**Title Page**

**Ineffective of lopinavir/ritonavir and chloroquine for a COVID-19 treatment: A perspective of physiologically-based pharmacokinetic and pharmacodynamic modelling**

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**Word count**

**Total abstract:** 250

**Maintext:** 3336

**ABSTRACT.**

Ineffective selection of therapeutic drugs during an urgent situation leads to failure for COVID-19 treatment in large clinical trials, resulting in wasting time and cost. We aimed to demonstrate the utility of physiologically-based pharmacokinetic (PBPK)/pharmacodynamic (PD) modeling to support the withdrawal of chloroquine and ritonavir-boosted lopinavir (LPV/r) for COVID-19 treatment. The developed whole-body PBPK models were validated against clinical data. Model validation was performed using acceptable methods. The inhibitory effect was calculated to demonstrate drug efficacy. Various regimens of chloroquine and LPV/r for COVID-19 treatment in different clinical trials were used for a simulation. The risk of cardiotoxicity following high dose chloroquine administration was assessed. The effect of lung pH on drug concentrations in epithelial lining fluid (ELF) following a high dose of chloroquine and LPV/r was evaluated. The whole-body PBPK models were successfully developed (AAFEs of 1.2-fold). The inhibitory effect (%E) of chloroquine following high dose regimens in both ELF and bronchial epithelial cells (BEC) were lower than 2 and 1%, respectively. The corresponding values for the high dose of LPV/r were 40 and 2%, respectively. The risk of prolonged QTc in the population was higher than 20%. In addition, the %E of chloroquine was increased to 76% at pH 5.6 and decreased to 0.13% at pH 7.5. The change in pH in ELF had no influence on LPV/r concentrations.

PBPK/PD modelling supports the withdrawal of chloroquine and LPV/r for COVID-19 treatment as an effective tool for the selection of therapeutic drug regimens in urgent situation.

*Keywords:*COVID-19, SARs-CoV-2, lopinavir, chloroquine, PBPK

1. **BACKGROUND**

During the early pandemic of COVID-19, the rapid development of novel antiviral drugs to combat the infection to meet the urgent need was limited. Repurposing approved drugs under advanced phases of clinical trials would be a preferable fast-track. Ritonavir-boosted lopinavir (LPV/r) and chloroquine were initially selected as repurposing drugs for COVID-19 therapy based on *in vitro* evidence but were finally withdrawn due to unsatisfactory treatment outcomes in large clinical trials 1. In such an emergency situation, conducting large clinical trials may not be a cost- and time-effective solution. Physiologically-based pharmacokinetic (PBPK) modelling is a pharmacokinetic model mimicking human physiology to explain drug disposition in the human body for a dose-finding in various diseases 2.PBPK/PD modelling has been successfully applied for the selection of appropriate drug regimens and dose optimization in various diseases and conditions. It is also accepted by regulatory agencies as supplementary information for drug registration 3.The aim of the study was to demonstrate the utility of the PBPK and pharmacodynamic (PD) modelling as a tool to predict clinical effectiveness of chloroquine and LPV/r for COVID-19 therapy.

1. **Material and methods**

*2.1 Model construction*

The whole-body PBPK modellings for LPV/r, chloroquine and rifampicin (used for model validation for the developed lung compartments) were constructed based on the information reported from previous studies 4,5 using Simbiology® (version 5.8.2), a product of MATLAB® (version 2019a) (MathWorks, Natick, MA, USA). The lung compartments were divided to pulmonary circulation, lung-blood circulation, bronchial epithelial cells (BEC), and epithelial lining fluid (ELF). Model assumptions included blood-flow limited model (except the lung compartment), immediate drug dissolution, absence of drug absorption in the stomach and large intestine, and absence of enterohepatic recirculation. The physicochemical and biochemical parameters of each drug are shown in **Table S1**.

*2.2 Model validation*

The constructed models were validated using the eight clinically published articles 6-13. Model accuracy was evaluated based on absolute average-folding errors (AAFEs) (a comparison between predictive results and observed data) and a virtual predictive check (VPC). The accepted AAFEs value was < 2-fold 4.The AAFEs equation is as follow:

(1).

Where n is the number of samples; predicted and observed PK/PD parameters are simulated and clinically observed data, respectively. The AAFEs value is reported as mean (±range).

*2.3 Sensitivity analysis*

Sensitivity analysis (a sensitivity coefficient) was performed to determine the effect of model parameters on the plasma drug concentrations following the 250 mg once-daily dose of chloroquine for 14 days. The model parameters for sensitivity analysis were absorption rate constant (Ka), fraction of unbound drug in plasma (fu,p), and blood-to-plasma partition ratio (Rb:p). In addition, the fraction of unbound drug in tissue (fu,t), pH in BEC and ELF, apparent permeability from apical-to-basolateral (Papp, A-to-B) and apparent permeability from basolateral-to-apical (Papp, B-to-A) were used for a sensitivity analysis following a single 600 mg dose of rifampicin and twice-daily dose of 400/100 mg LPV/r for 14 days. Each model parameter was varied by ±20% from its original value. One hundred virtual populations were simulated with the fixed values of other model parameters (constant values). The equation for sensitivity analysis is as follow:

(2)

Where %Y and %X are the percent changes of the AUC312:336h and model parameter, respectively.

*2.4 PBPK-PD model*

A Pharmacodynamic (PD) model (Emax model) was constructed to assess the inhibitory effect of each study drug on SARs-COV-2 according to the equation:

(3)

Where E is inhibitory effect; Emax is maximal inhibition; EC50,u is the half-maximal effective concentration (unbound drug); and Au, lung,tiss is the amount of unbound drug in BEC (lung tissue) (mol/L or M). The Emax and EC50 of LPV/rwere 0.89 and 21.7 µM, respectively 14. The corresponding values for chloroquine were 0.9 and 64.7 µM, respectively 14,15. The EC50 values were selected to calculate the EC90 using the Hill function and then multiplied by the fraction of unbound drug. The fu for both chloroquine and lopinavir (LPV/r) was assumed to be one due to low protein concentration in the experimental environment compared with blood/plasma or tissue. The inhibitory effect (%E), amount of unbound drug in BEC and ELF are presented as a mean (±95% confident interval (CI). The %E total is the inhibitory effect of a combination of LPV/r and chloroquine.

*2.5 Dose simulation using clinical trial information*

*2.5.1 Clinical scenarios*

One hundred virtual populations (50 males and 50 females, aged 18-60 years, weighing 60 kg, fasting state) witheight clinical scenarioswere simulated as follows:

*Chloroquine:* multiple oral doses of 300 mg base chloroquine given twice daily for (i) 7 days in patients with mild COVID-19 (scenario-I) (average negative PCR on day 16) 16; (ii) 10 days for patients with mild COVID-19 (average negative PCR on day 14) (scenario-II) 17; (iii) 14 days in patients with moderate COVID-19 (average negative PCR on day 14) (scenario-III) 18; and (iv) 600 mg base chloroquine given twice daily for 14 days in patients with severe COVID-19 (average negative PCR on day 13) (scenario-IV) 19.

*Lopinavir/ritonavir:*multiple oral doses of 400/100 mg LPV/r given twice daily for (i) 7 days in patients with mild/moderate COVID-19 (average negative PCR on day 21) (scenario-V) 20; (ii) 14 days in patients with mild/moderate COVID-19 (average negative PCR on day 14) (scenario-VI) 17; and (iii) 10 days in patients with mild/moderate COVID-19 (median hospital discharge on day 11) (scenario-VII) 21; and (iv) loading doses of 800/200 mg LPV/r for two doses, followed by 600/150 mg twice daily on day 2 for 9 days in patients with mild/moderate COVID-19 (scenario-VIII) 22.

*2.5.2 Effect of ELF pH on chloroquine and LPV/r concentrations*

The pH in normal ELF in healthy airways is acidic (5.5 to 7.5) 23, which may influence the degree of ionization of chloroquine and LPV/r, resulting in the change in the amount of unbound drug in ELF. The pH in the ELF was set as 6.9. The effects of pH on the amount of unbound chloroquine and LPV/r were simulated at the four pH, *i.e.,* 5.6, 6.5, 6.9, and 7.5.

*2.5.3 Simulation of the risk of cardiac arrhythmia corrected QT (QTc) interval*

Since chloroquine is associated with the risk of cardiac arrythmia with prolongation of corrected QT (QTc) interval [24], the effects of various dosage regimens of chloroquine on QTc were evaluated following the previously published PK-QTc relationship 24. The equation to describe the relationship between chloroquine concentration and QTc is shown below:

QTprediction =QTbaseline + 0.006 \* Total blood chloroquine concentration (3)

The data from one published article was used to validate the risk of QTc prolongation. One hundred virtual populations (50 males and 50 females, aged 18-60 years, weighing 40-70 kg) were simulated. Results are reported as the percentage of predicted peak QTc following 600 mg base of chloroquine given twice daily for 14 days 14 and 300 mg base of chloroquine given twice daily for 14 days in COVID-19 patients with severe symptoms.

**3 Results**

*3.1 Model validation and sensitivity analysis*

The AAFEs are summarized in **Table 1,** and the VPCs are shown in **Figure S1.** The AAFEs [mean (range)] for all regimens was 1.2 (1.08-1.59) 6-13. The AAFEs for ritonavir, chloroquine, LPV/r and rifampicin in plasma were 1.29 (1.18-1.59) 8, 1.21 (1.15-1.26) 9,10 , 1.18 (1.08-1.27) 6,7 and 1.16 (1.13-1.18) 11,13, respectively. The AAFEs of ELF (rifampicin and LPV/r) and BEC (rifampicin) were 1.09 (1.02-1.17) 6,13 and 1.19 13, respectively. For chloroquine, sensitivity coefficient values for Ka, fu and Rb:p  were +0.13, -0.90 and +0.06, respectively. The sensitivity coefficient of rifampicin ELF for fu,t, pH in BEC, pH in ELF, Papp, A-to-B and Papp B-to-A were -0.53, -0.16, -0.05, -0.07, and -0.60, respectively. The corresponding values of rifampicin for BEC were +0.56, -0.18, -0.08, -0.10 and -0.61, respectively.

*3.2 Clinical scenarios*

*3.2.1 Chloroquine*

*Scenario-I:* The Au, chloroquine in BEC and ELF were 253 (246-261) (**Fig. 1**) and 1,800 (1,747-1,852) (**Fig. 2**) nM, respectively. The %E for BEC and ELF were 0.035 (0.035-0.036) and 0.740 (0.710-0.770)%, respectively.

*Scenario-II:* The Au, chloroquine in BEC and ELF were 329 (321-337) (**Fig. 1**) and 2,338 (2,281-2,395) (**Fig 2**) nM, respectively. The %E for BEC and ELF were 0.045 (0.043-0.046) and 0.950 (0.920-0.980)%, respectively.

*Scenario-III:* The Au, chloroquine in BEC and ELF were 345 (334-356) (**Fig. 1**) and 2,453 (2,376-2,530) (**Fig. 2**) nM, respectively. The %E for BEC and ELF were 0.053 (0.051-0.055)% and 1.120 (1.080-1.150)%, respectively.

*Scenario-IV:* The Au, chloroquine in BEC and ELF were 729 (710-747) (**Fig. 1**) and 5,174 (5,042-5,307) nM, respectively (**Fig. 2**). The %E for BEC and ELF were 0.102 (0.098-0.105) and 2.120 (2.050-2.190)%, respectively.

*3.2.2 LPV/r*

*Scenario-V:* The Au, LPV/r in BEC and ELF were 532 (492-571) **(Fig. 3)** and 263 (243-283) **(Fig. 4)** nM, respectively. The %E for BEC and ELF were 0.470 (0.440-0.510) and 11.06 (10.54-11.58)%, respectively.

*Scenario-VI:*  The Au, LPV/r in BEC and ELF were 1524 (1,407-1,640) **(Fig. 3)** and 754 (697-811) **(Fig. 4)** nM, respectively. The %E for BEC and ELF were 1.37 (1.26-1.47) and 30.57 (29.14-32.0)%, respectively.

*Scenario-VII:* The Au, LPV/r in BEC and ELF were 1486 (1378-1594) **(Fig. 3)** and 735 (682-789) **(Fig 4)** nM, respectively. The %E for BEC and ELF were 1.34 (1.24-1.43) and 30.09 (28.84-31.33)%, respectively.

*Scenario-VIII:* The Au, LPV/r in BEC and ELF were 2658 (2449-2867) **(Fig. 3)** and 1,315 (1,212-1,419) **(Fig 4)** nM, respectively. The %E for BEC and ELF were 2.35 (2.18-2.53) and 41.47 (39.96-42.98)%, respectively.

*3.3 Effect of ELF pH on chloroquine and LPV/r concentrations*

*3.3.1 Chloroquine*

For pH 5.6, the Au, chloroquine in BEC and ELF were 638 (615-660) and 1,649,912 (1,591,718-1,708,106) nM, respectively. The %E for BEC and ELF were 0.097 (0.093-0.10) and 76.19 (75.88-76.50)%, respectively. For pH 6.5, the Au, chloroquine in BEC and ELF were 660 (636-683) and 29,543 (28,488-30,599) nM, respectively. The %E for BEC and ELF were 0.101 (0.098-0.105) and 11.63 (11.27-11.99)%, respectively. For pH 7.5, the Au, chloroquine in BEC and ELF were 639 (618-660) and 286 (276-295) nM, respectively. The %E for BEC and ELF were 0.098 (0.095-0.10) and 0.132 (0.128-0.137)%, respectively. The Au, chloroquine values are shown in **Fig 5**

*3.3.2 LPV/r*

For pH 5.6, the Au, LPV/r in BEC and ELF were 2,481 (2,278-2,684) and 1,228 (1,128-1,329) nM, respectively. The %E for BEC and ELF were 2.20 (2.03-2.38) and 40.07 (38.56-41.58)%, respectively. For pH 6.5, Au, LPV/r in BEC and ELF were 2,737 (2,711-2,762) and 1,355 (1,230-1,479) nM, respectively. The %E for BEC and ELF were 2.41 (2.20-2.63) and 41.43 (39.77-43.10)%, respectively. For pH 7.5, Au, LPV/r in BEC and ELF were 2,625 (2,430-2,820) and 1,299 (1,203-1,396) nM, respectively. The %E for BEC and ELF were 2.33 (2.17-2.49) and 41.37 (39.90-42.84)%, respectively.

*3.3.2 Simulation of QTc interval*

The percentage of QTc prolongation (over 500 milliseconds) following 600 mg base of chloroquine given twice daily for 14 days for an average patient’s body weight of 70 kg was 21%. This result was in agreement with the reported clinical study 19. The corresponding value for an average patient’s body weight of 40 kg was 47%.

The percentage of QTc prolongation following 300 mg base of chloroquine given twice daily for 14 days for an average patient’s body weight of 40 kg was 5%. There was no change in QTc (0%) for an average patient’s body weight of 70 kg.

**4. Discussion**

*4.1 Model validity*

The study successfully developed PBPK/PD models with acceptable AAFEs. None of the sensitivity coefficients was greater than one, indicating insensitivity of the model parameters to drug concentrations in plasma, BEC, and ELF. The developed models were valid and applicable for supporting drug selection for COVID-19 therapy in emergency situations.

*4.2 Clinical scenarios*

*Chloroquine:*  Chloroquine is, therefore, ineffective against SARs-COV-2 neither through the blockage of viral entry (ELF) nor viral replication (BEC). Results of the PBPK-PD modeling support the decision on withdrawing chloroquine for COVID-19 treatment since the inhibitory effect (%E) of the drug for both BEC and ELF in all scenarios were lower than 2% and 1%, respectively. As SARs-CoV-2 particles most likely enter the human body through the human epithelial airway or bronchial mucosa, the epithelial lining fluid (ELF) are likely to be first exposed to the viral particles before entering the lungs. Such low chloroquine concentration (<EC90) in ELF (**Fig 2**) is inadequate to prevent viral entry. Besides, the inhibitory effect of chloroquine on viral entry blockage (ELF) was low. This is explained by the weak binding affinity (-3.3 to -5.9 kcal/mol) of chloroquine to angiotensin-converting enzyme 2 (ACE-2) 25, a crucial receptor for a Severe Acute Respiratory Syndrome coronavirus 2 (SARs-CoV-2) infection. As the binding affinity of SARs-CoV-2 (wild type) to ACE-2 is about 10-fold higher (up to -49.94 kcal/mol) 26, chloroquine therefore, cannot compete with SARs-COV-2 virus for the binding site and thus, the blockage of viral entry. Furthermore, the drug also cannot prevent viral replication in the lung epithelial cells when the viral particles infect BEC since the concentrations in the BEC is about 10-fold lower than the EC90 (**Fig 1**). Capthesin L (CTSL) is an endosomal protease that plays an important role in the viral endocytosis to glycoprotein processing of SARs-CoV-2 in the cell. Results of the docking simulation also suggest that this endosomal protease is a poor target for chloroquine (high binding affinity of -5.4 kcal/mol) 27. In addition, results of the *in vitro* study in Calu-3 cell lines also showed the weak inhibitory effect of chloroquine on SARs-CoV-2 (IC50 up to 64.7 µM) 14. Since chloroquine is ineffective for both the blockage of viral entry and replication, the drug is likely to be ineffective for both prevention and treatment of COVID-19.

With increasing knowledge of the time-course of SARs-COV-2 infection, antiviral therapy plays an essential role during the first ten days of symptoms onset when the viral load is high to prevent the development of severe symptoms [28]. Ineffective treatment outcomes following multiple doses of 300 mg base chloroquine given twice daily for 7 to 14 days (**scenario-I to III**) in shortening of the time to PCR-negative results and duration of hospitalization, as well as to prevent disease progression could be due to the delay in chloroquine treatment after 5 days of symptoms onset. Antiviral therapy during this critical period is essential in COVID-19 patients with mild to moderate symptoms. Nevertheless, chloroquine was shown in this study to be ineffective against SARs-CoV-2 even with the doubling dose regimen (loading doses of 600 mg base chloroquine twice daily for 14 days) in patients with severe COVID-19 (**Scenario-IV**). Rather, with such severe symptoms, therapy with anti-inflammatory inhibitors (*e.g.,* inhibitors of IL-6 and IL-1, and dexamethasone) is required. Understanding the time-course of SARs-CoV-2 infection is a critical issue for the implementation of treatment plan.

**LPV/r:** The simulated results also support the decision to withdrawLPV/r for COVID-19 treatment since the inhibitory effect (%E) of LPV/r in ELF was lower than 20% (**Scenario-V**). Any dose regimens of LPV/r are therefore inadequate to provide sufficient concentrations of LPV in plasma, ELF, and BEC to inhibit viral entry or replication. Ineffective treatment efficacy of LPV/r in **Scenario-V** could be explained by the delay and inadequate treatment duration (termination of the drug after the 9 days course of treatment) before confirmation of negative COVID-19 test (16 days). Too early termination of drug administration would lead to insufficient maintenance of drug concentrations and inhibitory effects. Furthermore, the %E of LPV/r in BEC was as low as <2% and would be inadequate to inhibit viral replication in lung epithelial cells. Although the full standard course of LPV/r (400/100 mg LPV/r given twice daily for 10 to 14 days) was given (**Scenario-VI-VII**), LPV concentrations at the target sites (ELF and BEC) were still inadequate. This regimen provided LPV concentrations 60- and 128-fold lower than the EC90 values for SARs-CoV-2 inhibition in the BEC and ELF (**Figure 3, 4**), respectively. It is clear that the standard regimen of LPV/r was ineffective for patients with mild to moderate COVID-19 (%E of LPV/r for the BEC and ELF was lower than 1.5% and 30 %. With an increased dose of LPV/r up to 800/200 mg twice daily for two doses on day 1, followed by 600/150 mg twice daily for up to 10 days (Scenario-VIII), despite the increase in %E for both BEC and ELF, the inhibitory effects were still too low (2 and 40%, respectively) and would be ineffective for both prevention and treatment of COVID-19.

The transmembrane serine protease-2 (TMPRSS-2) has been reported as a crucial receptor for SARs-CoV-2 entry [29]. During the early outbreak of COVID-19, LPV/r was proposed as an effective inhibitor of TMPRSS-2 and ACE-2 with moderate affinity (binding scores of -7.261 and -7.890, respectively) 29 and a potential drug candidate for a COVID-19 treatment. The trough plasma concentration of LPV at steady-state following the standard dose regimen was 13.6 mg/L 22 , while the EC50 of LPV for inhibition of SARs-CoV-2 was 13.64 mg/L (21.7 µM, Calu-3 cell lines) 14. Corrected unbound LPV/r level in plasma was only 0.136 mg/L (fu=0.01), while unbounded EC50 was 13.64 mg/L (low protein binding in the *in vitro* studies) 14. However, the plasma concentration-time profiles could not represent the drug concentration at target sites *e.g.*, bronchial epithelial cell lines (BECs) and epithelial lining fluids (ELF). In addition to TMPRSS-2 and ACE-2, LPV is a potent inhibitor of SARs-COV-2 replication *via* 3CLpro with the binding affinity of -8 kcal/mol 30.

Altogether, results suggest that the screening methods for repurposing drugs based on information from the *in vitro* studies and molecular are not reliable to conclude the efficacy of LPV/r or any other repurposing drugs for the treatment of new emerging diseases. PBPK/PD modeling, on the other hand, is an effective tool to select a potential drug candidate to meet the requirement during the urgent circumstance.

*4.3 Effect of ELF pH on chloroquine and LPV/r concentrations*

Generally, the changes of ELF pH dramatically affected chloroquine concentration in ELF, but not in BEC. The ELF pH of 5.6 was reported in patients with bacterial pneumonia [REF]. With an acidic condition, the % E (ELF) of chloroquine to prevent viral entry appears adequate to prevent SARs-CoV-2 infection to lung epithelial cells. Nonetheless, patients with pneumonia are in a critical situation, and chloroquine is unlikely to provide effective therapy. Rather, these patients require treatment with immunomodulators. Unlike chloroquine, the changes of ELF pH had no influence on LPV/r concentrations in both ELF and BEC.

*4.4 Cardiotoxic effect*

Chloroquine is cardiotoxic, inducing QTc prolongation even when administered in a short duration [31]. At a low dose (300 mg base twice daily for 7 to 14 days) in **Scenario-I to III**, the were no evidence of QTc prolongation (<500 milliseconds) in patients with normal body weight (70 kg). Increased risk of QTc prolongation was however, found in patients with an average body weight of 40 kg without any benefit from COVID-19 treatment. At a higher dose (**Scenario-IV**), an increased risk of QTc prolongation was found in both groups of patients but with a higher risk in patients with an average body weight of 40 kg. Besides the lack of antiviral effect on SARs-COV-2, the anti-inflammatory effect of chloroquine is also weak. There has been no strong evidence to support the use of chloroquine in the late stage of COVID-19. For LPV/r, gastrointestinal adverse events are generally the most commonly reported adverse effects of LPV/. LPV/r also has been shown to increase risk of QTc and PR prolongation in COVID-19 patients 32. At high dose, an increase in alanine-amino-transferase (ALAT) and ALAT of ≥ 3 times of the upper limits and ≥ 5 times of upper normal limits reports for a COVID-19 were 24%, and 12%, respectively 22.Furthermore, as a potent CYP3A inhibitor, LPV/r may lead to drug-drug interactions when administered with other drugs. Combination therapy of LPV/r and chloroquine is not recommended due to ineffective treatment and an increased risk of QTc prolongation.

**5. Conclusion**

In summary, the results of this study support the decision of withdrawing chloroquine and LPV/r for COVID-19 treatment. The application of PBPK/PD modelling could assist in selecting appropriate drug regimens with maximizing efficacy and minimizing risks prior to confirmation in clinical trials to save time and resources.

**CONTRIBUTORS**

T.S. performed data analysis, interpretation, and wrote the draft manuscript. K.N. and J.K. designed the study and was responsible for a conception of study and revising critical important for manuscript. All authors did a final approval of the version to be submitted.

**FUNDINGS**

This study was supported by Thammasat Postdoctoral Fellowship. Also, it was received funding from Thammasat University under the project Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma (No. 1/2556, dated 12 October 2013), and the National Research Council of Thailand (No. 45/2561, dated 10 September 2018). K.N. is supported by the National Research Council of Thailand under the Research Team Promotion grant (grant number NRCT 820/2563, dated 12 November 2020).

**COMPETING INTERESTS**

The authors declared no competing interest for this work

**ACKNOWlEDGEMENT**

We thanked Dr. Marco Siccardi and Mr. Rajith Kumar Reddy Rajoli, department of molecular and clinical pharmacology, University of Liverpool, for his supports and advice.

**DATA AVAILAVILITY STATMENT**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**What is already known abouth this subject**

* An early physiologically-based pharmacokinetic approach predicted lopinavir/ritonavir and chloroquine as effective drugs for COVID-19 treatment.
* Large clinical trials concluded that lopinavir/ritonavir is ineffective for a COVID-19 treatment.
* Later on, lopinavir/ritonavir and chloroquine have been withdrawl for a COVID-19 treatment according to WHO criteria.
* Lopinavir/ritonavir have been used as a clinical practice guideline for a COVID-19 treatment in some countries e.g., Thailand.

**What is already adds**

* This study showed concrete evidences to support a withdrawal of lopinavir/ritonavir and chloroquine for a COVID-19 treatment due to ineffective either for a therapy or prevention of COVID-19 infection.
* The application of PBPK/PD modelling could assist in selecting appropriate drug regimens with maximizing efficacy and minimizing risks prior to confirmation in clinical trials to save time and resources

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**Figure legends**

**Figure 1.** Comparisons the amount of chloroquine in bronchial epithelial cells (BEC) with different scenarios (I-IV) and the cut-off level.

**Figure 2.** Comparisons the amount of chloroquine in extracellular lining fluid (ELF) with different scenarios (I-IV) and the cut-off level.

**Figure 3.** Comparisons the amount of lopinavir (scenario-V-VIII) in bronchial epithelial cells (BEC) and the cut-off level.

**Figure 4.** Comparisons the amount of lopinavir (scenario-V-VIII) in extracellular lining fluid (ELF) and the cut-off level.

**Figure 5.** Comparison the effect of different extracellular lining fluid (ELF) pH values on amount of chloroquine in ELF with cut-off level.

**Tables legends**

**Table 1.** Model validation