



ELSEVIER

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

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

Hot Topic

Chloroquine and hydroxychloroquine as available weapons to fight COVID-19

Repositioning of drugs for use as antiviral treatments is a critical need [1]. It is commonly very badly perceived by virologists, as we experienced when reporting the effectiveness of azithromycin for Zika virus [2]. A response has come from China to the respiratory disease caused by the new coronavirus (SARS-CoV-2) that emerged in December 2019 in this country. Indeed, following the

very recent publication of results showing the in vitro activity of chloroquine against SARS-CoV-2 [3], data have been reported on the efficacy of this drug in patients with SARS-CoV-2-related pneumonia (named COVID-19) at different levels of severity [4,5]. Thus, following the in vitro results, 20 clinical studies were launched in several Chinese hospitals. The first results obtained from more than

Table 1
Main results of studies on the activity of chloroquine or hydroxychloroquine on coronaviruses^a

Reference	Compound(s)	Targeted virus	System used for antiviral activity screening	Antiviral effect
[12]	Chloroquine	SARS-CoV	Vero (African green monkey kidney) E6 cells	EC ₅₀ = 8.8 ± 1.2 μM
[16]	Chloroquine	SARS-CoV (four strains)	Vero E6 cells	EC ₅₀ = 4.4 ± 1.0 μM
[17]	Chloroquine, chloroquine monophosphate, chloroquine diphosphate		Vero 76 cells	Chloroquine: EC ₅₀ = 1–4 μM Chloroquine monophosphate: EC ₅₀ = 4–6 μM Chloroquine diphosphate: EC ₅₀ = 3–4 μM
			BALB/c mice	Intraperitoneal or intranasal chloroquine administration, beginning 4 h prior to virus exposure: 50 mg/kg but not 10 mg/kg or 1 mg/kg reduced for the intranasal route (but not the intraperitoneal route) viral lung titres from mean ± S.D. of 5.4 ± 0.5 to 4.4 ± 1.2 in log ₁₀ CCID ₅₀ /g at Day 3 (considered as not significant)
[18]	Chloroquine, hydroxychloroquine	SARS-CoV	Vero cells	Chloroquine: EC ₅₀ = 6.5 ± 3.2 μM Hydroxychloroquine: EC ₅₀ = 34 ± 5 μM
		Feline coronavirus	Crandell-Reese feline kidney (CRFK) cells	Chloroquine: EC ₅₀ > 0.8 μM Hydroxychloroquine: EC ₅₀ = 28 ± 27 μM
[19]	Chloroquine	HCoV-229E	Human epithelial lung cells (L132)	Chloroquine at concentrations of 10 μM and 25 μM inhibited HCoV-229E release into the culture supernatant
[20]	Chloroquine	HCoV-OC43	HRT-18 cells Newborn C57BL/6 mice; chloroquine administration transplacentally and via maternal milk	EC ₅₀ = 0.306 ± 0.0091 μM 100%, 93%, 33% and 0% survival rate of pups when mother mice were treated per day with 15, 5, 1 and 0 mg/kg body weight, respectively
[21]	Chloroquine	Feline infectious peritonitis virus (FIPV)	<i>Felis catus</i> whole fetus-4 cells	FIPV replication was inhibited in a chloroquine concentration-dependent manner
[22]	Chloroquine	SARS-CoV MERS-CoV HCoV-229E-GFP (GFP-expressing recombinant HCoV-229E)	Vero E6 cells Huh7 cells (human liver cell line) Huh7 cells (human liver cell line)	EC ₅₀ = 4.1 ± 1.0 μM EC ₅₀ = 3.0 ± 1.1 μM EC ₅₀ = 3.3 ± 1.2 μM
[3]	Chloroquine	SARS-CoV-2	Vero E6 cells	EC ₅₀ = 1.13 μM

CCID₅₀, 50% cell culture infectious dose; CoV, coronavirus; EC₅₀, 50% effective concentration (mean ± S.D.); GFP, green fluorescent protein; HCoV, human coronavirus; MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome; S.D., standard deviation.

^a See also [1] (Table 1) for additional references.

100 patients showed the superiority of chloroquine compared with treatment of the control group in terms of reduction of exacerbation of pneumonia, duration of symptoms and delay of viral clearance, all in the absence of severe side effects [4,5]. This has led in China to include chloroquine in the recommendations regarding the prevention and treatment of COVID-19 pneumonia [4,6].

There is a strong rationality for the use of chloroquine to treat infections with intracellular micro-organisms. Thus, malaria has been treated for several decades with this molecule [7]. In addition, our team has used hydroxychloroquine for the first time for intracellular bacterial infections since 30 years to treat the intracellular bacterium *Coxiella burnetii*, the agent of Q fever, for which we have shown in vitro and then in patients that this compound is the only one efficient for killing these intracellular pathogens [8,9]. Since then, we have also shown the activity of hydroxychloroquine on *Tropheryma whipplei*, the agent of Whipple's disease, which is another intracellular bacterium for which hydroxychloroquine has become a reference drug [10,11]. Altogether, one of us (DR) has treated ~4000 cases of *C. burnetii* or *T. whipplei* infections over 30 years (personal data).

Regarding viruses, for reasons probably partly identical involving alkalisation by chloroquine of the phagolysosome, several studies have shown the effectiveness of this molecule, including against coronaviruses among which is the severe acute respiratory syndrome (SARS)-associated coronavirus [1,12,13] (Table 1). We previously emphasised interest in chloroquine for the treatment of viral infections in this journal [1], predicting its use in viral infections lacking drugs. Following the discovery in China of the in vitro activity of chloroquine against SARS-CoV-2, discovered during culture tests on Vero E6 cells with 50% and 90% effective concentrations (EC₅₀ and EC₉₀ values) of 1.13 μ M and 6.90 μ M, respectively (antiviral activity being observed when addition of this drug was carried out before or after viral infection of the cells) [3], we awaited with great interest the clinical data [14]. The subsequent in vivo data were communicated following the first results of clinical trials by Chinese teams [4] and also aroused great enthusiasm among us. They showed that chloroquine could reduce the length of hospital stay and improve the evolution of COVID-19 pneumonia [4,6], leading to recommend the administration of 500 mg of chloroquine twice a day in patients with mild, moderate and severe forms of COVID-19 pneumonia. At such a dosage, a therapeutic concentration of chloroquine might be reached. With our experience on 2000 dosages of hydroxychloroquine during the past 5 years in patients with long-term treatment (>1 year), we know that with a dosage of 600 mg/day we reach a concentration of 1 μ g/mL [15]. The optimal dosage for SARS-CoV-2 is an issue that will need to be assessed in the coming days. For us, the activity of hydroxychloroquine on viruses is probably the same as that of chloroquine since the mechanism of action of these two molecules is identical, and we are used to prescribe for long periods hydroxychloroquine, which would be therefore our first choice in the treatment of SARS-CoV-2. For optimal treatment, it may be necessary to administer a loading dose followed by a maintenance dose.

Funding: This work was supported by the French Government under the 'Investments for the Future' program managed by the National Agency for Research (ANR) [Méditerranée Infection 10-IAHU-03]. The funding sources had no role in the preparation, review or approval of the manuscript.

Competing interests: None declared.

Ethical approval: Not required.

Philippe Colson, Jean-Marc Rolain, Jean-Christophe Lagier, Philippe Brouqui, Didier Raoult*
Aix-Marseille Université, Institut de Recherche pour le Développement (IRD), Assistance Publique-Hôpitaux de Marseille (AP-HM), MEPHI, 27 boulevard Jean Moulin, 13005 Marseille, France
IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France

*Corresponding author. Present address: IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France.
Tel.: +33 4 13 732 401; fax: +33 4 13 732 402.
E-mail address: didier.raoult@gmail.com (D. Raoult)

References

- Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. *Int J Antimicrob Agents* 2007;30:297–308. doi:10.1016/j.ijantimicag.2007.05.015.
- Bosseboeuf E, Aubry M, Nhan T, de Pina JJ, Rolain JM, Raoult D, et al. Azithromycin inhibits the replication of Zika virus. *J Antivir Antiretrovir* 2018;10:6–11. doi:10.4172/1948-5964.1000173.
- Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020 Feb 4 [Epub ahead of print]. doi:10.1038/s41422-020-0282-0.
- 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 Feb 19 [Epub ahead of print]. doi:10.5582/bst.2020.01047.
- Chinese Clinical Trial Registry. <http://www.chictr.org.cn/searchproj.aspx?title=%E6%B0%AF%E5%96%B9&officialname=&subjectid=&secondaryid=&applier=&studyleader=ðicalcommitteesanction=&sponsor=&studyaimentcode=&studyaimentcode=&studytype=0&studestage=0&studydesign=0&minstudyexecutetime=&maxstudyexecutetime=&recruitmentstatus=0&gender=0&agreetosign=&secsponsor=®no=®status=0&country=&province=&city=&institution=&institutionlevel=&measure=&intercode=&sourceofspends=&createtime=0&isuploadrf=&whetherpublic=&btngo=btn&verifycode=&page=1>.
- Multicenter Collaboration Group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for Chloroquine in the Treatment of Novel Coronavirus Pneumonia Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia [in Chinese]. *Zhonghua Jie He He Hu Xi Za Zhi* 2020;43:E019. doi:10.3760/cma.j.issn.1001-0939.2020.0019.
- Al-Bari MA. Chloroquine analogues in drug discovery: new directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. *J Antimicrob Chemother* 2015;70:1608–21. doi:10.1093/jac/dkv018.
- Raoult D, Drancourt M, Vestris G. Bactericidal effect of doxycycline associated with lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. *Antimicrob Agents Chemother* 1990;34:1512–14. doi:10.1128/aac.34.8.1512.
- Raoult D, Houppikian P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. *Arch Intern Med* 1999;159:167–73. doi:10.1001/archinte.159.2.167.
- Boulos A, Rolain JM, Raoult D. Antibiotic susceptibility of *Tropheryma whipplei* in MRC5 cells. *Antimicrob Agents Chemother* 2004;48:747–52. doi:10.1128/aac.48.3.747-752.2004.
- Fenollar F, Puéchal X, Raoult D. Whipple's disease. *N Engl J Med* 2007;356:55–66. doi:10.1056/NEJMr062477.
- Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun* 2004;323:264–8. doi:10.1016/j.bbrc.2004.08.085.
- Savarino A, Di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. *Lancet Infect Dis* 2006;6:67–9. doi:10.1016/S1473-3099(06)70361-9.
- Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. *Int J Antimicrob Agents* 2020:105923. doi:10.1016/j.ijantimicag.2020.105923.
- Lagier JC, Fenollar F, Lepidi H, Giorgi R, Million M, Raoult D. Treatment of classic Whipple's disease: from in vitro results to clinical outcome. *J Antimicrob Chemother* 2014;69:219–27. doi:10.1093/jac/dkt310.
- Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology* 2005;2:69. doi:10.1186/1743-422X-2-69.
- Barnard DL, Day CW, Bailey K, Heiner M, Montgomery R, Lauridsen L, et al. Evaluation of immunomodulators, interferons and known in vitro SARS-coV inhibitors for inhibition of SARS-coV replication in BALB/c mice. *Antivir Chem Chemother* 2006;17:275–84. doi:10.1177/095632020601700505.
- Biot C, Daher W, Chavain N, Fandeur T, Khalife J, Dive D, et al. Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities. *J Med Chem* 2006;49:2845–9. doi:10.1021/jm0601856.

- [19] Kono M, Tatsumi K, Imai AM, Saito K, Kuriyama T, Shirasawa H. Inhibition of human coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: involvement of p38 MAPK and ERK. *Antiviral Res* 2008;77:150–2. doi:[10.1016/j.antiviral.2007.10.011](https://doi.org/10.1016/j.antiviral.2007.10.011).
- [20] Keyaerts E, Li S, Vijgen L, Rysman E, Verbeeck J, Van Ranst M, et al. Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice. *Antimicrob Agents Chemother* 2009;53:3416–21. doi:[10.1128/AAC.01509-08](https://doi.org/10.1128/AAC.01509-08).
- [21] Takano T, Katoh Y, Doki T, Hohdatsu T. Effect of chloroquine on feline infectious peritonitis virus infection in vitro and in vivo. *Antiviral Res* 2013;99:100–7. doi:[10.1016/j.antiviral.2013.04.016](https://doi.org/10.1016/j.antiviral.2013.04.016).
- [22] de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, et al. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob Agents Chemother* 2014;58:4875–84. doi:[10.1128/AAC.03011-14](https://doi.org/10.1128/AAC.03011-14).