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Original article

Antimalarial drugs inhibit the replication of SARS-CoV-2: An *in vitro* evaluation

Mathieu Gendrot [a,b,c,1], Julien Andreani [c,d,1], Manon Boxberger [c,d], Priscilla Jardot [c,d], Isabelle Fonta [a,b,c,e], Marion Le Bideau [c,d], Isabelle Duflot [c,d], Joel Mosnier [a,b,c,e], Clara Rolland [c,d], Hervé Bogreau [a,b,c,e], Sébastien Hutter [b,c], Bernard La Scola [c,d,*], Bruno Pradines [a,b,c,e,**]

[a] Unité Parasitologie et Entomologie, Département Microbiologie et Maladies Infectieuses, Institut de Recherche Biomédicale des Armées, Marseille, France
[b] Aix Marseille Univ, IRD, SSA, AP-HM, VITROME, Marseille, France
[c] IHU Méditerranée Infection, Marseille, France
[d] Aix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France
[e] Centre National de Référence Du Paludisme, Marseille, France

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ABSTRACT

In December 2019, a new severe acute respiratory syndrome coronavirus (SARS-CoV-2) causing coronavirus diseases 2019 (COVID-19) emerged in Wuhan, China. African countries see slower dynamic of COVID-19 cases and deaths. One of the assumptions that may explain this later emergence in Africa, and more particularly in malaria endemic areas, would be the use of antimalarial drugs. We investigated the *in vitro* antiviral activity against SARS-CoV-2 of several antimalarial drugs. Chloroquine (EC_{50} = 2.1 μM and EC_{90} = 3.8 μM), hydroxychloroquine (EC_{50} = 1.5 μM and EC_{90} = 3.0 μM), ferroquine (EC_{50} = 1.5 μM and EC_{90} = 2.4 μM), desethylamodiaquine (EC_{50} = 0.52 μM and EC_{90} = 1.9 μM), mefloquine (EC_{50} = 1.8 μM and EC_{90} = 8.1 μM), pyronaridine (EC_{50} = 0.72 μM and EC_{90} = 0.75 μM) and quinine (EC_{50} = 10.7 μM and EC_{90} = 38.8 μM) showed *in vitro* antiviral effective activity with IC_{50} and IC_{90} compatible with drug oral uptake at doses commonly administered in malaria treatment. The ratio C_{lung}/EC_{90} ranged from 5 to 59. Lumefantrine, piperaquine and dihydroartemisinin had IC_{50} and IC_{90} too high to be compatible with expected plasma concentrations (ratio $C_{max}/EC_{90} < 0.05$). Based on our results, we would expect that countries which commonly use artesunate-amodiaquine or artesunate-mefloquine report fewer cases and deaths than those using artemether-lumefantrine or dihydroartemisinin-piperaquine. It could be necessary now to compare the antimalarial use and the dynamics of COVID-19 country by country to confirm this hypothesis.

1. Introduction

In December 2019, a new severe acute respiratory syndrome coronavirus (SARS-CoV-2) causing coronavirus diseases 2019 (COVID-19) emerged in Wuhan, China [1]. Despite containment measures, SARS-CoV-2 spread in Asia, Southern Europe, then in America and in Africa. The global number of cumulative cases in world was 13,646,660 and 809,747 deaths in August 24, 2020 (https://www.coronavirus-statistiques.com/stats-globale/covid-19-cases-europe/). Currently, 54 countries are affected in Africa with 1,003,435 cumulative cases and 20,398 reported deaths (August 24, 2020) (https://who.maps.arcgis.com/apps/opsdashboard/index.html#/0c9b3a8b68d0437a8cf28581e9c063a9). African countries see slower dynamic of COVID-19 cases and deaths. Several hypotheses could explain the later emergence and spread of COVID-19 pandemic in Africa, like delay in systematic SARS-CoV-2 detection and appropriate epidemiological surveillance, limited international air traffic, climate conditions, demographic conditions with less people above 65 years old, genetic polymorphisms of the cell entry

* Corresponding author. Unité Parasitologie et Entomologie, Institut de Recherche Biomédicale des Armées, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005, Marseille, France.
** Corresponding author. MEPHI, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005, Marseille, France.
E-mail addresses: bruno.pradines@gmail.com (B. La Scola), bernard.la-scola@univ-amu.fr (B. Pradines).
[1] Authors contributed equally to this work.

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receptor for the SARS-CoV-2 (angiotensin converting enzyme 2, ACE-2) [2]. Another hypothesis that may explain this later emergence in Africa, and more particularly in malaria endemic areas, would be the use of antimalarial drugs. Antimalarial drugs could be effective against SARS-CoV-2. In 2002, the World Health Organization (WHO) recommended the use of artemisinin-based combination therapy (ACT) in the treatment of uncomplicated falciparum malaria in Asia, South America and Africa. The combinations artesunate-amodiaquine (Burundi, Cameroon, Democratic Republic of Congo, Gabon, Ivory Coast), artesunate-mefloquine (Cambodia, Brazil), artemether-lumefantrine (Benin, Central African Republic, Malawi, South Africa) or dihydroartemisinin-piperaquine (Thailand, Vietnam) are currently used. Although chloroquine is no longer used to treat falciparum malaria due to high level of resistance, it remains the first-line treatment in combination with primaquine for vivax malaria in some African countries, such as Ethiopia, South Africa and Sudan, in American countries, such as Brazil, Colombia, Guyana, Nicaragua, Peru, Venezuela), in Eastern Mediterranean countries, such as Afghanistan, Pakistan, Sudan, in south east Asian countries, such as India, Myanmar. Antimalarial drugs are potential candidates to be repurposed in both COVID-19 prophylaxis and therapy [3]. Are antimalarial drugs effective against SARS-CoV-2?

Chloroquine, a quinoline, has been shown to be effective *in vitro* against SARS-CoV-2 in Vero E6 cells (African green monkey kidney cells) with median effective concentration (EC_{50}) at micromolar range [4–6]. Hydroxychloroquine, an analogue of chloroquine used in autoimmune diseases such as rheumatoid arthritis and lupus, has also demonstrated *in vitro* antiviral activity against SARS-CoV-2 with EC_{50} at micromolar range [4,6,7]. Twenty-three clinical trials have been conducted in China to investigate the efficacy and safety of chloroquine and hydroxychloroquine in the treatment of COVID-19 [8–10]. Although the clinical assay was not performed according to the randomized double blind method, preliminary data indicated that chloroquine phosphate has demonstrated efficacy in treatment of COVID-19 with few severe adverse reactions in more than 100 patients by shortening hospital stay and improving the clinical evolution [9]. Hydroxychloroquine could shorten time to clinical recovery [11,12]. Effects of hydroxychloroquine were potentiated *in vitro* and *in vivo* by azithromycin [7,13]. Ferroquine, a ferrocenic analogue of chloroquine with anti-malarial activity [14], was shown to be an effective inhibitor of SARS-CoV-1 replication with EC_{50} of 1.4 μM [15].

In 2002, the World Health Organization (WHO) recommended the use of artemisinin-based combination therapy (ACT) in the treatment of uncomplicated *P. falciparum* malaria (artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperaquine or artesunate-mefloquine). Marketed artemisinin derivatives exhibited *in vitro* anti-viral activity at micromolar concentrations against human cytomegalovirus [16–18]. Amodiaquine, a quinoleine antimalarial and one of the partner of artemisinin derivative in ACT, was found to be active *in vitro* at micromolar concentration against SARS-CoV-1 (2.5 μM) but was inactive *in vivo* on SARS-CoV-1 in BALB/c mice [19]. Another quinoline, mefloquine, which has been used in combination with artesunate for the treatment of uncomplicated falciparum malaria, exerts *in vitro* cytopathic effects on Vero cells infected by SARS-CoV-2 at 10 μM [20]. Pyronaridine, a quinoline component of the EU-approved antimalarial Pyramax (pyronaridine-artesunate), was effective *in vitro* against Ebola virus with EC_{50} of 1.14 μM and protected mice when administered 1 h after infection [21].

Taken together, these reports suggest that antimalarial drugs may have antiviral effects and be effective against SARS-CoV-2. Chloroquine, hydroxychloroquine, ferroquine, quinine, mefloquine, desethylamodiaquine (the metabolite of amodiaquine), lumefantrine, pyronaridine, piperaquine and dihydroartemisinin (the metabolite of artemisinin derivatives) were assessed *in vitro* against a clinically isolated SARS-CoV-2 strain.

2. Material and methods

2.1. Antimalarial drugs, virus and cells

All the drugs were provided by Sigma (Saint Louis, MO, USA). Stock solution of chloroquine diphosphate, hydroxychloroquine and ferroquine were prepared in water, in methanol for quinine, mefloquine, desethylamodiaquine, pyronaridine, piperaquine and dihydroartemisinin and in DMSO for lumefantrine. All the stock solutions were diluted in Minimum Essential Media (MEM, Gibco, ThermoFischer) in order to have 7 final concentrations ranging from 0.1 μM to 100 μM. The clinically isolated SARS-CoV-2 strain (IHUMI-3) [12] was maintained in production in Vero E6 cells (American type culture collection ATCC® CRL-1586™) in MEM with 4% of fetal bovine serum and 1% glutamine (complete medium).

2.2. Cytotoxicity assay

In vitro cell viability evaluation on the VERO E6 cell line was performed according to the method described by Mosmann with slight modifications [22]. Briefly, 10^5 cells in 200 μl of complete medium were added to each well of 96-well plates and incubated at 37 °C in a humidified 5% CO_2. After 24 h incubation, 25 μl of complete medium and 25 μl of each concentration of antimalarial drugs were added and the plates were incubated 48 h at 37 °C. After removal of the surpernatant, 100 μL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, Sigma Aldrich, France) solution (0.5 mg/ml in MEM without FBS) were then added to each well. Cells were incubated for 2 h at 37 °C. After incubation, the MTT solution was removed and 100 μl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Then, plates were shaken at 700 rpm for 10 min at 37 °C. The absorbance was measured at 570 nm using a TECAN Infinite F200 Microplate Reader. DMSO was used as blank. The 50% cytotoxicity concentration (CC_{50}) was calculated with the inhibitory sigmoid E_{max} model, which estimated the CC_{50} through nonlinear regression by using a standard function of the R software (ICEstimator version 1.2, http://www.antimalarial-icestimator.net). CC_{50} value resulted in the mean of 6 different experimentations.

2.3. Antiviral activity assay

Briefly, 96-well plates were prepared with 5.10^5 cells/mL of Vero E6 (200 μL per well), as previously described [7]. Antimalarial concentrations were added 4 h before infection. Vero E Cells were infected with IHUMI-3 strain at an MOI of 0.25. After 48h post-infection, the replication was estimated by RT-PCR using the Superscrit III platinum one step with Rox kit (Invitrogene) after extraction with the BIoExtract SuperBall kit (Biosellal, Dardilly, France). The primers used were previously described [23]. EC_{50} and EC_{90} were calculated with the inhibitory sigmoid E_{max} model, which estimated the EC_{50} and EC_{90} through nonlinear regression by using a standard function of the R software (ICEstimator version 1.2). EC_{50} value resulted in the mean of 6 different experimentations.

2.4. Data analysis and interpretation

Selectivity index (SI) as ratio of CC_{50}/EC_{50} was estimated for each antimalarial drug. The expected maximum blood concentration (C_{max}) was estimated from literature for each drug at doses commonly administered in malaria treatment. The ratios C_{max}/EC_{50} and C_{max}/EC_{90} were estimated to find out if the effective concentration in plasma to cure SARS-CoV-2 is achievable in human. If data on drug accumulation into lung was available, the ratios C_{lung}/EC_{50} and C_{lung}/EC_{90} were calculated.

3. Results

CC_{50}, EC_{50}, EC_{90} and SI for each antimalarial drug are presented in Table 1. Chloroquine, hydroxychloroquine, ferroquine, desethylamodiaquine, mefloquine and pyronaridine showed EC_{50} and EC_{90} at low micromolar range (Table 1). The ratio C_{max}/EC_{90} in blood ranged from 0.22 to 0.5 for chloroquine, hydroxychloroquine, mefloquine, pyronaridine and quinine (Fig. 1). But, these drugs were known to accumulate in lungs. The expected concentrations in lungs after an oral uptake at doses commonly administered in malaria treatment were sufficient to exert antiviral effects on SARS-CoV-2 (Fig. 2).

4. Discussion

Chloroquine and its analogues ferroquine and hydroxychloroquine showed *in vitro* activities at low-micromolar range with EC_{50} of 2.1 ± 0.7 μM (SI > 47.6), 1.5 ± 0.3 μM (SI > 66.7) and 1.5 ± 0.3 μM (SI = 13.6), respectively. The EC_{50} values for chloroquine and hydroxychloroquine are lower than those obtained in a previous work on Vero E6 cells at MOI of 0.2 (7.1 and 17.3 μM, respectively) [4]. EC_{50} values depend on several methodological conditions like MOI, duration of incubation [6]. Ferroquine has already shown *in vitro* anti-coronavirus activity against feline coronavirus with EC_{50} of 2.9 μM and SARS-CoV-1 virus with EC_{50} of 1.4 μM [15]. These concentrations were consistent with concentrations observed in human plasma and lungs. Chloroquine given at 100 mg day in the prophylaxis of malaria leads to a plasma concentration of 0.01–0.4 mg/l, ie 0.03–1.25 μM [24]. Chloroquine has an excellent diffusion and tissue concentration which would lead to chloroquine levels 200 to 700 times higher in the lung than in the blood (a concentration which can go up to 280 mg/kg in the lung) [25]. An oral uptake of 400 mg of hydroxychloroquine led to a C_{max} of 1.22 μM [26]. Hydroxychloroquine accumulated 30 times more in lungs than in blood (around 0.3 μM vs 7.8 μM at 6 h) [27]. An oral uptake of 800 mg of ferroquine led to a mean C_{max} (maximum blood concentration) value of 155 ng/ml (around 0.6 μM) and $t_{1/2}$ (elimination half-life) of 10.9 days [28]. No data is available on ferroquine accumulation in lungs. However, as ferroquine is an analogue of chloroquine, we can assume that it may accumulate at least 10 times than in blood. The antiviral activity of chloroquine and its analogues against SARS-CoV-2 are compatible with oral uptake at doses administered in malaria treatment. Chloroquine and hydroxychloroquine inhibited SARS-CoV-2 entry [4,5]. Chloroquine impaired the terminal glycosylation of ACE-2 receptor required for virus entry that resulted in reduced binding affinities between SARS-CoV-1 and its ACE-2 receptor and blocked SARS-CoV-1 entry in human cells [29]. The spike viral protein of SARS-CoV-2 used the ACE-2 receptor for entry, but also sialic acids and gangliosides. In silico analyses showed that the viral spike protein was not able to bind gangliosides in the presence of chloroquine or hydroxychloroquine [30]. Additionally, the ORF8 viral protein could bind to the porphyrin. At the same time, viral orflab, ORF10 and ORF3a proteins could attack the heme to dissociate the iron to form the porphyrin and inhibit the human heme metabolism leading to a decrease of hemoglobin amount which carry oxygen and carbon dioxide. Chloroquine could inhibit the binding of ORF8 to porphyrin and prevent the attack of the 1-beta chain of hemoglobin by orflab, ORF10 and ORF3a proteins [31]. Besides its antiviral activity, chloroquine and hydroxychloroquine have anti-inflammatory effects by decreasing the expression of various pro-inflammatory cytokines including interleukin 6 (IL6), tumor necrosis factor-alpha (TNF) and interferon gamma (INFγ) by mononuclear cells [32]. These cytokines were considerably increased in the cytokine storm due to COVID-19 [33]. Many clinical trials on hydroxychloroquine alone or in combination with azithromycin to treat COVID-19 are in progress. The efficacy of hydroxychloroquine alone or in combination with azithromycin has been controversial. Hydroxychloroquine showed antiviral activity Vero E6 cells (African green monkey kidney cells) [4–6,34] but not in a model of reconstituted airway epithelium [34]. Moreover, neither hydroxychloroquine alone or in combination with azithromycin showed significant effect on the viral load levels in comparision with placebo [34]. Some studies showed that early treatment with hydroxychloroquine alone or in combination with azithromycin was associated with a reduced risk of hospitalization, reduced risk of death and shorter duration of viral presence [35–39]. Early treatment with hydroxychloroquine decreased the level of secreted inflammatory cytokines (IL6, TNF and INFγ) [40]. Conversely, some studies showed that treatment of mild-to-moderate or mild-to-severe COVID-19 with hydroxychloroquine alone or in combination with azithromycin did not improve clinical status or duration of viral shedding in comparison with standard care [41–45]. Moreover, therapeutic interventions using high dosage chloroquine and/or in combination with macrolides may have severe side-effects including

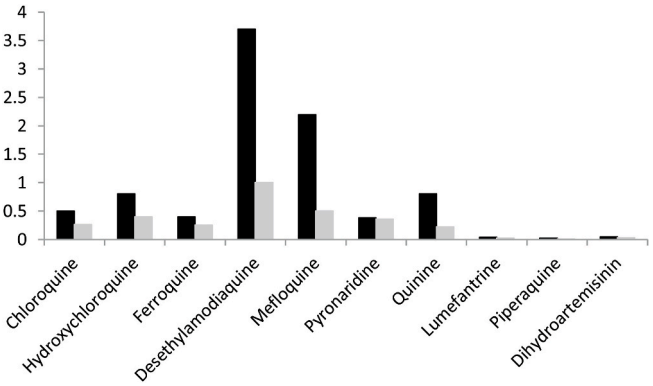

Fig. 1. Bar chart displaying C_{max}/EC_{50} (in black) and C_{max}/EC_{90} (in grey) for antimalarial drugs evaluated for *in vitro* activity againt SARS-CoV-2.

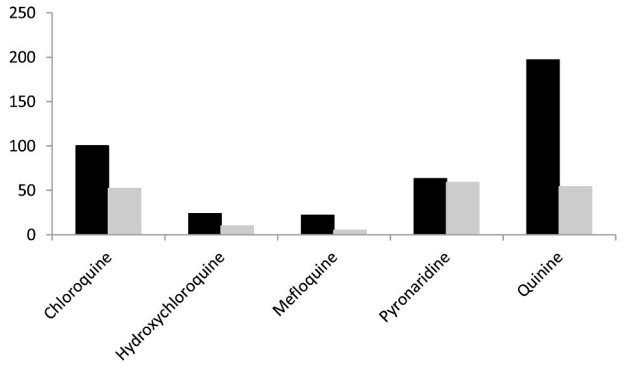

Fig. 2. Bar chart displaying C_{lung}/EC_{50} (in black) and C_{lung}/EC_{90} (in grey) for antimalarial drugs evaluated for *in vitro* activity againt SARS-CoV-2 for which data on lung accumulation were available in literature.

Table 1
Median effective concentration (EC_{50}), 90% effective concentation (EC_{90}) against SARS-CoV-2, 50% cytotoxicity concentation (CC_{50}) and selectivity index (SI) for antimalarial drugs.

Drug	EC_{50} in μM	EC_{90} in μM	CC_{50} in μM	SI
Chloroquine	2.1 ± 0.7	3.8 ± 1	>100	>47
Hydroxychloroquine	1.5 ± 0.3	3.0 ± 1.9	20.4 ± 1.4	11
Ferroquine	1.5 ± 0.3	2.4 ± 0.9	>100	>67
Desethyamodiaquine	0.52 ± 0.2	1.93 ± 1.0	86.1 ± 10.5	166
Quinine	10.7 ± 3.0	38.8 ± 34	>100	>9
Mefloquine	1.8 ± 1.0	8.1 ± 3.7	14.4 ± 2.1	8
Pyronaridine	0.72 ± 0.6	0.75 ± 0.4	15.9 ± 1.6	22
Lumefantrine	24.7 ± 3.6	59.8 ± 26.8	87.7 ± 11.9	4
Piperaquine	33.4 ± 3.8	65.4 ± 25.6	55.0 ± 4.8	2
Dihydroartemisinin	20.1 ± 4.5	41.9 ± 18.0	58.9 ± 7.4	3

cardiac toxicity.

A prophylactic approach with chloroquine at lower dosage could be administrated in vulnerable persons with comorbidities at-risk of severe COVID-19 or in health workers [46]. Chloroquine at 100 mg daily was used for decades for the antimalarial chemoprophylaxis. Several trials on prophylaxis with chloroquine are currently in progress (NCT04349371, NCT04303507). Chloroquine could be evaluated alone or in combination with antibiotics like doxycycline in prophylactic trials. Indeed doxycycline showed *in vitro* antiviral activity against SARS-CoV-2 ($EC_{50} = 5.6$ μM) and low toxicity [47]. Doxycycline at 100 mg daily was used for many years for the antimalarial chemoprophylaxis and combining chloroquine to doxycycline in daily prophylaxis did not increase the risk of adverse effects [48].

Desethylamodiaquine, the metabolite of amodiaquine, showed the best *in vitro* efficacy with EC_{50} of 0.52 ± 0.2 μM (SI = 166). Amodiaquine was used in combination with artesunate in the treatment of uncomplicated malaria in Africa (306 mg amodiaquine base and 100 mg artesunate). Amodiaquine given at 612 mg day led to a plasma concentration of 753 ng/ml of desethylamodiaquine (around 1.9 μM) and a $t_{1/2}$ of 8.9 days [49]. About 0.07% of the administered dose was found in rat lung [50]. This suggests that for an uptake of 612 mg in human, 428 μg would be found in lungs. Amodiaquine, was found to be active also *in vitro* against SARS-CoV-1 with EC_{50} of 2.5 μM but was inactive *in vivo* on SARS-CoV-1 in BALB/c mice [19]. Chloroquine, effective *in vitro* with EC_{50} of 2.5 μM, was also inactive *in vivo* on SARS-CoV-1 in BALB/c mice. Amodiaquine also inhibited dengue virus type 2 replication with EC_{50} of 1.08 μM and EC_{90} of 2.69 μM [51]. The antiviral activity of desethylamodiaquine against SARS-CoV-2 is compatible with oral uptake of amodiaquine at doses commonly administered in malaria treatment. Amodiaquine can only be recommended as treatment and not as prophylaxis due to risk of hepatitis and agranulocytosis during long-term administration [52].

Mefloquine showed anti-SARS-CoV-2 activity with EC_{50} of 1.8 μM and EC_{90} of 8.1 μM. These results are consistent with previous study which showed that mefloquine at 10 μM inhibited completely cytopathic effect onVero E6 cells infected by SARS-CoV-2 [20]. Mefloquine administered at malaria therapeutic dose (1250 mg) lead to a blood concentration of 1648 ng/ml (around 4 μM) in healthy males [53]. A study on postmortem cases showed that mefloquine levels are 10 times higher in the lung than in the blood (a concentration which can go up to 180 mg/kg in the lung) [54]. The antiviral activity of mefloquine against SARS-CoV-2 is compatible with malaria oral therapeutic doses. But mefloquine can cause neuropsychiatric adverse effects [55].

Pyronaridine showed effective antiviral activity with EC_{50} of 0.72 μM and EC_{90} of 0.75 μM. Pyronaridine tetraphosphate given at 720 mg day led to a plasma concentration of 271 ng/ml (around 0.3 μM) in human and a $t_{1/2}$ of 33.5 days [56]. A single oral dose of 2 mg (10 mg/kg) in rats led to a blood C_{max} of 223 ng/ml and a lung C_{max} of 36.4 μg/g (165 more concentrated) [57]. The antiviral activity of pyronaridine against SARS-CoV-2 is compatible with malaria oral therapeutic doses. Acute and sub-acute toxicity was less than that of chloroquine. Cardiovascular toxicity was also less than that of chloroquine [58]. Pyronaridine was well tolerated: around 38% of adverse events *versus* 56% for chloroquine [59].

Quinine showed medium antiviral *in vitro* activity with EC_{50} of 10.7 ± 3.0 μM and EC_{90} of 38.8 ± 34 μM. A 600 mg single oral dose of quinine sulphate led to blood C_{max} around 3.5 mg/l (around 8.5 μM) [60]. In rat, after intravenous dose of 10 mg/kg of quinine, the observed concentration lung/blood ratio was 246 [61]. The *in vitro* effective concentration in lungs to cure SARS-CoV-2 is achievable in human. If its clinical efficacy in human would be confirmed, quinine could be administered in intravenous in patients before cytokine storm. Quinine can cause haemolytic anemia in patients with G6PD deficiency and severe side-effects including cardiac toxicity [52]. Additionally, quinine could be associated with doxycycline against COVID-19, as is done in malaria treatment [62].

Lumefantrine, piperaquine and dihydroartemisinin showed low antiviral activity with EC_{50} of 24.7, 33.4 and 20.1 μM, respectively. A single oral dose of lumefantrine (480 mg) led to C_{max} of 1.1 μM [63]. A single oral dose of 1280 mg of piperaquine and 160 mg of dihydroartemisinin in fed participants led to plasma C_{max} of 596 ng/ml and 324 ng/ml, respectively [64]. No data is available on drug accumulation in lungs for these antimalarial. The ratio C_{max}/EC_{50} or C_{max}/EC_{90} were too low to reach effective concentrations to inhibit SARS-CoV-2 in human. However, ACT (artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperaquine, artesunate-mefloquine or artesunate-pyronaridine), evaluated at plasma concentrations expected after oral uptake at recommended doses used in uncomplicated malaria treatment, showed an *in vitro* inhibition of SARS-CoV-2 replication that ranged from 30 to 70% [65]. The combination mefloquine-artesunate was found to be the most effective *in vitro* against SARS-CoV-2.

5. Conclusion

Chloroquine, hydroxychloroquine, ferroquine, desethylamodiaquine, mefloquine, pyronaridine and quinine showed *in vitro* antiviral effective activity against SARS-CoV-2 with IC_{50} and IC_{90} compatible with drug oral uptake at doses commonly administered in malaria treatment. These *in vitro* activities are higher than those obtained with drugs which are evaluated in clinical trials worldwide like remdisivir (23 μM), lopinavir (26.6 μM) or ritonavir (>100 μM) [66]. However, these results must be taken with caution regarding the potential use of antimalarial drugs in SARS-CoV-2 infected patients: it is difficult to translate *in vitro* study results to actual clinical treatment in patients. Experts agree on the *in vitro* activity of chloroquine or hydroxychloroquine against SARS-CoV-2 but disagree on hydroxychloroquine efficacy in COVID-19 treatment, which remains controversial [67,68]. *In vivo* evaluation in animal experimental models is now required to confirm the antiviral effects of these antimalarial drugs on SARS-CoV-2. The antiviral effects of some antimalarial drugs could partially explain the later emergence and spread of COVID-19 pandemic in Africa. It could be necessary now to compare the antimalarial use and the dynamics of COVID-19 country by country to confirm the potential effects of antimalarial drugs. Based on our results, we would expect that countries which commonly use artesunate-amodiaquine or artesunate-mefloquine report fewer cases and deaths than those using artemether-lumefantrine or dihydroartemisinin-piperaquine.

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Ethical approval

Not required.

CRediT authorship contribution statement

Mathieu Gendrot: Conceptualization, Investigation, Writing - review & editing. **Julien Andreani:** Investigation, Writing - review & editing. **Manon Boxberger:** Investigation, Writing - review & editing. **Priscilla Jardot:** Investigation, Writing - review & editing, Writing review and editing. **Isabelle Fonta:** Investigation, Writing - review & editing. **Marion Le Bideau:** Investigation, Writing - review & editing. **Isabelle Duflot:** Investigation, Writing - review & editing. **Joel Mosnier:** Investigation, Writing - review & editing. **Clara Rolland:** Investigation, Writing - review & editing. **Hervé Bogreau:** Methodology, Investigation, Writing - review & editing. **Sébastien Hutter:** Conceptualization, Investigation, Writing - review & editing. **Bernard La Scola:** Conceptualization, Supervision, Writing original and revised draft.

Bruno Pradines: Conceptualization, Supervision, Writing original and revised draft.

Declaration of competing interest

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