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## Antimalarial artemisinin-based combination therapies (ACT) and COVID-19 in Africa: *In vitro* inhibition of SARS-CoV-2 replication by mefloquine-artesunate

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### ARTICLE INFO

#### Article history:

Received 29 June 2020

Received in revised form 5 August 2020

Accepted 10 August 2020

#### Keywords:

SARS-CoV-2

COVID-19

Antiviral

Antimalarial drug

*in vitro*

malaria

### ABSTRACT

**Objectives:** At the end of November 2019, a novel coronavirus responsible for respiratory tract infections (COVID-19) emerged in China. Despite drastic containment measures, this virus, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spread in Asia and Europe. The pandemic is ongoing with a particular hotspot in Southern Europe and America; many studies predicted a similar epidemic in Africa, as is currently seen in Europe and the United States of America. However, reported data have not confirmed these predictions. One of the hypotheses that could explain the later emergence and spread of COVID-19 pandemic in African countries is the use of antimalarial drugs to treat malaria, and specifically, artemisinin-based combination therapy (ACT).

**Methods:** The antiviral activity of fixed concentrations of ACT at concentrations consistent with those observed in human plasma when ACT is administered at oral doses for uncomplicated malaria treatment was evaluated *in vitro* against a clinically isolated SARS-CoV-2 strain (IHUMI-3) in Vero E6 cells.

**Results:** Mefloquine-artesunate exerted the highest antiviral activity with % inhibition of  $72.1 \pm 18.3\%$  at expected maximum blood concentration ( $C_{max}$ ) for each ACT drug at doses commonly administered in malaria treatment. All the other combinations, artesunate-amodiaquine, artemether-lumefantrine, artesunate-pyronaridine, or dihydroartemisinin-piperaquine, showed antiviral inhibition in the same ranges (27.1 to 34.1%).

**Conclusions:** Antimalarial drugs for which concentration data in the lungs are available are concentrated from 10 to 160 fold more in the lungs than in blood. These *in vitro* results reinforce the hypothesis that antimalarial drugs could be effective as an anti-COVID-19 treatment.

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### Highlights

- Declared data on COVID-19 to WHO are lower than estimated by several model analyses
- The use of antimalarial drugs is one hypothesis to explain the later emergence and spread of COVID-19 pandemic in Africa

- Artemisinin-based combination therapy (ACT) at blood concentrations expected after clinical use at recommended doses in malaria, showed *in vitro* inhibition of SARS-CoV-2 replication
- Mefloquine-artesunate at 550 mg/250 mg (equivalent blood concentration 8.3 and 5  $\mu\text{M}$ ) led to 72.1% inhibition
- Mefloquine concentrated ten times more in lungs than in blood

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### Introduction

In December 2019, a new severe acute respiratory syndrome coronavirus (SARS-CoV-2), causing coronavirus diseases 2019

(COVID-19), emerged in Wuhan, China (Wu F et al., 2020). Despite containment measures, SARS-CoV-2 spread in Asia, Southern Europe, then in America and currently in Africa. Currently, 54 countries are affected in Africa, with 332,820 cumulative confirmed cases and 8,733 reported deaths (June 25, 2020) (<https://www.afro.who.int/health-topics/coronavirus-covid-19>). The five most affected African countries are South Africa with 111,796 cases and 2,205 deaths, Nigeria with 22,020 cases and 542 deaths, Ghana with 15,013 cases and 95 deaths, Cameroon with 12,270 cases and 313 deaths, and Algeria with 12,248 cases and 869 deaths. The first African cases are supposed to have occurred on February 14, 2020, in Egypt, and on February 27, 2020, in Nigeria for Sub-Saharan Africa. Gilbert et al. (2020) estimated that countries with the highest importation risk of COVID-19 cases from China were Egypt, Algeria, South Africa, Nigeria, and Ethiopia, according to air travel flows from infected provinces of China to Africa and African country's capacity to detect COVID-19. Another study estimated the number of cases of COVID-19 in each African country, and in particular, the timing of reporting 10,000 cases for all African countries (Pearson et al., 2020). For instance, the timing of reporting 10,000 cases was between April 11 and 18 (6,129 cases on June 25, 2020) in Senegal, April 17 and 23 (12,270 cases) in Cameroon, May 7 to 21 (197 cases) in Angola and April 23 and May 3 (5,034 cases) in Ethiopia. The declared data to WHO indicated the spread of COVID-19 in Africa but with lower confirmed cases than expected. Several hypotheses could explain the later emergence and spread of the COVID-19 pandemic in Africa, like delay in systematic SARS-CoV-2 detection and appropriate epidemiological surveillance (Kobia and Gitaka, 2020), quick implementation of lockdown measures, physical distancing and enhanced hygiene, limited international air travel flows (Haider et al., 2020), climate conditions (Huang et al., 2020a; Wang et al., 2020), demographic conditions with fewer people above 65 years old (Diop et al., 2020), genetic polymorphisms of the cell entry receptor for the SARS-CoV-2 (angiotensin-converting enzyme 2 (ACE-2)) (Cao et al., 2020), cross-immunity to SARS-CoV-2 (Grifoni et al., 2020; Katoh et al., 2020).

Another hypothesis that may explain this later emergence in Africa, particularly in malaria-endemic areas, would be the use of antimalarial drugs (Izoulet, 2020). Since 2002, the World Health Organization (WHO) has recommended the use of artemisinin-based combination therapy (ACT) in the treatment of uncomplicated falciparum malaria (artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperaquine, or artesunate-mefloquine). The combination of artesunate-amodiaquine is preferentially used as first-line treatment in Burundi, Cameroon, Democratic Republic of Congo, Gabon, Ivory Coast, for instance, artesunate-mefloquine in Cambodia and Brazil, artemether-lumefantrine in Benin, Central African Republic, Malawi and South Africa and dihydroartemisinin-piperaquine in Thailand or Vietnam. Amodiaquine and mefloquine, two quinoline ACT partners, were active *in vitro* at micromolar concentration against SARS-CoV-1 at 2.5  $\mu$ M and SARS-CoV-2 at 10  $\mu$ M, respectively (Barnard et al., 2006; Fan et al., 2020).

Although *in vitro* activity is not necessarily linked to clinical efficacy, access to *in vitro* effectiveness of ACT against SARS-CoV-2 may provide some answers if antimalarial use may have been involved in the later emergence and spread of COVID-19 pandemic in Africa. This study aimed to evaluate the antiviral activity of ACT at concentrations consistent with those observed in human plasma when ACT is administered at oral doses for uncomplicated malaria treatment.

## Methods

### Antimalarial drugs

All the drugs were provided by Sigma (Saint Louis, MO, USA). A stock solution of pyronaridine and piperaquine was prepared

in water, in methanol for mefloquine, desethylamodiaquine, and dihydroartemisinin, and in DMSO for lumefantrine. All the stock solutions were diluted in Minimum Essential Media (MEM, Gibco, ThermoFischer). Fixed concentrations of drug combinations were prepared according to human plasma concentrations expected during oral intake of ACT. The expected maximum blood concentration ( $C_{max}$ ) was estimated from the literature for each drug at doses commonly administered in malaria treatment. The artemisinin compound (artemether, artesunate)  $C_{max}$  was determined as a bioequivalent concentration of its active metabolite, dihydroartemisinin. Indeed, either artesunate or artemether was metabolized rapidly in dihydroartemisinin after oral administration of fixed-dose combinations (30 min to 3 h). A fixed-dose of artesunate-amodiaquine led to plasma  $C_{max}$  of dihydroartemisinin and desethylamodiaquine (200 mg/540 mg) around 802 and 879 ng/ml (experimental fixed-dose estimated at 5 and 4  $\mu$ M) (Navaratnam et al., 2009). A fixed-dose of artemether-lumefantrine (80 mg/280 mg) led to plasma  $C_{max}$  of dihydroartemisinin and lumefantrine around 126 ng/ml and 6.98  $\mu$ g/ml (experimental fixed-dose estimated at 1 and 33  $\mu$ M) (Ali et al., 2010; Ashley et al., 2007). A fixed-dose of dihydroartemisinin-piperaquine (120 mg/960 mg) led to plasma  $C_{max}$  of dihydroartemisinin and piperaquine around 387 ng/ml and 539 ng/ml (experimental fixed-dose estimated at 3.1 and 1  $\mu$ M) (Hanboonkunupakarn et al., 2019). A fixed-dose of artesunate-mefloquine (250 mg/550 mg) led to plasma  $C_{max}$  of dihydroartemisinin and piperaquine around 698 ng/ml and 1392 ng/ml (experimental fixed-dose estimated at 5 and 8.3  $\mu$ M) (Valea et al., 2014). A fixed-dose of artesunate-pyronaridine (180 mg/540 mg) led to plasma  $C_{max}$  of dihydroartemisinin and pyronaridine around 109 ng/ml and 130 ng/ml (experimental fixed-dose estimated at 1 and 0.5  $\mu$ M) (Jittamala et al., 2015). ACT was evaluated *in vitro* at 0.5  $\times$  .1X and 2X of experimental fixed-dose estimated for each combination.

### Virus and cells

The clinically isolated SARS-CoV-2 strain (IHUMI-3) (Gautret et al., 2020) was maintained in production in Vero E6 cells (American type culture collection ATCC<sup>®</sup> CRL-1586<sup>™</sup>) in MEM with 4% of fetal bovine serum and 1% glutamine (complete medium).

### Antiviral activity assay

Briefly, 96-well plates were prepared with 5.10<sup>5</sup> cells/mL of Vero E6 (200  $\mu$ L per well), as previously described (Andreani et al., 2020). ACT concentrations were added 4 h before infection. Vero E Cells were infected with IHUMI-3 strain at an MOI of 0.25. Controls 0% (Vero E cell infected without drug) and 100% inhibition (Vero E cell infected with 100  $\mu$ M of ferroquine, Sigma, Saint Louis, MO, USA) were included. After 48 h post-infection, the replication was estimated by RT-PCR using the Superscript III platinum one step with Rox kit (Invitrogen) after extraction with the BloExtract SuperBall kit (Biosellal, Dardilly, France). The primers used were previously described (Amrane et al., 2020).

### Data analysis and interpretation

The percentage of inhibition of SARS-CoV-2 replication was estimated for each combination as following:  $(\text{mean CT}_{\text{combination}} - \text{mean CT}_{\text{control 0\%}}) / (\text{mean CT}_{\text{control 100\%}} - \text{mean CT}_{\text{control 0\%}}) * 100$ . Data were expressed as the mean and standard deviation of five different experiments.

**Table 1**

Estimation of the % of inhibition of the SARS-CoV-2 replication by fixed-doses of ACT (1x corresponds to expected maximum blood concentration ( $C_{max}$ ) for each ACT drug at doses commonly administered in malaria treatment)

Combination	Inhibition % at 2x plasma $C_{max}$		Inhibition % at 1x plasma $C_{max}$		Inhibition % at 0.5x plasma $C_{max}$	
	Concentrations	Mean $\pm$ SD	Concentrations	Mean $\pm$ SD	Concentrations	Mean $\pm$ SD
Mefloquine-dihydroartemisinin	8.3–5 $\mu$ M	99.6 $\pm$ 0.7	4.1–2.5 $\mu$ M	72.1 $\pm$ 18.3	2.0–1.25 $\mu$ M	30.9 $\pm$ 14.1
Desethylamodiaquine-dihydroartemisinin	4.0–5.0 $\mu$ M	85.8 $\pm$ 9.9	2.0–2.5 $\mu$ M	32.3 $\pm$ 9.9	1.0–1.25 $\mu$ M	17.2 $\pm$ 6.5
Pyronaridine-dihydroartemisinin	0.5–1.0 $\mu$ M	38.2 $\pm$ 5.7	0.25–0.5 $\mu$ M	34.1 $\pm$ 7.1	0.12–0.25 $\mu$ M	16.3 $\pm$ 5.0
Lumefantrine-dihydroartemisinin	33.0–2.0 $\mu$ M	37.7 $\pm$ 3.4	16.5–0.5 $\mu$ M	27.1 $\pm$ 6.0	8.2–0.25 $\mu$ M	12.3 $\pm$ 4.4
Piperaquine-dihydroartemisinin	1.0–3.1 $\mu$ M	29.7 $\pm$ 16.8	0.5–1.5 $\mu$ M	29.3 $\pm$ 4.6	0.25–0.75 $\mu$ M	14.0 $\pm$ 4.2

SD = standard deviation

## Results

Estimations of the % of inhibition of the SARS-CoV-2 replication by fixed-doses of ACT were summarized in Table 1. Mefloquine-artesunate exerted the highest antiviral activity with % inhibition of  $72.1 \pm 18.3$  % at expected maximum blood concentration ( $C_{max}$ ) for each ACT drug at doses commonly administered in malaria treatment. All the other combinations showed antiviral inhibition in the same ranges (27.1 to 34.1 %).

## Discussion

A patient treated with fixed-doses of ACT at commonly recommended doses for uncomplicated malaria shows maximum blood concentrations ( $C_{max}$ ) of the two drugs, which can inhibit 27.1 to 72.1 % of the Vero E Cells infected with the SARS-CoV-2 IHUMI-3 strain. Treatment with artesunate-amodiaquine, artemether-lumefantrine, piperaquine-dihydroartemisinin, or artesunate-pyronaridine leads to replication inhibition around 30%. Additionally, some of these antimalarial drugs are concentrated in the lungs. A single oral dose of 2 mg (10 mg/kg) of pyronaridine in rats led to a blood  $C_{max}$  of 223 ng/ml and a lung  $C_{max}$  of 36.4  $\mu$ g/g (163 more concentrated in the lungs than blood) (Park and Pradeep, 2010). About 0.07% of the administered oral dose (8.6 mg/kg) of amodiaquine was found in rat lung (Winstanley et al., 1988). Treatment with artesunate-mefloquine (expected blood  $C_{max}$  at 8.3 and 1  $\mu$ M) leads to replication inhibition above 70%. This is consistent with the *in vitro* antiviral activity of mefloquine previously reported at 10  $\mu$ M (Fan et al., 2020). A study on human postmortem cases showed that mefloquine levels are ten times higher in lung than in blood (concentration can go up to 180 mg/kg in the lungs) (Jones et al., 1994). No data is available on drug accumulation in the lungs for the other antimalarial drugs, particularly dihydroartemisinin. The data on pyronaridine and mefloquine suggest that the concentrations expected in the lungs allow a 100% replication inhibition of SARS-CoV-2.

Additionally, artesunate exerts anti-inflammatory effects by decreasing the secretion of various pro-inflammatory cytokines including interleukin 6 (IL6), tumor necrosis factor-alpha (TNF), interleukin 1 beta (IL1beta) and interleukin 6 (IL6) through inhibition of nuclear factor kappa B (NF-kB) (Xu et al., 2007). The secretions of IL1beta, IL6, and interferon-gamma (INF $\gamma$ ) were considerably increased in the cytokine storm due to COVID-19 (Huang et al., 2020b; Qin et al., 2020). The combination of antiviral activity and anti-inflammatory effects could lead to a better clinical efficacy of mefloquine-artesunate.

These *in vitro* results reinforce the hypothesis that antimalarial drugs could be effective as an anti-COVID-19 treatment. Based on our results, we would expect that countries that commonly use ACT report fewer cases and deaths during malaria season. It could be necessary now to evaluate the ACT efficacy clinically to treat COVID-19, particularly that of mefloquine-artesunate, and the

potential intervention of ACT against SARS-CoV-2 by comparing its antimalarial use and the dynamics of COVID-19 country by country.

## Author contribution

**Mathieu Gendrot** carried out *in vitro* and molecular investigations, analyzed the data, and participated in drafting the paper. **Isabelle Duflot** participated in cellular and viral cultures. **Manon Boxberger** carried out molecular investigations. **Océane Delandre** participated *in vitro* and molecular investigations. **Priscilla Jardot**, **Marion Le Bideau**, **Julien Andreani**, and **Clara Rolland** participated in virus isolation and its maintenance in culture. **Isabelle Fonta**, **Joel Mosnier** and **Sébastien Hutter** participated *in vitro* investigations. **Bernard La Scola** supervised the study and participated in drafting the paper. **Bruno Pradines** designed and supervised the study, analyzed the data, and drafted the manuscript.

## Ethical approval

No ethical approval is required.

## Declaration of interest

M Boxberger received a PhD grant supported by L'Occitane Society. All the other authors have no conflict of interest to declare.

## Funding

This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the program « Investissements d'avenir », reference ANR-10-IAHU-03. M Boxberger was supported by L'Occitane Society.

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