**Title:**  Physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling as a tool for antiviral drug dose regimens for COVID-19

**Running title:** *In silico* COVID-19 therapeutic supports

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**AUTHOR CONTRIBUTIONS:**

**CONFLICT OF INTEREST:** All authors have no conflict of interest to declare.

**What is already know**

* Chloroquine and lopinavir were recommended by WHO for COVID-19 treatment.
* Lopinavir has been used in a clinical practice guideline for COVID-19 treatment without supportive evidence.

**What this study adds**

* Lopinavir is effective against SARs-CoV-2 with high inhibitory effect.
* The inhibitory effect of lopinavir in airway epithelial cells is lower than extracellular lung fluid.

**What is the clinical significance**

* Lopinavir should be administered during the early stage of SARs-CoV-2 infection when the COVID-19 test is positive.
* Late administration leads to clinical failure due to low efficacy in airway epithelial cells.

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**ABSTRACT**

**Background and Purpose:** Ritonavir-boosted lopinavir and chloroquine were withdrawn for COVID-19 treatment according to WHO recommendation. However, lopinavir is still being used for COVID-19 treatment in a clinical practice guideline without supportive evidence. We demonstrated the utility of physiologically-based pharmacokinetic (PBPK)/pharmacodynamic (PD) models to support clinical use of lopinavir and the withdrawal of chloroquine for COVID-19 treatment.

**Experimental approach:** The developed whole-body PBPK models were validated against clinical data. Model validation was performed using acceptable methods. The inhibitory effect (%E) was calculated to demonstrate drug efficacy. The recommended drug regimen for COVID-19 was the combination of 400/100 mg lopinavir/ritonavir given twice daily and 300 mg base chloroquine given twice daily for 14 days.

**Key Results:** This study successfully developed whole-body PBPK models (AAFEs of 1.2-fold). For patients with a 70 kg body weight, %E for chloroquine in epithelial lining fluid (ELF) and bronchial epithelial cells (BEC) were about 2% and 12%, respectively. The corresponding values for lopinavir were 66% and 87.4%, respectively. With the increased body weight to 90 kg, %E for lopinavir in BEC dramatically dropped to lower than 60%, while that in ELF was slightly decreased (86.87%).

**Conclusion and Implications:** The results support the decision of withdrawing chloroquine and using lopinavir in asymptomatic (with positive antigen kit test) or mild COVID-19 cases. In addition, results support the administration of antiviral drugs within the ten days of infection to prevent treatment failure.

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

1. **INTRODUCTION**

Since the outbreak of the Coronavirus Disease of 2019 (COVID-19) in China in 2019, over 18 million active cases remain hospitalized for treatment, with over 400 thousand new cases being reported weekly (Worldometer, 2020). Favipiravir is an alternative COVID-19 medicine in an oral formulation with reduced treatment cost (Hill et al., 2020; Reddy & Lai, 2020). It is effective against SARs-COV-2, although the use of this drug alone is unlikely a preferable choice. Combination of favipiravir with other antiviral drugs that act on different targets would be a promising approach. Ritonavir-boosted lopinavir (LPV/r), a previously repurposed drug, had been used to treat COVID-19 but was eventually withdrawn according to the World Health Organization (WHO) recommendation (WHO, 2020). It is however, still being used in clinical practice guidelines for COVID-19 treatment in several countries, including Thailand (Department of Disease Control, Thailand, 2021). Recent studies suggested that antiviral drugs for COVID-19 treatment should be administered within ten days of infection (Perazzolo et al., 2021; Yan et al., 2020). The lack of benefits on the treatment of COVID-19 with LPV/r reported in previous studies are likely to be due to the late start of treatment. As LPV (LPV/r) targets the transmembrane serine protease 2 enzyme (blockage of SARs-COV-2 entry), co-administration of LPV/r with favipiravir would be expected to provide synergistic efficacy. Thailand is the country that successfully controlled COVID-19 during the early phase of disease outbreak. LPV/r is recommended in the Thai clinical practice guideline for COVID-19 treatment (Department of Disease Control, Thailand, 2021). Chloroquine, in addition to LPV/r, had been included in the clinical practice guideline (Department of Disease Control, Thailand, 2020), but was later removed from the guideline according to the WHO’s recommendation. The aim of the study was to demonstrate the utility of the physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) modelling as a tool to find suitable drugs and dose regimens for COVID-19 clinical practice guideline. PBPK/PD modelling has been successfully applied for selection of appropriate drug regimens and dose optimisation in various diseases (Perry et al., 2020).

1. **MATERIALS AND METHODS**
   1. **Model construction**

The whole-body PBPK modellings for LPV/r, chloroquine and rifampicin were constructed based on the information reported from previous studies (Saeheng et al., 2019; Siccardi et al., 2015) using Simbiology® (version 5.8.2), a product of MATLAB® (version 2019a) (MathWorks, Natick, MA, USA). Rifampicin was used for model validation due to the availability of information on drug concentrations in the lung compartments. The lung compartments were divided to the 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 were obtained from the published articles (**Table S1**) (Baneyx et al., 2014; Ernest et al., 2005; Katneni et al., 2018; Kigen et al., 2018; Kirby et al., 2011; Olafuyi et al., 2019; Projean et al., 2003; Rasool et al., 2019; Seng et al., 2015; Stigliani et al., 2016; Varma et al., 2012; Wagner et al., 2017; Wilkins et al., 2008; Xu et al., 2017; Zhang et al., 2012).

**2.2 Model validation**

The constructed models were validated using the eight clinically published articles relating to chloroquine, ritonavir, LPV/r, and rifampicin. 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). AAFEs value of < 2-fold is considered acceptable (Saeheng et al., 2019). 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. AAFEs is reported as mean (±ranges).

**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 included 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 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/r (one value from Yamamoto et al., 2020) and chloroquine (five values from Ko et al., 2020; Liu et al., 2020). 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 chloroquine was assumed to be one due to low protein concentration in the experimental environment compared with blood/plasma or tissue. The fu for LPV/r was 0.03 (Sheahan et al., 2020). The multiplicity of infections (MOI) represents disease severity, *i.e.,* mild (0.01 and 0.02), moderate (0.1 and 0.2), and severe (0.8). 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 and clinical practice guideline**

**2.5.1 Clinical scenarios**

One hundred virtual populations (50 males and 50 females, aged 18-60 years, weighing 60 kg, during fasting state) withsix 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) (Gao et al., 2020); (ii) 14 days in patients with moderate COVID-19 (scenario-II) (Huang et al., 2020); and (iii) 10 days in patients with moderate/severe COVID-19 (scenario-III) (Huang et al. 2020).

*Lopinavir/ritonavir:* multiple oral doses of 400/100 mg LPV/r given twice daily for (i) 7 to 14 (average of 9 days) days in patients with mild/moderate COVID-19 (scenario-IV) (Li et al., 2020); and (ii) 7 days in patients with mild/moderate COVID-19 (scenario-V) (Gao et al. 2020).

*Lopinavir/ritonavir plus chloroquine:* multiple oral doses of 300 mg base of chloroquine co-administered with 400/100 mg of LPV/r given twice daily for 10 days in patients with mild COVID-19 (scenario-VI) (Prasithsirikul et al. 2020).

**2.5.2 Simulations of new regimens**

LPV/r in combination with a fixed-dose (300 mg base given twice daily) of chloroquine was selected for simulations of new alternative regimens. One hundred virtual populations (50 males and 50 females, aged 18-60 years, weighing 70 kg, during fasting state) were simulated for the four different LPV/r dosage regimens as follows: (i) 400/100 mg LPV/r given orally twice daily given for 14 days (regimen-I); (ii) 400/100 mg LPV/r given orally once daily given for 14 days (regimen-II); (iii) 200/50 mg LPV/r given orally twice daily for 14 days (regimen-III); and (iv) 200/50 mg LPV/r given orally once daily for 14 days (regimen-IV).

The effects of pateint’s body weight on %E and amount of drug concentrations following all regimens were evaluated. One hundred virtual populations (50 males and 50 females, aged 18-60 years) with four levels of weighting (*i.e.,* 40, 50, 70, and 90 kg) were simulated for %E and amount of drugs in each lung compartment.

**2.6 Statistical analysis**

Shapiro-Wilk test was used for normality testing. Comparisons of %E for LPV/r in different regimens were performed using one-way ANOVA for normally distributed data and one-way ANOVA with Kruskal-Wallis test for non-normally distributed data. *Post hoc* test was performed using the Dunn test (normally distributed data) or Games-Howells (non-normally distributed data). The statistical significance level was set at =0.05.

1. **RESULTS**

**3.1 Model validation and sensitivity analysis**

The AAFEs [mean (ranges)] for all regimens was 1.2 (1.08-1.59) (Atzori et al., 2002; Eron et al., 2004; Gustafsson et al., 1983; Hsu et al., 1997; Mzayek et al., 2007; Na-Bangchang et al., 1994; Rafiq et al., 2010; Rasool et al., 2019; Ziglam et al., 2002). The AAFEs for ritonavir, chloroquine, LPV/r, and rifampicin in plasma were 1.29 (1.18-1.59) (Hsu et al., 1997), 1.21 (1.15-1.26) (Mzayek et al., 2007; Na-Banchang et al., 1994), 1.18 (1.08-1.27) (Atzori et al., 2003; Eron et al., 2004) and 1.16 (1.13-1.18) (Rafiq et al 2010; Rasool et al. 2019; Ziglam et al., 2002), respectively. The AAFEs of ELF (rifampicin and LPV/r) and BEC (rifampicin) were 1.09 (1.02-1.17) (Atzori et al., 2003; Ziglam et al., 2002) and 1.19 (Ziglam et al., 2002). The AAFEs are summarized in **Table 1** and the VPCs are shown in **Figure S1 (A, B, and C)**. 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.

**3.2 Clinical scenarios and clinical practice guideline**

*Chloroquine*

Scenario-I: Au, chloroquine in BEC, ELF and plasma were 243 (234-252) 1,726 (1,662-1,791), and 125 (121-128) nM, respectively. With the MOI of 0.01, %E for BEC and ELF were 0.01, 0.88 (0.85-0.92) and 5.90 (5.70-6.10)%, respectively. The corresponding values for the MOI of 0.02 were 0.65 (0.63-0.68) and 4.41 (4.24-4.58)%, respectively.

Scenario-II: Au, chloroquine in BEC, ELF and plasma were 330 (317-343), 2,342 (2,250-2,435) and 166 (162-170) nM, respectively. With the MOI of 0.1, %E for BEC and ELF were 0.45 (0.43-0.47) and 3.09 (2.98-3.21)%, respectively. The corresponding values for the MOI of 0.2 were 0.48 (0.46-0.50) and 3.26 (3.14-3.40)%, respectively.

Scenario-III: Au, chloroquine in BEC, ELF, and plasma were 339 (325-352), 2,406 (2,309-2,504) and 193 (188-198) nM, respectively. With the MOI of 0.8, %E for BEC and ELF were 0.46 (0.44-0.47) and 3.14 (3.02-3.26)%, respectively.

*LPV/r*

Scenario-IV: Au, LPV in BEC, ELF and plasma were 1,659 (1,529-1,789), 821 (757-885) and 146 (146-147) nM, respectively. The %E for BEC and ELF were 63.7 (62.68-64.80) and 87.02 (86.16-87.88)%, respectively.

Scenario-V: Au, lopinavir Au in BEC, ELF and plasma were 584 (454-715), 289 (225-353) and 76 (71-81) nM, respectively. %E for BEC and ELF were 23.18 (22.10-24.25) and 35.67 (34.81-36.53)%, respectively.

*Chloroquine plus LPV/r*

Scenario-VI: With the MOI of 0.01, Au, chloroquine in BEC, ELF and plasma were 738 (715-761), 5,240 (5,079-5,401) and 234 (229-239) nM, respectively. The corresponding values for LPV/r were 2,491 (2,383-2,599), 1,233 (1,180-1,286) and 136 (135-137) nM, respectively. For chloroquine, %E for BEC and ELF were 2.63 (2.55-2.71) and 15.52 (15.15-15.89) %, respectively. The corresponding values for LPV/r were 71 (70-72) and 88 (87-89) nM, respectively. %E total (LPV/r plus chloroquine) for BEC and ELF were 72.14 (71.63-72.65) and 90.37 (90.31-90.42)%, respectively.

**3.3 Simulations of new regimens**

*Body weight of 70 kg:* For chloroquine, %E for BEC and ELF of all regimens were about 2% and 12%, respectively. %E for LPV/r for BEC ranged from 36.30% to 65.99% (**Figure 1**), of which regimen-I showed the highest %E (65.99%, p<0.0001) compared with others (**Figure 1**). Similarly, these values in ELF ranged from 84.17% to 87.40% (**Figure 1**). The %E total of LPV/r for each regimen for BEC and ELF was approximately 37 to 66% and 86 to 89%. (**Figure S2**). Au, chloroquine for BEC of all regimens ranged from 524 to 560 nM. The corresponding values for ELF ranged from 3,719 nM to 3,978 nM. In addition, Au, lopinavir of all regimens for BEC ranged from 368 nM to 1,776 nM. The corresponding values for ELF ranged from 181 nM to 879 nM.

*Body weight of 90 kg:* For chloroquine, %E for BEC and ELF of all regimens dropped to less than 2% and 10%, respectively. For LPV/r, %E for BEC dramatically decreased compared with 70 kg body weight (28.45-60.84%) (**Figure 2**). However, the values for ELF were maintained, ranging from 83.82% to 86.95% (**Figure 2**). Regimen-I also provided the highest %E compared with others (p<0.0001) for both BEC and ELF (**Figure 2**). Furthermore, %E total for BEC and ELF for all combination regimens were comparable to LPV/r (**Figure S3**). Au, chloroquine for BEC of all regimens ranged from 403 nM to 456 nM. The values for ELF ranged from 2,865 nM to 3,242 nM. For BEC, Au, lopinavir of all regimens ranged from 258 nM to 1,317 nM. The corresponding values for ELF were 128 nM to 652 nM.

*Body weight of 50 kg:* For chloroquine, %E for all regimens was less than 3%. These values in ELF were around 15%. For LPV/r, the corresponding values in BEC ranged from 40.96 nM to 71.52% (**Figure 3**). These values slightly increased to 86% in ELF (**Figure 3**). It was noted that regimen-I also provided the greatest %E compared to others (p<0.0001), while regimen-IV showed the lowest %E (**Figure 3**). Additionally, %E total values for all regimens in BEC and ELF were approximate to be equal to %E for LPV/r (**Figure S4**). Au, chloroquine for all regimens in BEC ranged from 661 nM to 769 nM. These values increased in ELF, ranging from 4,691 to 5,463 nM. For LPV/r, Au, lopinavir for all regimens in BEC ranged from 476 nM to 2,567 nM. The corresponding range for ELF was 236 nM to 1,271 nM.

*Body weight of 40 kg:* For chloroquine, overall, %E of all regimens were about 3%. The values were increased up to 17% for ELF. For LPV/r, these values for BEC ranged from 44.50% to 73.40% (**Figure 4**). Regimen-I provided the highest %E compared with others (p<0.0001). Similarly, the values for ELF were higher than BEC (86.06 to 88.05%) (**Figure 4**). For ELF, regimen-I provided the highest %E compared with others (p<0.0001). In contrast, regimen-IV provided the lowest %E but was still higher than 85% (**Figure 4**). For the combination regimens, the %E total of all regimens for BEC and ELF were close to that of LPV/r (**Figure S5**). For BEC, Au, chloroquine of all regimens ranged from 770 to 873 nM. The corresponding values for ELF were 5,465 nM to 6,198 nM. Additionally, overall, Au, lopinavir of all regimens ranged from 562 nM to 2,886 nM. For ELF, the values ranged from 278 nM to 1,428 nM.

The relationship between %E and time after administration of each drug regimen for each body weight (40, 50, 70 and 90 kg) are shown in **Figure S6-S13.**

1. **DISCUSSION AND CONCLUSION**

The study successfully developed PBPK/PD models with acceptable AAFEs of 1.2-fold (<2-fold). None of the sensitivity coefficients was greater than one, indicating insensitivity of the model parameters to plasma drug concentrations, BEC, and ELF. The developed models are considered valid and applicable for supporting dose regimen selection for a clinical practice guideline. The utility of PBPK/PD modeling as a tool for rationale drugs and dose selection is well demonstrated with chloroquine and LPV/r for COVID-19 treatment according to Thailand practical guidelines.

**Clinical scenarios**

*Chloroquine:* The results support the decision on withdrawing chloroquine for COVID-19 treatment (WHO, 2020) since the inhibitory effect (%E) of the drug for both BEC and ELF in all disease severity were lower than 1% and 10%, respectively. As SARs-CoV-2 particles most likely enter the human body through the human epithelial airway or bronchial mucosa (Perazzolo et al., 2020), the epithelial lining fluid (ELF) are likely to be exposed to these viral particles before entering the lung. Such low chloroquine concentration (<EC90) in ELF is inadequate to prevent viral entry. In addition, when the viral particles infect BEC, chloroquine concentrations in the BEC should be higher than EC90 to prevent viral replication in the cells. Similarly, chloroquine concentrations in the BEC were approximately 10-fold lower than EC90, and is, therefore, ineffective against SARs-COV-2 neither through the blockage of viral entry (ELF) nor viral replication (BEC).

*LPV/r:* While the WHO recommends withdrawing lopinavir (WHO, 2020), simulated results from the study support the use of this drug for COVID-19 treatment. Ineffective treatment efficacy of LPV/r in the scenarios-IV and V is likely to be due to delayed treatment and inadequate treatment duration before confirmation of negative COVID-19 test. Too early termination of drug administration would lead to insufficient maintenance of drug concentrations or a decrease in inhibitory effect (dose-response relationship). Based on the results of this study, it was clear that LPV/r was effective against SARs-CoV-2 for asymptomatic or mild symptoms (MOI=0.01), as the inhibitory effect (%E) of LPV/r for BEC and ELF was higher than 85% (scenario-IV), and the concentrations in both BEC and ELF were 2- to 3-fold higher than the EC90. LPV/r concentrations are adequate to prevent viral entry (ELF) and suppression of replication (BEC). Since LPV/r is a short half-life drug (2-3 hours), the lower %E for both ELF and BEC in scenario-V could be due to early termination of LPV/r administration before the negative COVID-19 test, resulting in a dramatic drop in %E for both ELF and BEC. Additionally, the delay of LPV/r dose administration also contributes to ineffective therapy (Yan et al., 2020).

*LPV/r plus chloroquine:* In patients with mild symptoms (MOI=0.01), simulated results of the LPV/r plus chloroquine combination support the decision of withdrawing chloroquine and LPV/r for a COVID-19 treatment (Department of Disease Control, Thailand, 2020; WHO, 2020). It is noted that the %E of chloroquine for both ELF and BEC are inadequate to prevent viral entry as well as to suppress viral replication. On the other hand, the %E of LPV/r in both lung compartments are sufficient to block viral entry and replication (%E>85%). Moreover, %E total of the combination for both ELF and BEC is slightly increased by about 2% when compared with the administration of LPV/r alone. Chloroquine in combination with LPV/r is, therefore, not necessary. LPV/r should be combined with other antiviral drugs with synergistic action, *e.g*., favipiravir. Interestingly, the concentrations of both chloroquine and LPV/r in ELF are higher than BEC. This is due to the more significant difference of drug permeability from basolateral to apical (Papp, B-to-A) compared with apical to basolateral (Papp, A-to-B), resulting in accumulation of drugs in ELF; Papp, B-to-A/Papp, A-to-B ratios for chloroquine and LPV/r are 1.38-fold (Katneni et al., 2018) and 7-fold (Kigen et al., 2017), respectively. LPV is a neutral drug that can freely diffuse through the cell membrane. In contrast, only the non-ionic charge of chloroquine (diprotic drug) can diffuse through the cell membrane, resulting in a lower accumulation of chloroquine. It is noted that the amounts of both lopinavir and chloroquine in ELF or BEC are totally different from plasma. Therefore, the plasma drug concentration is not a good surrogate of the amount of drug in ELF or BEC. The use of plasma concentrations as an indicator (plasma concentration/EC90) for dose optimization may not be appropriate.

These results support the use of LPV/r during the early infection stage, mainly when the Antigen Test Kit (ATK) or PCR test is positive. In addition, the results support the optimal time to start antiviral treatment within ten days of infection (Perazzolo et al., 2021; Yan et al., 2020) in patients with asymptomatic or mild symptoms (*e.g*., fever, cough, sputum, and chest distress). Early treatment of LPV/r reduces the time of viral shedding (Yan et al., 2020), but delayed treatment (> 10 days) may lead to clinical failure of antiviral therapy. About 81% of infected patients recover without treatment, risk-benefit assessment of antiviral drugs use for COVID-19 should be critically assessed. Notably, 39% of patients presented with persistent post-COVID-19 interstitial lung disease after infection, resulting in persistent physiological and functional lungs deficits (Myall et al, 2021). These irreversible complications remain a great concern; early treatment of COVID-19 using antiviral drugs is, therefore, likely to provide therapeutic benefits. Remarkably, patients with underlying diseases, *e.g.*, diabetes type-I, diabetes type-II, hypertension and cardiovascular diseases, are significantly associated with severity and mortality rate for COVID-19 (Mishra et al., 2021). The administration of antiviral drugs, *e.g.,* LPV/r and favipiravir combination, should provide beneficial therapy to these patients. It is noted that LPV/r (protease inhibitor) increases the risk of acute myocardial infarction (odds ratios: 1.16 (95%CI: 1.10-1.23)) (DAD Study Group et al., 2007) and diabetes (insulin resistance) (Chandwani et al., 2008). However, the risk of myocardial infarction and diabetes (Lee et al., 2004) results from long-term use of LPV/r (DAD study Group et al., 2007). The short-term treatment of COVID-19 using LPV/r should be safe. In addition, the current Thai clinical practice guideline for COVID-19 treatment recommends the administration of favipiravir and LPV/r in combination to treat patients with asymptomatic and/or mild symptoms with positive ATK test *via* home isolation (Department of disease control, Thailand, 2021).

**Simulations of new regimens**

In general, chloroquine was not found to be effective in patients with various body weights (%E both in BEC and ELF were lower than 20%). As a result, the combination of chloroquine with LPV/r is not recommended. It appeared that the %E total (a combination of LPV/r with chloroquine) was slightly increased compared with the administration of LPV/r alone.

With the patient’s body weight of 70 kg, regimen-I was the best option since %E values for LPV/r both in BEC (65.10%) and ELF (87.34%) were highest compared with other regimens (p<0.0001). However, gastrointestinal side effects may decrease drug tolerance and adherence. A once-daily dose with dose reduction would be a preferable choice (regimen-II and IV). It was clear that regimen-II provided superior %E than regimen-IV both in BEC and ELF (p<0.0001). Regimen-II seemed to be the suitable choice. Notably, %E of regimen-II for BEC dropped to around 50%. This regimen may not be effective for viral suppression. With an increasing body weight to 90 kg, regimen-I is still the best option, but %E for BEC was dramatically dropped to lower than 60%, while %E for ELF was slightly decreased (86.87%). Dose reduction (regimen-II, III, and IV) led to the decrease in %E (below 50%) in the BEC compartment. In cases when the patient’s adherence was of concern, regimen-II was recommended. In contrast, %E both for BEC and ELF of regimen-I was slightly increased when the body weight was decreased to 50 kg (70.88% and 87.77% for BEC and ELF, respectively). In cases when the amount of dose is a great concern, regimen-IV (200/50 mg LPV/r once-daily) is an alternative choice. This regimen, however, may not be appropriate for the inhibition of viral replication (%E for the BEC =42%.) Otherwise, regimen-II or III would be more suitable. With the lowest body weight of 40 kg, regimen-I was also the best option as it provided the highest %E both in BEC and ELF. However, patients with low body weight are likely to experience toxicity due to a decrease in the apparent volume of distribution of the drug (Cressey et al., 2015). In such a case, regimen-II or III (a dose reduction) is recommended.

PBPK/PD modelling could be applied for dose optimisation for a clinical practice guideline. However, the clinical application based on the applied PBPK/PD models in this study for LPV/r administration may be limited due to limited information of LPV/r; only the MOI of 0.01 (asymptomatic or mild cases) was available. Further details of LPV/r with various MOI values (*e.g*., 0.1 and 0.2) is required to predict the suitable dose regimens for moderate cases. Furthermore, the EC50 values in this study for both chloroquine and LPV/r were obtained from the experiments using Vero-E6 cell lines which were derived from the kidney of a green African monkey. The results might not represent the characteristics of the main targets of SARs-CoV-2 in humans. Calu-3 is the more preferable cell line, but this human cell line is an immortalized cell line, which may not represent the main targets of SARs-CoV-2. A recent study revealed that TMPRSS2 and cathepsin-L (CTSL) receptors have moderate to high expression in nasal, trachea, large, and small human airways (Aguiar et al., 2020). CTSL receptor is also expressed in Vero-E6 cell line with high expression level (Mellott et al., 2021), while low expression in Calu-3 cell line has been reported (Mellott et al., 2021). Additionally, this receptor is likely to be a target for chloroquine action in SARs-CoV-2. (Braz et al., 2020). The use of Vero-E6 and Vero-E6/TMPRSS2 cell lines for chloroquine and LPV/r is more preferrable.

In conclusion, PBPK/PD modelling is an effective tool to assist drug and dose regimen selection for a clinical practice guideline. The results of this study support the decision of withdrawing chloroquine and the use of LPV/r. In addition, results support early treatment with antiviral drugs within the ten days of infection. The standard dose regimen of LPV/r administration (400/100 mg LPV/r twice daily) is suitable for patients weighing over 70 kg. Dose reduction (400/100 mg LPV/r once daily or 200/50 mg of LPV/r twice daily) is recommended for individuals weighing lower than 50 kg.

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**Figure Legends9**

**Figure 1.** Comparisons of the inhibitory effect (%E) of different regimens of lopinavir in bronchial epithelial cell (BEC) and extracellular lining fluid (ELF) in patients weighed 70 kg.

**Figure 2.** Comparisons of the inhibitory effect (%E) of different regimens of lopinavir in bronchial epithelial cell (BEC) and extracellular lining fluid (ELF) in patients weighed 90 kg.

**Figure 3.** Comparisons of the inhibitory effect (%E) of different regimens of lopinavir in bronchial epithelial cell (BEC) and extracellular lining fluid (ELF) in patients weighed 50 kg.

**Figure 4.** Comparisons of the inhibitory effect (%E) of different regimens of lopinavir in bronchial epithelial cell (BEC) and extracellular lining fluid (ELF) in patients weighed 40 kg.