J Antimicrob Chemother
doi:10.1093/jac/dkaa191

Journal of Antimicrobial Chemotherapy

Rationale of a loading dose initiation for hydroxychloroquine treatment in COVID-19 infection in the DisCoVeRy trial

Minh Patrick Lê[1,2]*, Nathan Peiffer-Smadja[3,4], Jeremie Guedj[4], Nadège Néant[4], France Mentré[4], Florence Ader[5,6], Yazdan Yazdanpanah[3,4,] and Gilles Peytavin[1,4] on behalf of the C-20–15 DisCoVeRy French Steering Committee†

[1]AP-HP, Bichat Claude Bernard Hospital, Pharmacology-Toxicology Department, 75018 Paris, France; [2]INSERM, UMRS-1144, Université de Paris, 75006 Paris, France; [3]AP-HP, Bichat Claude Bernard Hospital, Tropical and infectious diseases Department, 75018 Paris, France; [4]IAME, INSERM, UMRS1137, Université de Paris, 75018 Paris, France; [5]Hospices Civils de Lyon, Department of Infectious Diseases, Croix-Rousse Hospital, 104 Grande-Rue de la Croix-Rousse, Lyon 69004, France; [6]Université Claude Bernard Lyon 1, Inserm, 1111, Centre International de Recherche en Infectiologie (CIRI) UCBL1, Lyon, France

*Corresponding author. E-mail: minh.le@aphp.fr
†Members are listed in the Acknowledgements section.

Around the world, several dose regimens of hydroxychloroquine have been used for COVID-19 infection treatment, with the objective of identifying a short-term course. Hydroxychloroquine was found to decrease the viral replication in a concentration-dependent manner *in vitro* and to be more active when added prior to the viral challenge. A loading dose is used to rapidly attain a target drug concentration, which is usually considered as approximately the steady-state concentration. With a loading dose, the minimum effective concentration is reached much more rapidly than when using only the maintenance dose from the start. Thus, we propose a hydroxychloroquine sulphate dose regimen of 400 mg twice daily at Day 1 then 400 mg once daily from Day 2 to Day 10. We aim to evaluate this in the C-20-15 DisCoVeRy trial.

In France and all around the world, many different dose regimens (200 to 1200 mg daily, one to three times daily, with or without a loading dose) of hydroxychloroquine have been used and are recommended for COVID-19 infection treatment (Table 1). In many sites and countries, clinicians use dose regimens recommended for chronic autoimmune disease treatment, such as rheumatoid arthritis or systemic lupus erythematosus. The objective and exigencies of a short-term course of hydroxychloroquine treatment against COVID-19 infection are, however, quite different from the usual chronic autoimmune disease treatment. A large number of clinical trials are being initiated all around the world to evaluate the efficacy and toxicity of this drug in this infection. It is important for these trials to use hydroxychloroquine doses based on a specific scientific rationale.

Based on the severe acute respiratory distress syndrome (ARDS) reported, marked by an uncontrolled cytokine release, eradication of the novel coronavirus (SARS-CoV-2) that causes COVID-19[1] requires rapid drug penetration into pulmonary tissues, intracellular drug uptake and anti-inflammatory[2] and immunomodulatory effects.[1]

Among the different candidates for COVID-19 infection treatment, hydroxychloroquine is a racemic 4-aminoquinoline derivative, chemically related to chloroquine but less toxic in animals,[3] used as the sulphate salt for oral administration (200 mg hydroxychloroquine sulphate is equivalent to 155 mg hydroxychloroquine base) and demonstrating favourable *in vitro* antiviral activity against SARS-CoV-2.

Hydroxychloroquine was found to decrease viral replication in a concentration-dependent manner *in vitro* [EC_{50} of 0.72 μM (242 ng/mL) at 48 h] and to be more active when added prior to the viral challenge.[4] Hydroxychloroquine effectively inhibited the entry step, as well as the post-entry stages of SARS-CoV-2 infection, by changing the glycosylation of the ACE2 receptor and the spike protein.[5]

Besides its antiviral activity, hydroxychloroquine is also known for its modulation of the immune response. In particular, hydroxychloroquine can increase the intracellular pH and inhibit lysosomal activity in antigen-presenting cells, reducing T cell activation, differentiation and expression of co-stimulatory proteins (e.g. CD154 on CD4+ T cells) and cytokines produced by T cells and B cells (e.g. IL-1, IL-6 and TNF). Hydroxychloroquine can also interrupt binding between toll-like receptors (TLR7 and TLR9) and their RNA/DNA ligands and interfere with the interaction between cytosolic DNA and the nucleic acid sensor cyclic GMP-AMP synthase, attenuating the subsequent pro-inflammatory signalling activation and production of cytokines, such as IL-1 and TNF.[1]

Following oral dosing, hydroxychloroquine is rapidly and extensively absorbed, with a time to maximum blood concentration

Table 1. Clinical trials evaluating hydroxychloroquine in COVID-19 prevention or treatment using a loading dose

Study identifier	Sponsor	Phase	Primary purpose	Day 1	Maintenance dose	Maintenance daily dose	Duration of treatment
NCT04323631	Rambam Health Care Campus	1	treatment	400 mg twice daily	200 mg twice daily	400 mg	10 days
NCT04318444	Columbia University	2	prevention	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04303507 (COPCOV)	University of Oxford	NA	prevention	10 mg base/kg	200 mg once daily	200 mg	90 days
NCT04321616 (SOLIDARITY)	Oslo University Hospital	2	treatment	800 mg twice daily	400 mg twice daily	800 mg	10 days
NCT04315948 (DISCOVERY)	Institut National de la Santé Et de la Recherche Médicale, France	3	treatment	400 mg twice daily	400 mg once daily	400 mg	10 days
NCT04304053 (HCQ4COV19)	Fundacio Lluita Contra la SIDA	3	prevention	800 mg once daily	400 mg once daily	400 mg	7 days
NCT04308668 (COVID-19 PEP)	University of Minnesota	3	treatment	1400 mg once daily	600 mg once daily	600 mg	5 days
NCT04325893 (HYCOVID)	University Hospital, Angers	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	8 days
2020-001113-21 (RECOVERY)	University of Oxford	2/3	treatment	800 mg twice daily	400 mg twice daily	800 mg	10 days
NCT04342221 (COV-HCQ)	University Hospital Tübingen	3	treatment	800 mg once daily	600 mg once daily	600 mg	7 days
NCT04342156 (SHARP COVID-19)	Tan Tock Seng Hospital	3	prevention	800 mg once daily	400 mg once daily	400 mg	5 days
NCT04342169	University of Utah	2	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04341441 (WHIP COVID-19)	Henry Ford Health System	3	prevention	400 mg once daily	200 mg once daily	200 mg	8 weeks
NCT04339816 (AZIQUINE-ICU)	Charles University, Czech Republic	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04338906 (CLOCC)	Heinrich-Heine University, Duesseldorf	4	treatment	400 mg twice daily	200 mg twice daily	400 mg	7 days
NCT04335552	Duke University	2	treatment	800 mg once daily	600 mg once daily	600 mg	5 days
NCT04334967	Providence Health & Services	4	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04334382 (HyAzOUT)	Intermountain Health Care, Inc.	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04332991 (ORCHID)	Massachusetts General Hospital	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04332094 (TOCOVID)	Fundació Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau	2	treatment	400 mg twice daily	200 mg twice daily	400 mg	7 days
NCT04330144 (HOPE Trial)	Gangnam Severance Hospital	3	prevention	800 mg once daily	400 mg once daily	400 mg	5 days
NCT04329832 (HAHPS)	Intermountain Health Care, Inc.	2	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04329611	University of Calgary	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04329572	Azidus Brasil	1	treatment	400 mg twice daily	400 mg once daily	400 mg	5 days
ChiCTR2000029898	Peking University Third Hospital	4	treatment	600 mg twice daily	200 mg twice daily	400 mg	5 days
IRCT20100228003449N30	Tehran University of Medical Sciences	2/3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04328012 (COVIDMED)	Bassett Healthcare	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5–14 days
NCT04328272	Ayub Medical College, Abbottabad	3	treatment	600 mg twice daily	400 mg twice daily	800 mg	7 days

Continued

Table 1. *Continued*

Study identifier	Sponsor	Phase	Primary purpose	Day 1	Maintenance dose	Maintenance daily dose	Duration of treatment
NCT04345861 (COVIDOC)	University Hospital, Montpellier	2/3	treatment	800 mg once daily	600 mg once daily	600 mg	10 days
NCT04328285 (COVIDAXIS)	Centre Hospitalier Universitaire de Saint Etienne	3	prevention	400 mg twice daily	200 mg once daily	200 mg	60 days
NCT04347512 (TEACHCOVID)	University Hospital, Strasbourg, France	3	treatment	400 mg once daily	200 mg once daily	200 mg	5 days
NCT04345692 (OAHU-COVID19)	Queen's Medical Centre	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04346667 (PEACE)	Government of Punjab, Specialized Healthcare and Medical Education Department	4	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04344444	LCMC Health	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04341727	Washington University School of Medicine	3	treatment	400 mg twice daily	200 mg twice daily	400 mg	5 days
NCT04334148 (HERO-HCQ)	Duke University	3	prevention	600 mg twice daily	400 mg once daily	400 mg	30 days
NCT04333654	Sanofi	1	treatment	800 mg + 400 mg	200 mg thrice daily	600 mg	10 days
2020-001270-29	Sanofi	2/3	treatment	800 mg + 400 mg	200 mg thrice daily	600 mg	10 days

NA, not applicable.

achieved in approximately 4 h. Oral absorption of hydroxychloroquine sulphate tablets is not stereoselective and comparable fractions of each enantiomer are absorbed (74% of the dose for the R enantiomer compared with 77% for the S enantiomer).[6] According to the Plaquenil label information,[7] under normal circumstances and because they are film-coated, tablets should not be crushed or cut in half. In the exceptional case of a nasogastric tube for patients in the ICU, tablets might be crushed after removal of the film coating and dispersed slowly in water before administration.[8] Absorption of any altered hydroxychloroquine formulation should be verified with therapeutic drug monitoring when available.

Hydroxychloroquine, as a weak base, demonstrates an extensive volume of distribution attributed to tissue uptake by ion trapping rather than tissue binding.[9] It enters erythrocytes via passive diffusion and accumulates in blood cells, with a mean (SD) blood to plasma ratio of 7.2±4.2.[10] Approximately 50% of hydroxychloroquine is bound to plasma proteins.

Hydroxychloroquine is mainly metabolized to *N*-desethylhydroxychloroquine and two other metabolites in common with chloroquine, desethyl-chloroquine and bidesethyl-chloroquine.[3] The metabolism of hydroxychloroquine is known to be stereoselective, favouring the metabolism of (+)-(S)-hydroxychloroquine. The hepatic extraction ratio of racemic hydroxychloroquine from blood is very low (0.06), suggesting that first-pass hepatic extraction accounts for only 6% of the administered dose.[10] Based on animal studies, the rest of the hydroxychloroquine is extensively distributed in tissues such as muscles, eyes, heart, kidneys, liver, spleen and lungs, where sequestration is prolonged.[3]

To date, the specific metabolic pathways of hydroxychloroquine are unknown and considered likely to be similar to those of chloroquine. By extrapolation, human P450 enzymes such as CYP2D6, -2C8, -3A4 and -3A5 are believed to be the major enzymes responsible for the *N*-desethylation of hydroxychloroquine.[11] Whether genotypic polymorphism in these oxidative enzymes affects the antiviral efficacy or toxicity of hydroxychloroquine is still unknown.

The very long mean ± SD terminal elimination half-life of hydroxychloroquine (50±16 days)[12] appears to be mainly due to its extensive volume of distribution (5522 L, estimated from blood data),[10] which is consistent with the lipophilicity of the hydroxychloroquine molecule (logP = 3.84).[4] Based on pharmacokinetic predictions in healthy volunteers, a period of 6 months on oral hydroxychloroquine sulphate 200 mg tablets once daily is required to achieve 96% steady-state concentrations of hydroxychloroquine.[12] Elimination is predominantly via the faeces, with approximately 20%–25% of the dose excreted as unchanged drug in the urine.

A loading dose is used to rapidly attain a target drug concentration, which is usually considered as approximately the steady-state concentration. With a loading dose, the minimum effective concentration is reached much more rapidly than when using only the maintenance dose from the start. With some drugs, especially those with a large volume of distribution such as hydroxychloroquine, it may be necessary to give a large dose initially to get above the minimum effective concentration and get the beneficial effect quickly. However, the loading dose will not maintain the target

concentration unless an appropriate maintenance dose is also used. Theoretically, this loading dose is calculated by multiplying the desired target drug concentration by the volume of distribution of the drug.

In the case of hydroxychloroquine, the desired target concentration might be the EC_{50} against SARS-CoV-2 (242 ng/mL) and we used the volume of distribution (5522 L) for a calculated loading dose of approximately 1336 mg at Day 1. According to the within-subject variability, this volume of distribution is very close to the apparent volume of distribution (V_{ss}/F) estimated by Ducharme *et al.*[13] (7760±4480 L). Based on the oral galenic formulation available for hydroxychloroquine sulphate (200 mg film-coated tablets), the loading dose might be 600 mg twice daily to approach the calculated loading dose.

Based on results obtained by simulation using a physiologically based pharmacokinetic (PBPK) model that can predict hydroxychloroquine concentrations in human lung tissues *in silico*,[4] several dosing regimens for hydroxychloroquine sulphate including a loading dose were evaluated. They showed that plasma concentrations of hydroxychloroquine rapidly increased after each of the three different regimens of loading dose (800+400 mg versus 600+600 mg versus 400+400 mg at Day 1) followed by different maintenance doses. Moreover, the free lung tissue inhibitory quotients (IQs) for hydroxychloroquine (ratio of free lung tissue trough concentration to EC_{50}) were calculated for the different regimens. The regimen consisting of 400 mg twice daily at Day 1 (loading dose) then 200 mg twice daily from Day 2 to Day 5 (maintenance dose) was expected to achieve a free lung IQ of 21 on the first day, 39 on the third day and 85 on the tenth day. Owing to the uncertainty surrounding the *in vitro* susceptibility of SARS-CoV-2, particularly in pulmonary tissue, we decided to choose the longer option of treatment, given the best ratio of free lung tissue trough concentration to EC_{50} of 85 for 5 day and 154 for 10 day treatment durations.

The same maintenance dose was previously tested by Furst *et al.*[14] as the daily dose indicated in active rheumatoid arthritis, where mostly gastrointestinal adverse events occurred during the first 6 weeks of treatment. According to the discontinuations for adverse effects being proportionally related to the cumulative dose (16 800 mg) in that study, the lower loading dose of 400 mg q12h and a maintenance dose of 400 mg q24h for a 10 day treatment duration (cumulative dose of 4400 mg) were chosen in the C-20-15 DisCoVeRy study (NCT04315948).

In the light of these pharmacological, virological and safety data from the literature, we believe that our hydroxychloroquine dosing regimen should be evaluated in a multicentre, adaptive, randomized clinical trial versus lopinavir/ritonavir, with or without β-1b IFN, versus remdesivir versus standard of care for the treatment of adults hospitalized with COVID-19.

Acknowledgements

C-20–15 DisCoVeRy (NCT04315948) French Steering Committee

Principal investigator: Florence Ader. Scientific coordinator: Yazdan Yazdanpanah. Chief methodologist: France Mentre. Infectious diseases specialists: François-Xavier Lescure, Nathan Peiffer-Smadja. Intensivists: Lila Bouadma, Julien Poissy, Jean-François Timsit. Virologists: Bruno Lina, Florence Morfin-Sherpa. Pharmacologist: Gilles Peytavin. Clinical Trial Unit: Methodologists: Charles Burdet, Cedric Laouenan. Statisticians: Drifa Belhadi, Axelle Dupont. E-CRF designers and data managers: Basma Basli, Anissa Chair, Samira Laribi, Julie Level, Marion Schneider, Marie-Capucine Tellier. Project manager: Aline Dechanet. INSERM sponsor: Sandrine Couffin-Cadiergues, Christelle Delmas, Hélène Esperou. Monitoring: Claire Fougerou, Ambre Gelley, Laëtitia Moinot, Linda Wittkop. ANRS coordination and Pharmacovigilance: Carole Cagnot, Alpha Diallo, Soizic Le Mestre, Delphine Lebrasseur-Longuet, Noemie Mercier, Ventzislava Petrov-Sanchez. Sample handling: Vinca Icard, Benjamin Leveau. Drug supply: Johanna Guillon, Anne-Marie Taburet. RENARCI: Marion Noret. REACTing: Eric d'Ortenzio, Oriane PuechaL, Juliette Saillard, Caroline Semaille.

Funding

This trial C-20–15 DisCoVeRy (NCT04315948) was funded by the Institut National de la Santé Et de la Recherche Médicale (INSERM), France.

Transparency declarations

M.P.L. has received travel grants from Bristol-Myers-Squibb and Janssen. F.M. has consulted for Novartis, Ipsen, Servier, and Da Volterra, and received research grants from Roche, and Sanofi (outside the submitted work). G.P. has received travel grants, consultancy fees, honoraria, or study grants from various pharmaceutical companies, including Bristol-Myers-Squibb, Gilead Sciences, Janssen, Merck and ViiV Healthcare. All other authors: none to declare.

References

1 Zhou D, Dai S-M, Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. *J Antimicrob Chemother* 2020; **75**: doi:10.1093/jac/dkaa114.

2 Lu L-H, Chao C-H, Yeh T-M. Inhibition of autophagy protects against sepsis by concurrently attenuating the cytokine storm and vascular leakage. *J Infect* 2019; **78**: 178–86.

3 McChesney EW. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. *Am J Med* 1983; **75**: 11–8.

4 Yao X, Ye F, Zhang M *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; doi: 10.1093/cid/ciaa237.

5 Liu J, Cao R, Xu M *et al*. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov* 2020; **6**: doi:10.1038/s41421-020-0156-0.

6 McLachlan AJ, Tett SE, Cutler DJ *et al*. Disposition and absorption of hydroxychloroquine enantiomers following a single dose of the racemate. *Chirality* 1994; **6**: 360–4.

7 US FDA. Drugs@FDA: FDA Approved Drug Products. https://www.accessdata.fda.gov/.

8 Pesko L. Compounding: hydroxychloroquine. *Am Drug* 1993; 207–57.

9 Macintyre AC, Cutler DJ. The potential role of lysosomes in tissue distribution of weak bases. *Biopharm Drug Dispos* 1988; **9**: 513–26.

10 Tett S, Cutler D, Day R *et al*. A dose-ranging study of the pharmacokinetics of hydroxy-chloroquine following intravenous administration to healthy volunteers. *Br J Clin Pharmacol* 1988; **26**: 303–13.

11 Tanaka E, Taniguchi A, Urano W *et al*. Pharmacogenetics of disease-modifying anti-rheumatic drugs. *Best Pract Res Clin Rheumatol* 2004; **18**: 233–47.

12 Tett S, Cutler D, Day R *et al*. Bioavailability of hydroxychloroquine tablets in healthy volunteers. *Br J Clin Pharmacol* 1989; **27**: 771–9.

13 Ducharme J, Fieger H, Ducharme M *et al*. Enantioselective disposition of hydroxychloroquine after a single oral dose of the racemate to healthy subjects [see comments]. *Br J Clin Pharmacol* 1995; **40**: 127–33.

14 Furst DE, Lindsley H, Baethge B *et al*. Dose-loading with hydroxychloroquine improves the rate of response in early, active rheumatoid arthritis: a randomized, double-blind six-week trial with eighteen-week extension. *Arthritis Rheum* 1999; **42**: 357–65.