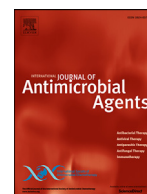




Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Reply to Gautret et al: hydroxychloroquine sulfate and azithromycin for COVID-19: what is the evidence and what are the risks? ☆,☆☆

Julian D Machiels^a, Chantal P Bleeker-Rovers^b, Rob ter Heine^c,
Janette Rahamat-Langendoen^a, Quirijn de Mast^b, Jaap ten Oever^b, Teun Bousema^a,
Reinout van Crevel^b, Heiman FL Wertheim^{a,*}

^a Department of Medical Microbiology & Radboudumc Center for Infectious Diseases, the Netherlands

^b Department of Internal Medicine & Radboudumc Center for Infectious Diseases, the Netherlands

^c Department of Pharmacy & Radboudumc Center for Infectious Diseases, the Netherlands

ARTICLE INFO

Article history:

Received 22 May 2020

Accepted 12 June 2020

Keywords:

COVID-19

SARS-CoV-2

2019-nCoV

(Hydroxy)chloroquine

Azithromycin

ABSTRACT

The severity of COVID-19 has resulted in a global rush to find the right antiviral treatment to conquer the pandemic and to treat patients. This requires reliable studies to support treatment. In a recently published study by Gautret et al. the authors concluded that hydroxychloroquine monotherapy and hydroxychloroquine in combination with azithromycin reduced viral load. However, this trial has several major methodological issues, including the design, outcome measure and the statistical analyses. In this paper we discuss the background, clinical evidence, pharmacology and methodological issues related to this clinical trial. We understand the rush to release results, however in case conclusions are far reaching the evidence needs to be robust.

© 2020 Elsevier B.V. and International Society of Chemotherapy. All rights reserved.

1. Introduction

The severity of COVID-19 has resulted in a global rush to find the right antiviral treatment that reduces the risk of complications and improves patient outcome [1]. This requires reliable studies to support treatment recommendations and improve clinical care with as little toxicity as possible. Despite the sense of urgency, we need to restrain ourselves from publishing premature results as this may cause more harm than good. An example of a study with considerable concerns about scientific rigor was a recent publication on the treatment with hydroxychloroquine and azithromycin by Gautret et al. [2] The authors concluded that their study showed that 'hydroxychloroquine treatment is significantly associated with viral load reduction/disappearance in COVID-19 patients and its effect is reinforced by azithromycin'. There is a rationale for studying these compounds, and they deserve clinical evaluation for COVID-19, but there are important methodological issues related to this study and potential risks in the choice for this drug combination. Several critical reviews have been published in response to Gautret et al. [3,4] In this paper we review method-

ological issues related to this clinical trial and we provide a discussion on the background of the potential antiviral activity of (hydroxy)chloroquine and azithromycin, clinical evidence and pharmacology. We advocate adequately powered, well-designed studies with clinically relevant outcome measures to support treatment strategies for COVID-19.

2. (Hydroxy)chloroquine and azithromycin antiviral activity

Despite the strong claim by Gautret et al., no solid clinical data were reported that demonstrated a relevant clinical effect of (hydroxy)chloroquine. The authors refer to an expert opinion on chloroquine, claiming that an early clinical trial in China showed a significant effect of chloroquine on clinical outcome and viral clearance without reporting the data itself [5,6]. *In vitro* antiviral effects of (hydroxy)chloroquine make this agent a logical choice to investigate as a treatment for COVID-19, with hydroxychloroquine appearing to be the most potent of the two [7]. Broad *in vitro* antiviral activity of chloroquine has been known for decades and gained recent attention due to the COVID-19 pandemic [8,9]. Inhibition of *in vitro* growth of several different corona and other viruses by (hydroxy)chloroquine is thought to be related to interaction of the drug with several steps of the viral replication cycle among which modifying the endosomal acidity and the subsequent effects on virus entry and exit [10–14]. For COVID-19, an

☆ On behalf of the Radboudumc Center of Infectious diseases COVID-19 team.

☆☆ All authors contributed equally to this paper.

* Corresponding author

E-mail address: Heiman.wertheim@radboudumc.nl (H.F.L. Wertheim).

additional mechanism has been identified by interaction with the binding of the spike protein with the Angiotensin converting enzyme 2 receptor [15]. Furthermore, it has been postulated that (hydroxy)chloroquine exerts antiviral effects by activating the innate immune system [16]. However, despite the encouraging results of the antiviral potential of (hydroxy)chloroquine for different viruses *in vitro*, this so far is not supported by *in vivo* data [17–20]. The antiviral effects of the macrolide antibiotic azithromycin are questionable. Although preclinical models suggest activity against Zika and Ebola virus, no *in vivo* activity has yet been demonstrated against these viruses [21,22]. Furthermore, a study in patients infected with MERS-CoV showed that macrolide therapy was not associated with a reduction in mortality or viral clearance [23]. The study by Gautret et al. does not provide the much-needed high quality data on the efficacy of hydroxychloroquine alone or in combination with azithromycin against COVID-19.

3. Methodological considerations

In addition to the chosen outcome measure, described in detail in section 5, there are several methodological concerns with the reported study by Gautret et al. [2–4]. Controls comprised of patients who refused treatment and an unknown number of patients from a different hospital that may differ in diagnostic guidelines, performance and treatment policies. The fact that quantitative PCR (qPCR) was used to estimate viral clearance was recorded differently among patients is concerning. Whilst all patients on experimental treatment had a semi-quantitative assessment of viral load (i.e. a CT-value), 8/16 controls had only a qualitative outcome and 2/16 even had no qPCR result available at baseline. With initial viral load and day since symptom onset being strong determinants of the kinetics of viremia, this is a clear concern [24]. Variation in viral load also complicates comparisons between hydroxychloroquine and hydroxychloroquine/azithromycin since CT-values appear lower (and thus viral load appears higher) in hydroxychloroquine-treated individuals (median 26, IQR 22–29.8) compared to hydroxychloroquine/azithromycin (median 27.5, IQR 24.8–28.8). Given the uncertainties in the interpretation of CT-values as viral load, it is impossible to determine whether this reflects a relevant difference in viral load, but this may have contributed to a shorter time to qPCR negativity in this arm. Control patients were also younger (median 34, IQR 14.5–64.3) than patients undergoing treatment (median 51.5, IQR 41.5–59.3). Whilst age is an obvious determinant of clinical outcome of COVID-19 infection and older age is associated with higher peak viral load, the effect of age on clearance of viremia is currently unknown [25]. The fact that the difference in age between intervention and control populations did not reach statistical significance reflects the small study population – markedly smaller than the 48 individuals proposed in sample size calculations – and does not mean that age may not have been a relevant determinant of the kinetics of viremia.

The large proportion of individuals who were lost to follow-up in the experimental arm (6/26, 23%) is another red flag for the interpretation of study findings, especially since for some of the dropouts the inability to complete six days of follow-up was plausibly associated with treatment outcome (four transferred to intensive care, one died) or tolerability of medication (one stopped because of nausea) and one patient decided to leave the hospital. Lastly, reanalysis of the data with more appropriate statistical techniques showed that hydroxychloroquine treatment resulted in no to only modest effect [26,27].

4. Pharmacological considerations

After oral dosing, the bioavailability of hydroxychloroquine is high. It has a very high volume of distribution due to accumulation

in tissue and red blood cells and a terminal half-life of approximately 40 days [28]. Hydroxychloroquine is partly cleared from the body by metabolism to desethyl-hydroxychloroquine, which is also likely to exert antiviral activity, and partly by renal excretion [29,30]. The clearance may be prolonged by strong inhibitors of hepatic metabolism and renal dysfunction, but considering the general short treatment period for COVID-19, this may be of limited relevance [31]. Azithromycin is considered a relatively weak inhibitor of cytochrome P450 isoenzyme 3A and the pharmacokinetic interaction of azithromycin with hydroxychloroquine is not considered clinically relevant [32]. A substantial part of the hospitalized patients with COVID-19 become critically ill [33]. In the latter population, oral hydroxychloroquine absorption may be impaired and distribution may be altered [34]. There are no pharmacokinetic data of hydroxychloroquine in COVID-19 patients yet and it remains unknown whether effective exposure can be reached.

One of the pivotal studies suggesting hydroxychloroquine as a potential treatment of COVID-19 concluded that, based on *in silico* pharmacokinetic explorations, clinically feasible doses of hydroxychloroquine yield drug concentrations above the half-maximal antiviral concentration as obtained from *in vitro* experiments [7]. However, this bench-to-bedside translation should be interpreted with caution. It is unknown whether the pharmacologically active (plasma protein unbound) concentrations in the *in vitro* experiments are comparable to those observed in humans. The *in vitro* experiments were performed in a medium of a different constitution than that of human blood. Furthermore, hydroxychloroquine is administered as a racemic mixture of R- and S- hydroxychloroquine sulfate, and plasma protein binding and clearance are stereospecific. This complicates the extrapolation from the static *in vitro* experiments to the dynamic situation *in vivo* [35–37].

5. Clinical considerations

Importantly, a study reporting on the clearance of viremia alone as surrogate endpoint is insufficient to justify the use of hydroxychloroquine and azithromycin as COVID-19 treatment. The clinical efficacy of hydroxychloroquine is difficult to determine in the study by Gautret et al. Only 22% of the small number of patients included had a lower respiratory tract infection and 17% were even asymptomatic – a population not representative of the average hospitalized patient with COVID-19. A subsequent study of 80 patients receiving the combination of hydroxychloroquine and azithromycin by the same research group also does not allow a conclusion on effectiveness [38]. Whilst the initial study had a small control group, smaller than justified by power calculations, the follow-up study missed a control group and again mainly consisted of moderately ill patients with a high chance of spontaneous recovery. Huang et al. found similar results in a small randomized study with 22 patients. Ten patients were treated with chloroquine, the controls (n=12) were treated with lopinavir/ritonavir. Endpoints were viral clearance by qPCR and improvement on CT scan, but no significant differences were shown up to 14 days. On day 14, 100% of the patients receiving chloroquine were discharged from the hospital, compared to only 50% in the lopinavir/ritonavir group (risk ratio 2 (CI 1.33–4.00) and rate ratio 2.21 (CI 0.81–6.22) [39]. However, patients in the chloroquine group were younger (41.5 vs 53, p=0.19) and started significantly earlier with treatment (2.5 vs 6.5 days, p<0.001). A recent controlled study among 181 patients showed no benefit of hydroxychloroquine [40]. In another controlled study among 150 patients, treating with hydroxychloroquine did not improve outcome [41]. An uncontrolled observational study among ten evaluable patients did not suggest hydroxychloroquine and azithromycin to impact rapid viral clearance or clinical benefit [42]. The results of a non-peer-reviewed open-label randomized trial of the effect of hydroxychloroquine in 62 moderately

ill patients have recently been reported on a preprint server [43]. Cough and fever remission occurred on average one day earlier in the intervention group. In addition, treated patients more often had improved CT imaging on day 6 (81 versus 55%). Toxicity weighs heavily with uncertain effectiveness.

Recently, the results of several large studies have been published. In the observational study by Geleris et al. including 1376 patients, the association between hydroxychloroquine use and intubation or death was studied. During a median follow-up of 22.5 days, 25.1% of the patients reached one end point. There was no significant association between the use of hydroxychloroquine and one of the endpoints [44]. In a randomized controlled trial comparing hydroxychloroquine and placebo as postexposure prophylaxis in 821 patients, there was no difference in incidence of COVID-19 between the two groups after high-risk or moderate-risk exposure to COVID-19 [45]. A press release on the results of the RECOVERY trial (4674 patients) preceding publication also concluded that there is no beneficial effect of hydroxychloroquine [46]. A systematic review by Hernandez et al. also concluded that evidence was conflicting and insufficient regarding the effect of hydroxychloroquine on all-cause mortality, progression to severe disease, clinical symptoms, and upper respiratory virologic clearance with antigen testing [47]. Another study published in the Lancet has been retracted.

Both (hydroxy)chloroquine and azithromycin can lead to severe cardiac disorders (e.g. increased QT length) and cardiotoxicity that could lead to arrhythmia and/or cardiac arrest. Furthermore, azithromycin increases exposure to other cardiotoxic drugs [48]. Lane et al. recently published the results of a multinational, network cohort and self-controlled case series study showing that short-term hydroxychloroquine treatment for other reasons than COVID-19 is safe, but addition of azithromycin appeared to induce conduction disorders and increased cardiovascular mortality (RR 2.19; 95% confidence interval 1.02–1.45), potentially due to synergistic effects on QT length [49]. This effect is important in patients with COVID-19 because of the high incidence of acute cardiac injury, myocarditis and cardiac arrhythmias [50]. In fact, in a recent study conducted by Chorin et al. in 84 COVID-19 patients an increase in QT interval of greater than 40ms was found in 30% and greater than 500ms in 11% [51]. The preliminary safety results of the PREPINT study (NCT04323527) showed that a higher dose of chloroquine for 10 days compared to a lower dose of chloroquine for 5 days showed increased QT lengths and increased mortality [52]. A recent database study also found that (hydro)chloroquine, azithromycin and a combination all resulted in increased QT interval, increased incidence of Torsades-de-Pointes and increased mortality [53]. Even if a larger randomized controlled trial (RCT) showed that the combination of (hydroxy)chloroquine and azithromycin would be effective in patients with COVID-19, safety would still be an issue.

6. Conclusion

To date, robust clinical evidence of the efficacy of (hydroxy)chloroquine is lacking, let alone the combination with azithromycin. The paper by Gautret et al. raised a lot of attention and contributed to a demand for the drug without the appropriate evidence of its benefit. The study by Gautret et al. showed important methodological issues and does not provide a suggestion of effectiveness. A lack of COVID-19 study subjects and a strong motivation to find a treatment is not an issue, but good quality studies are needed. The International Society of Antimicrobial Chemotherapy provided an official statement on the paper in their journal that it did not meet their standard [54]. They stated that 'it is important to help the scientific community by publishing new data

fast, this cannot be at the cost of reducing scientific scrutiny and best practices'. The authors of this paper fully agree.

Competing Interests

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval

Not required.

References

- [1] Ali I, Alharbi OML. COVID-19: Disease, management, treatment, and social impact. *Sci Total Environ* 2020;728:138861.
- [2] Gautret P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents* 2020;105949.
- [3] Taccone FS, Gorham J, Vincent JL. Hydroxychloroquine in the management of critically ill patients with COVID-19: the need for an evidence base. *Lancet Respir Med* 2020.
- [4] Ingraham NE, et al. Shining a light on the evidence for hydroxychloroquine in SARS-CoV-2. *Crit Care* 2020;24(1):182.
- [5] Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends* 2020;14(1):72–3.
- [6] Zhi, Z.J.H.H.X.Z., [Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia]. 2020.43(3): p. 185–188.
- [7] Yao X, et al. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis* 2020.
- [8] Inglot AD. Comparison of the antiviral activity in vitro of some non-steroidal anti-inflammatory drugs. *J Gen Virol* 1969;4(2):203–14.
- [9] Touret F, de Lamballerie X. Of chloroquine and COVID-19. *Antiviral Res* 2020;177:104762.
- [10] Savarino A, et al. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003;3(11):722–7.
- [11] Keyaerts E, et al. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun* 2004;323(1):264–8.
- [12] Vincent MJ, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J* 2005;2:69.
- [13] Tan YW, et al. An evaluation of Chloroquine as a broad-acting antiviral against Hand, Foot and Mouth Disease. *Antiviral Res* 2018;149:143–9.
- [14] Wang M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020;30(3):269–71.
- [15] Fantini J, et al. Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. *Int J Antimicrob Agents* 2020;105960.
- [16] Wang LF, et al. Hydroxychloroquine-inhibited dengue virus is associated with host defense machinery. *J Interferon Cytokine Res* 2015;35(3):143–56.
- [17] Tricou V, et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. *PLoS Negl Trop Dis* 2010;4(8):e785.
- [18] Paton NI, et al. Chloroquine for influenza prevention: a randomised, double-blind, placebo controlled trial. *Lancet Infect Dis* 2011;11(9):677–83.
- [19] Roques P, et al. Paradoxical Effect of Chloroquine Treatment in Enhancing Chikungunya Virus Infection. *Viruses* 2018;10(5).
- [20] Hraiech S, et al. Lack of viral clearance by the combination of hydroxychloroquine and azithromycin or lopinavir and ritonavir in SARS-CoV-2-related acute respiratory distress syndrome. *Annals of Intensive Care* 2020;10(1):63.
- [21] Madrid PB, et al. Evaluation of Ebola Virus Inhibitors for Drug Repurposing. *ACS Infect Dis* 2015;1(7):317–26.
- [22] Retalack H, et al. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. *Proc Natl Acad Sci U S A* 2016;113(50):14408–13.
- [23] Arabi YM, et al. Macrolides in critically ill patients with Middle East Respiratory Syndrome. *Int J Infect Dis* 2019;81:184–90.
- [24] Wolfel R, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020.
- [25] To KK, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020.
- [26] Hulme OJ, et al. Reply to Gautret et al. 2020: A Bayesian reanalysis of the effects of hydroxychloroquine and azithromycin on viral carriage in patients with COVID-19. *medRxiv* 2020 p. 2020.03.31.20048777.
- [27] Lover AA. Quantifying treatment effects of hydroxychloroquine and azithromycin for COVID-19: a secondary analysis of an open label non-randomized clinical trial (Gautret et al, 2020). *medRxiv* 2020 p. 2020.03.22.20040949.

- [28] Tett SE, et al. A dose-ranging study of the pharmacokinetics of hydroxy-chloroquine following intravenous administration to healthy volunteers. *Br J Clin Pharmacol* 1988;26(3):303–13.
- [29] Han Y, et al. Antimalarial drugs and their metabolites are potent Zika virus inhibitors. *Journal of Medical Virology* 2019;91(7):1182–90.
- [30] Ducharme J, et al. Enantioselective disposition of hydroxychloroquine after a single oral dose of the racemate to healthy subjects [see comments]. *British Journal of Clinical Pharmacology* 1995;40(2):127–33.
- [31] Browning DJ. Pharmacology of Chloroquine and Hydroxychloroquine. Hydroxy-chloroquine and Chloroquine Retinopathy. New York, NY: Springer; 2014.
- [32] Westphal JF. Macrolide - induced clinically relevant drug interactions with cytochrome P-450A (CYP) 3A4: an update focused on clarithromycin, azithromycin and dirithromycin. *Br J Clin Pharmacol* 2000;50(4):285–95.
- [33] Guan W-j, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine* 2020.
- [34] Power BM, et al. Pharmacokinetics of Drugs Used in Critically Ill Adults. *Clinical Pharmacokinetics* 1998;34(1):25–56.
- [35] McLachlan AJ, et al. Disposition of the enantiomers of hydroxychloroquine in patients with rheumatoid arthritis following multiple doses of the racemate. *Br J Clin Pharmacol* 1993;36(1):78–81.
- [36] McLachlan AJ, Cutler DJ, Tett SE. Plasma protein binding of the enantiomers of hydroxychloroquine and metabolites. *Eur J Clin Pharmacol* 1993;44(5):481–4.
- [37] Fan J, et al. Connecting hydroxychloroquine in vitro antiviral activity to in vivo concentration for prediction of antiviral effect: a critical step in treating COVID-19 patients. *Clinical Infectious Diseases* 2020.
- [38] Gautret P, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study. *Travel Med Infect Dis* 2020:101663.
- [39] Huang M, et al. Treating COVID-19 with Chloroquine. *Journal of Molecular Cell Biology* 2020;12(4):322–5.
- [40] Mahévas M, et al. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: observational comparative study using routine care data. *Bmj* 2020;369:m1844.
- [41] Tang W, et al. Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. *medRxiv* 2020 p. 2020.04.10.20060558.
- [42] Molina JM, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azitromycin in patients with severe COVID-19 infection. *Med Mal Infect* 2020.
- [43] Chen Z, et al. Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. *medRxiv* 2020 p. 2020.03.22.20040758.
- [44] Geleris J, et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* 2020.
- [45] Boulware DR, et al. A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. *New England Journal of Medicine* 2020.
- [46] Horby, P. and M. Landray. No clinical benefit from use of hydroxychloroquine in hospitalised patients with COVID-19. Available from: <http://www.ox.ac.uk/news/2020-06-05-no-clinical-benefit-use-hydroxychloroquine-hospitalised-patients-covid-19>.
- [47] Hernandez AV, et al. Hydroxychloroquine or Chloroquine for Treatment or Prophylaxis of COVID-19: A Living Systematic Review. *Annals of Internal Medicine* 2020.
- [48] Ray WA, et al. Azithromycin and the risk of cardiovascular death. *N Engl J Med* 2012;366(20):1881–90.
- [49] Lane JCE, et al. Safety of hydroxychloroquine, alone and in combination with azithromycin, in light of rapid wide-spread use for COVID-19: a multinational, network cohort and self-controlled case series study. *medRxiv* 2020 p. 2020.04.08.20054551.
- [50] Madjid M, et al. Potential Effects of Coronaviruses on the Cardiovascular System: A Review. *JAMA Cardiol* 2020.
- [51] Chorin E, et al. The QT interval in patients with COVID-19 treated with hydroxychloroquine and azithromycin. *Nat Med* 2020.
- [52] Borba M, et al. Chloroquine diphosphate in two different dosages as adjunctive therapy of hospitalized patients with severe respiratory syndrome in the context of coronavirus (SARS-CoV-2) infection: Preliminary safety results of a randomized, double-blinded, phase IIb clinical trial (CloroCovid-19 Study). *medRxiv* 2020 p. 2020.04.07.20056424.
- [53] Nguyen LS, et al. Cardiovascular Toxicities Associated with Hydroxychloroquine and Azithromycin: An Analysis of the World Health Organization Pharmacovigilance Database. *Circulation* 2020.
- [54] Voss, A. Official Statement from International Society of Antimicrobial Chemotherapy (ISAC) on: Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial (Gautret P et al. PMID 32205204). 2020; Available from: <https://www.isac.world/news-and-publications/official-isac-statement>.