint.

Conclusion

There is insufficient evidence to draw conclusions on the efficacy and safety of chloroquine and Hydroxychloroquine based on current evidence. Available studies have significant methodological limitations and the results are

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inconsistent.

Conflict of Interests

The authors declare that they have no competing interests.

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Appendix Table 1. Characteristics of Non-Randomized Controlled Human Studies

Study first author, Country	Design	Setting	Participants	Interventions/Exposure	Comparison	Outcome	Follow-up
Mahévas M. France	Retrospective cohort, Four centers	Inpatient	Hospitalized patients with COVID-19 Aged 18-80 Requiring>2 L/min of oxygen (48)	Hydroxychloroquine 600 mg/day within 48 hours of enrollment	Usual care (97)	– transfer to the ICU within 7 days of inclusion – transfer to the ICU within 7 days of inclusion and/or death from any cause	7 days
Gautret, Philippe France	open-label non-randomized clinical trial, single center	Inpatient	Hospitalized patients with RT-PCR-confirmed COVID-19 Age >12 years	Hydroxychloroquine sulfate 600mg with and without azithromycin for ten days (20/26)	components of usual care 16/16)(	Virological clearance at day-6 post-inclusion Virological clearance overtime during the study period clinical recovery side effects	14 days
Mazzanti, A Italy	ongoing, observational, prospective study	Inpatient	a diagnosis of COVID-19 confirmed by polymerase chain reaction	HCQ 400mg or 600mg (50)	Azithromycin (39), Lopinavir/ritonavir (52) or azithromycin+Lopinavir/ritonavir (9)	excessive QT prolongation, (defined as QTc interval ≥500 ms)	Not finished yet
Rosenberg, E. S US	Retrospective multicenter cohort	Inpatient	Inpatients with a laboratory-confirmed diagnosis of CoViD19	Oral HCQ alone (271) or HCQ+ AZI (735). The dosing differed across patients. The majority took HCQ 400 mg once to twice daily.	AZI alone (211) OR Neither of the mentioned drugs (221)	-in-hospital mortality -cardiac arrest -abnormal ECG (arrhythmia or prolonged QT interval)	21 days
Sbidian, E France	Retrospective cohort	Inpatient	adult inpatients with at least one PCR-documented SARS-CoV-2 RNA from a nasopharyngeal sample	HCQ alone : 600mg on the first day, 400mg daily for the next 9 days (623)	HCQ : 600mg first day, 400mg daily for the next 9 days + AZI :500mg on the first day followed by 250mg daily for the next 4 days (227) OR neither of HCQ or AZI (3792)	- all-cause 28-day mortality - 28-day discharge home	18 days
Singh, S US	Retrospective cohort	Inpatient	hospitalized adult patients (> 18 years) diagnosed with clinical and laboratory-confirmed COVID-19	HCQ (910) Varied dosing	Non-HCQ (910)	-7-,14-, and 30-day mortality -need for mechanical ventilation -incidence of new ventricular events (ie: fibrillation, tachycardia) or sudden cardiac death	30 days
Paccoud, O France	Retrospective cohort	Inpatient	All the patients hospitalized with a diagnosis of CoViD-19 via RT-PCR from a nasopharyngeal swab or sputum specimen	HCQ 200mg TID for 10 days (38)	Standard of care only (46)	-time to unfavorable outcome; e.g. death, need for ICU admission -time to death -time to hospital discharge to home or an aftercare unit -fever and cough at day 5 -adverse events in the HCQ group	10 days

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Appendix Table 1. Ctd

Study first author, Country	Design	Setting	Participants	Interventions/Exposure	Comparison	Outcome	Follow-up
Mallat, J UAE	Retrospective observational study	Inpatient	Hospitalized adult patients with confirmed SARS-CoV-2 infection (using RT-PCR for a nasopharyngeal swab)	HCQ 400 mg twice for day 1, followed by 400 mgs daily for 10 days (23)	No HCQ (11)	-Time to SARS-CoV-2 negativity test -turning negative on day 14 -Time course of inflammatory variables between admission and day seven or hospital discharge	14 days
Mehra, MR Six continents	Retrospective observational cohort study	Inpatient	All hospitalized patients with a PCR-confirmed COVID-19 infection	CQ: 765 mg [SD=308] for a mean of 6.6 [SD=2.4] days (1868) HCQ 596 [SD=126] mgs for a mean of 4.2 [SD=1.9] days (3016)	Neither drug (SOC) 81144	-in-hospital mortality -the first occurrence of a non-sustained [at least 6 sec] or Sustained ventricular tachycardia or ventricular fibrillation during hospitalization -rates of progression to mechanical ventilation use and the total and intensive care unit lengths of stay	Not mentioned
Geleris J US	Retrospective cohort	Inpatient	all admitted adults with a positive RT-PCR test for SARSCoV-2 from analysis of nasopharyngeal or oropharyngeal swab specimens obtained at any point during their hospitalization	HCQ: 600mg twice on day 1, followed by 400 mg daily for 4 additional Days (811)	SOC (565)	a composite of intubation or death in a time-to-event analysis	median follow-up of 22.5 days
Lagier, JC France	Retrospective cohort	Inpatient or day-care	all individuals >18 years of age with PCR-documented SARS-CoV-2 RNA from a nasopharyngeal sample	200 mg of oral HCQ, three times daily for ten days) HCQ alone (101)	HCQ+AZI (3337) AZI alone (137) Neither drug(162)	-death -transfer to ICU -more than 10 days of hospitalization -viral shedding	At least 9 days
Magagnoli US	Retrospective cohort	Inpatient	patients with laboratory confirmed SARS-CoV-2 infection	HCQ (97)	No HCQ (158	-the result of the hospitalization (discharge or death) -the result of hospitalization among patients requiring ventilation	Not mentioned
Ip, A	Retrospective multicenter cohort	Inpatient	Positive SARS-CoV-2 diagnosis by RT-PCR and not pregnant	HCQ (441)	SOC (342)	-death -side effects	8 days

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Appendix Table 2. Characteristics of Randomized Clinical Studies

Study first author, Country	Design	Setting	Participants	Interventions/Exposure (number of patients)	Comparison (number of patients)	Outcome	Follow-up
Chen, Zh China	double blinded RCT	Inpatient	Hospitalized patients with RT-PCR-confirmed COVID-19 Aged >18 years (Critical patients excluded)	Hydroxychloroquine oral 400mg daily between day 1 and 5 (31)	standard treatment ((oxygen therapy, antiviral agents, antibacterial agents, and immunoglobulin, with or without corticosteroids) (31)	Changes in time to clinical recovery (TTCR) Radiologic improvement	5 days
Chen, J China	Pilot clinical trial	Inpatient	Hospitalized Patients with Covid-19	Conventional treatment plus oral Hydroxychloroquine for 5 days (13/15)	only conventional treatment, including bed rest, oxygen inhalation, symptomatic support, antiviral therapy (14/15)	Viral clearance of sputum or lower respiratory tract secretions serious adverse drug event within 2 weeks the subject's condition changed to severe or critically ill	14 days
Wei Tang China	Open-label randomized trial	Inpatient	patients with covid-19 infection confirmed by RT-PCR	Hydroxychloroquine oral 1200 mg daily for three days followed by 800 mg daily for remaining days which is either two or three weeks depending on the severity (75/150)	Only standard of care (75/150)	SARS-CoV-2 RNA was assessed by real-time reverse transcription-PCR	14 days
Borba, MGS Brazil	randomized, double-blinded, phase IIb clinical trial	Inpatient	Hospitalized patients diagnosed with severe respiratory syndrome resulting from CoVid-19 ; clinically or PCR-confirmed	Hydroxychloroquine oral/via an NG-tube 600mg twice daily for 10 days or a total of 12 gr +standard of care which included azithromycin	450mg daily (only twice on the first day0 or a total of 2.7g +standard of care which included azithromycin	-mortality by D28; -mortality on day 13, participant's clinical status, laboratorial exams, and ECG on days 13 and 28, daily clinical status during hospitalization, duration of mechanical ventilation (if applicable) and supplementary oxygen (if applicable), and the time (in days) from treatment initiation to death -Adverse events and serious adverse events	14 days
Cavalcanti, AB Brazil	multicenter, randomized, open-label, three-group, controlled trial	Inpatient	consecutive patients who were 18 years of age or older and who had been hospitalized with suspected or confirmed Covid-19 with 14 or fewer days since symptom onset	HCQ 400mg BD for 7 days (221)	SOC (229)	-clinical status at 15 days -clinical status at 7 days -an indication for intubation within 15 days -the receipt of supplemental oxygen administered by a high-flow nasal cannula or noninvasive ventilation between randomization and 15 days -the receipt of supplemental oxygen administered by a high-flow nasal cannula or noninvasive ventilation between randomization and 15 days -in-hospital death -thromboembolic complications - acute kidney injury -and the number of days alive and free from respiratory support up to 15 days	15 days

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Appendix Table 2. Ctd

Study first author, Country	Design	Setting	Participants	Interventions/Exposure (number of patients)	Comparison (number of patients)	Outcome	Follow-up
Huang, M	Clinical study	inpatient	Adult patients with COVID 19 according to the diagnosis of WHO interim guidance (mild, moderate or severe CoViD-19)	CQ 500mg BD for 10 days (10)	Lopinavir/Ritonavir 400/100 mg BD for 10 days (12)	- viral negative transforming time and the negative conversion rate of SARS CoV 2 RT PCR at day 10, 14 of study period - the rate of hospital discharge at D ay 14, clinical recovery at day 10, CT scan improvement at D ay 10 and 14, and the frequency of adverse events	14 days

Appendix Table 3. Characteristics and Results of Animal Studies

ID	1
First Author, study year	Dale L Barnard, 2006
Interventions	Amodiaquine; Chloroquine
Animal model	Specific pathogen-free BALB/c female mice (11–18 g)
Virus model	SARS-CoV-1
Study design	4h before the virus exposure.
Dosage-forms	Chloroquine was used at 50, 10, and 1 mg/kg intraperitoneally and intranasally; and Amodiaquine was used at 150, 75, and 10 mg/kg intranasally, and 75, 37.5, 18.8, and 9.4 mg/kg intraperitoneally; twice a day for 3 days.
Comparison	PBS was used as placebo.
Number of subjects	15 mice per each concentration of the drugs used in the study
Tissue	Lung
Length of follow-up	3 days of the start of the treatment where virus was administered at 4 hours post treatment.
Outcomes	Virus titers (Duplicated Log10 CCID50/g) in homogenized lung tissue.
Results	Chloroquine showed no effect on virus titers in vivo when used intraperitoneally. However, it had a statistically non-significant effect in reducing the virus titers in lung tissues. Amodiaquine had no effect at the highest concentration used in the study (150mg/kg) where it did not reduce the virus titers at lung tissues.
Notes	The efficiency of the drugs was also tested in vitro in African green monkey cells using different strains of the SARS-Cov including Urbani, Toronto 2, Frankfurt 1, CHUK-W1 strains and showed no effect for Chloroquine and two other salts of it but were blocked by Amodiaquine in vitro. Also, both drugs were claimed to be well tolerated but the data was not shown.
ID	2
First Author, study year	Els Keyaerts, 2009
Interventions	Chloroquine diphosphate
Animal model	Newborn C57BL/6 mice; (El-evage Janvier, Le Genest Saint Isle, France)
Virus model	Coronavirus OC43 (HCoV-OC43)
Study design	In the study two parts were included. 200µl of different dilutions of Chloroquine (corresponding to 1, 5 or 15 mg/kg of body weight) daily starting from 1 days before/after labor was administered subcutaneously. Subsequently, 5-day suckling mice were infected intracerebrally with virus containing 1x103 copy numbers of the virus genome and were followed for the outcome as death. The follow up study involved administering chloroquine at the high dose of 15 mg/kg prepartum and switch the litters for breast feeding. The same way the pups were infected, and the survival was followed for 60 days post-infection. A negative control group included no drug intervention.
Dosage-forms	Test Prepartum; Group 15mg/kg (9mothers[m]-70pups[p]), Group 5mg/kg (5m-42p), Group 1mg/kg (4m-21p). Test Postpartum; Group 15mg/kg (11m-76p), Group 5mg/kg (6m-42p), Group 1mg/kg (4m-31p). Group placebo (19m-132p).
Comparison	No treatment
Number of subjects	9 mothers (m)-70 pups (p), 5m-42p, 4m-21p for different dose experiments. Test Postpartum groups 11m-76p, 6m-42p, 4m-31p for different drug concentrations. Group placebo 19m-132p.
Tissue	Not done
Length of follow-up	Up to 60 days varies between groups
Outcomes	Survival of the pups challenged with live virus 1000 TCID50 intracerebrally 5 days postpartum.
Results	A log rank test indicated that the survival curve for litters that were treated prepartum with 15mg/kg chloroquine was significantly different from the survival curves for the pups that were treated prepartum with 5mg/kg (P= 0.0237), 1mg/kg (P= 0.0001). 100% survived treated 15 mg/kg prepartum (97.4% treated postpartum). The survival was dose dependent. The results of the follow-up study showed that switching the litters between groups of mothers to detect the effect of the transplacental or milk delivered chloroquine. The drug was effective when transferred by milk and not transplacentally.
Notes	The efficiency of the drug was also tested in vitro using HRT-18 cells and concentration higher than 0.16 µM results in a decline in the number of HCoV-OC43 copies determined by qRT-PCR. Additionally, the 15mg/kg drug group survived 100% when used prepartum meaning no adverse effect was associated with the usage of the drug in this study.

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Appendix Table 3. Ctd

ID	3
First Author, study year	Junwei Niu, 2020
Interventions	Chloroquine
Animal model	BALB/c mice (12-days old)
Virus model	rOC43-ns2DelRluc replicative virus based on HCoV-OC43 virus.
Study design	Mice were inoculated with chloroquine and the virus then was administered intracerebrally at 100 TCID50 using rOC43-ns2DelRluc, and bioluminescence intensity was measured daily to quantify the virus replication. The tissues including brain and spinal cord were studied for the Photon flux and the presence of viral proteins by western blotting. The control mice did not get chloroquine before being infected with the virus.
Dosage-forms	Chloroquine was administered to mice 2 h before viral inoculation (day 0; 30 mg/kg) and then administered daily according to a previous study of HCoV-OC43-WT (Keyaerts et al., 2009).
Comparison	Drug to PBS as placebo
Number of subjects	3 mice in virus group and 3 mice in virus + drug group
Tissue	Whole brain and spinal cord
Length of follow-up	4 days post infection
Outcomes	Survival and the bioluminescence expressed by virus replication as well as the western blot analysis of the luciferase activity of the expressed protein in brain and spinal cord.
Results	No signals were detected in mice treated with Chloroquine, and all of them survived, whereas all mice receiving PBS displayed increased bioluminescence and died, demonstrating a significant difference relative to the individual controls. Also, western blot analysis supported the data of the mice being successfully infected when virus was inoculated.
Notes	The main aim of the study was to optimize and validate the detection of the virus infection in mice and drug treatment was used as a control for the whole experiment. The initial dose of 30 mg/kg chloroquine has been reported to be toxic in C57BL/6 mice as reported above but was used in BALB/c mice in here followed by half dose thereafter.

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