Short communication

**In vitro antiviral activity of doxycycline against SARS-CoV-2**

Mathieu Gendrot$^{a,b,c}$#, Julien Andreani$^{c,d}$#, Priscilla Jardot$^{c,d}$, Sébastien Hutter$^{b,c}$, Manon Boxberger$^{c,d}$, Joel Mosnier$^{a,b,c,e}$, Marion Le Bideau$^{c,d}$, Isabelle Duflot$^{c,d}$, Isabelle Fonta$^{a,b,c,e}$, Clara Rolland$^{c,d}$, Hervé Bogreau$^{a,b,c}$, 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.

#Authors contributed equally to this work.

*Co-corresponding author. Present address: Unité Parasitologie et Entomologie, Institut de Recherche Biomédicale des Armées, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France. Tel.: +33 4 13 732 231.

E-mail address: bruno.pradines@gmail.com

MEPHI, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France.

E-mail address: bernard.la-scola@univ-amu.fr
Abstract

In December 2019, a new severe acute respiratory syndrome coronavirus (SARS-CoV-2) causing coronavirus diseases 2019 (COVID-19) emerged in Wuhan, China. Despite containment measures, SARS-CoV-2 spread in Asia, Southern Europe, then in America and currently in Africa. Identifying effective antiviral drugs is urgently needed. An efficient approach to drug discovery is to evaluate whether existing approved drugs can be efficient against SARS-CoV-2. Doxycycline, which is a second-generation tetracycline with broad-spectrum antimicrobial, antimalarial and anti-inflammatory activities, showed \textit{in vitro} activity against SARS-CoV-2 with median effective concentration (EC$_{50}$) of 5.6 ± 0.4 µM.

Doxycycline, with its antiviral and anti-inflammatory activities, could be used in prophylaxis of COVID-19 at 100 mg day in combination with chloroquine, or in treatment at 200 mg day during 10 days in combination with hydroxychloroquine.

Keywords: COVID-19, SARS-CoV-2, Doxycycline, treatment, prophylaxis, antiviral, anti-inflammatory
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 currently in Africa. Currently, there is no antiviral treatment recommended by the French Health Ministry against SARS-CoV-2. Several drugs are tested in the context of the discovery trial [https://clinicaltrials.gov/ct2/show/NCT04315948] and a controversial protocol massively used worldwide associate a combination of hydroxychloroquine and azithromycin [2,3]. However, identifying effective low cost antiviral drugs with limited side effects affordable immediately especially for emerging countries is urgently needed. An efficient approach to drug discovery is drug repurposing that consists to evaluate whether existing approved drugs can be efficient against SARS-CoV-2.

Doxycycline is a second-generation tetracycline with broad-spectrum antimicrobial [4] and anti-inflammatory activities [5]. Additionally, doxycycline was approved as prophylaxis against malaria by the Food and Drug Administration in 1994 and has been used since 2006 at the dose of 100 mg/day by the French military forces deployed in malaria-endemic areas [6]. Doxycycline also shows antiviral activity in vitro. This tetracycline derivative significantly inhibited the replication of vesicular stomatitis virus in vitro [7], dengue virus by inhibition of NS2B-NS3 serine protease [8-10]. Doxycycline showed inhibition of entry and replication of Chikungunya virus in Vero cell at 11 µM [11]. Using in-silico method, doxycycline might be a potential inhibitor of Crimean-Congo hemorrhagic fever virus nucleoprotein, an essential protein in virus replication [12]. Additionally, doxycycline inhibited the early-stage replication of porcine reproductive and respiratory syndrome virus, which causes respiratory disease, with EC₅₀ (median effective concentration) of 0.25 µg/ml (about 0.5 µM) [13]. The current study evaluated the antiviral effect of doxycycline against SARS-CoV-2.
2. Methods & Materials

2.1. Agent, virus and cells

Stock solution of doxycycline hyclate (Sigma, Saint Louis, MO, USA) was prepared in
methanol and diluted in Minimum Essential Media (MEM, Gibco, ThermoFischer) in order to
have 7 final concentrations ranging from 0.1 µM to 100 µM. Chloroquine diphosphate
(Sigma, Saint Louis, MO, USA) was used as comparator. The clinically isolated SARS-CoV-
2 strain (IHUMI-3) [2] 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 [14]. 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 each concentration of doxycycline and
chloroquine were added and the plates were incubated 48h at 37 °C. After removal of the
surpernatant, 100 µL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-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 using 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<sub>50</sub> value resulted in the mean of 5 different experimentations.

2.3. Antiviral activity assay

Briefly, 96-well plates were prepared with 5.10<sup>5</sup> cells/mL of Vero E6 (200µL per well), as previously described [15]. Doxycycline and chloroquine concentrations were added 4 h before infection. Vero E Cells were infected with IHUMI-3 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 (Invitrogen) after extraction with the BioExtract SuperBall kit (Biosellal, Dardilly, France). The primers used were previously described [16]. The median effective concentration (EC<sub>50</sub>) was calculated with the inhibitory sigmoid E<sub>max</sub> model, which estimated the EC<sub>50</sub> through nonlinear regression by using a standard function of the R software (ICEstimator version 1.2). CC<sub>50</sub> value resulted in the mean of 4 different experimentations.

3. Results and discussion

The cytotoxicity evaluation of doxycycline and chloroquine showed that the CC<sub>50</sub> values were > 100 µM for 48h. The CC<sub>50</sub> value of chloroquine is consistent with those previously described [17,18]. The median effective concentration (EC<sub>50</sub>) was 5.6 ± 0.4 µM for doxycycline and 2.1 ± 0.8 µM for chloroquine (Figure 1). These EC<sub>50</sub> values depend on several methodological conditions like MOI, duration of incubation [17,19]. The EC<sub>50</sub> value for chloroquine is consistent previous results on Vero E6 cells at MOI of 0.2 [17]. Doxycycline was found to decrease the SARS-CoV-2 replication in a concentration-dependent manner. Besides its antiviral activity, doxycycline has anti-inflammatory effects by decreasing the expression of various pro-inflammatory cytokines including interleukins 1, 6 and 8 and tumor necrosis factor-alpha by macrophages [5] and chemokines including
monocyte chemotactic protein 1, macrophage inflammatory protein 1α and 1β [20]. The immunomodulatory activity of doxycycline improved survival of septic mice with pulmonary inflammation [21]. Doxycycline concentration was twice as high in lungs than in serum [22]. Doxycycline, used as malaria prophylaxis, was well tolerated at the dose of 100 mg/day during several months [23]. Additionally, doxycycline has been recommended at 100 mg twice a day during 10 days for post-exposure prophylaxis of anthrax. This dose can even been taken by children below 8 years old [24]. Doxycycline could be used in prophylaxis of COVID-19 at 100 mg day in combination with chloroquine at 100 mg day, which inhibits the entry of SARS-CoV-1 in cells [25], or in treatment at 200 mg day during 10 days in combination with hydroxychloroquine. At these doses currently used for other indications, doxycycline could be rapidly evaluated in clinical trials in COVID-19.

**Declarations**

**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.

**Competing interests:** The authors have no conflict of interest to declare.

**Ethical approval:** Not required.

**References**


Figure: Comparative antiviral efficacy of doxycycline and chloroquine against SARS-CoV-2 infection \textit{in vitro}