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Research Article

Simulation of pharmacokinetic drug-drug interaction and dosage regimens optimization of nelfinavir and cepharanthine as a potential combination against COVID-19

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ABSTRACT

The pharmacokinetic (PK) drug-drug interactions (DDIs) of nelfinavir and cepharanthine combination is limited information in human. In addition, the dosage regimen of this combination is not available for COVID-19 treatment. The objective of this study was to perform *in silico* simulations using GastroPlus™ software to predict physicochemical properties, PK parameters using the physiologically based pharmacokinetic (PBPK) model of healthy adults in different dosage regimens. The DDIs analysis of nelfinavir and cepharanthine combination was carried out to optimize the dosage regimens as a potential against COVID-19. The Spatial Data File (SDF) format of nelfinavir and cepharanthine structures obtained from PubChem database were used to carry out *in silico* predictions for physicochemical properties and PK parameters using several aspects of modules such as ADMET Predictor, Metabolism and Transporter, PBPK model. Subsequently, all data were utilized in the DDIs simulations. The dynamic simulation feature was selected to calculate and investigate the C_{max}, $AUC_{0\text{-}120}$, $AUC_{0\text{-}inf}$, C_{max} ratio, $AUC_{0\text{-}120}$ ratio, and $AUC_{0\text{-}inf}$ ratio. The victim or nelfinavir dosage regimens were used four oral administration regimens of 500 mg and 750 mg in every 8 and 12 hours for simulations. The perpetrator or cepharanthine oral dosage regimens were used in several regimens from 10 mg to 120 mg in every 8, 12, and 24 hours. From all predicted results, the dosage regimen as a potential combination against COVID-19 was nelfinavir 500 mg every 8 hours and cepharanthine 10 mg every 12 hours.

Keywords:
Coronavirus, COVID-19, Hydroxychloroquine, Monte Carlo simulation, SARS-CoV-2

1. INTRODUCTION

The coronavirus disease 2019 (COVID-19), the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, impacts more than 643,500,000 confirmed cases including more than 6,630,000 deaths reported on December 9, 2022 by the World Health Organization (WHO)[1]. Several drugs such as nirmatrevir-ritonavir, molnupiravir, remdesivir, lopinavir-ritonavir have been used to demonstrate benefits in patients with COVID-19 following to the WHO guideline[2] and approved by U.S. Food and Drug Administration (US-FDA)[3]. According to treatments against COVID-19 pay attention on preventing virus replication and managing inflammation and other comorbidity symptoms[4], the combination agents presenting different mechanism of actions were currently considered to provide synergistic antiviral effects. Ohashi H and colleagues reported the more potent antiviral activity of the combination agents of nelfinavir and cepharanthine compared to remdesivir demonstrated by *in vitro*, *in silico* and infected cell culture analysis to inhibit SARS-CoV-2 entry and RNA replication, respectively. The mathematical prediction using a classical Bliss independence method based on scaling parameter of C_{max} of nelfinavir 500 mg every 8 hours orally combined with cepharanthine 25 mg once weekly intravenously proposed that the

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combination further reduced the cumulative viral load, facilitated virus elimination and could limit the emergence of viral drug resistance[5]. Furthermore, this research group proposed the new mathematical prediction in combination of nelfinavir 500 mg every 8 hours orally and cepharanthine 100 mg once weekly intravenously reduced the viral RNA[6]. Nelfinavir is a protease inhibitor used to limit viral replication and improve immune function in HIV-infected individuals. It has been evaluated as first-line therapy with nucleoside reverse transcriptase inhibitors (NRTIs) in treatment-naive patients, or as an additional antiretroviral agent in protease inhibitor-naive patients already receiving NRTIs. When used in combination with NRTIs, nelfinavir 1,250 mg every 12 hours orally produced similar results to 750 mg every 8 hours orally. Nelfinavir is mediated metabolism by cytochrome P450 3A4 (CYP3A4) and CYP2C19 enzymes and P-glycoprotein transporter[7]. Hosogaya N and colleagues conducted the clinical trial to evaluate the antiviral efficacy, clinical efficacy and safety of nelfinavir in asymptomatic and mild COVID-19 patients in a multicenter randomized controlled trial in Japan by using nelfinavir 750 mg every 8 hours orally. However, this trial is under clinical evaluation[8]. Cepharanthine is a biscoclaurine alkaloid, extracted from the roots of *Stephania cepharantha* Hayata (Menispermaceae), known to have anti-inflammatory and immunomodulatory activities[9-10]. Cepharanthine has been reported a range of therapeutic potential including radiation induced leukopoenia[11], idiopathic thrombocytopenic purpura[12], alopecia areata and alopecia pityrodes[13]. Moreover, it mitigates lung injury induced by bilateral lower limb I/R in rats[14] and has antitumor activity, antitumor invasion and pro-apoptotic action in many cancer cells[15]. Cepharanthine is mediated metabolism by CYP3A4, CYP2E1 and CYP2C9 enzymes and P-glycoprotein transporter[16].

Drug-drug interactions (DDIs) are one of the commonest causes of adverse drug reactions (ADRs) when using at least two drugs or polypharmacy. The ADRs may cause from the pharmacokinetics interaction to reduce or increase the drug concentration of each other in blood circulation to change clinical efficacy. The pharmacokinetic drug-drug interactions are conducted to observe the change of plasma concentration-time profiles especially the multiple dosing to reach a steady state condition. The simplified disposition model at steady state allows comparisons of measurable parameters such as area under the curve (AUC), half-life ($t_{1/2}$), maximum concentration (C_{max}) and time to maximum concentration (T_{max}) following drug-drug interaction studies to characterize whether a drug level is lower or higher than using as a single drug. DDIs can change the volume of distribution (V_d) when transporters more than minimally affect drug disposition. The change of V_d will not impede the ability to accurately predict changes in exposure AUC when transporters are involved. However, if both clearance (CL) and V_d are changed, transporters being significantly involved in a DDIs[17]. Pharmacometrics properties of the small molecules facilitate the liberation (L), absorption (A), distribution (D), metabolism (M), excretion (E) and pharmacological action of the drugs. Physicochemical properties such as partition coefficient (logP), solubility, diffusion coefficient (Diff. Coeff.), permeation coefficient (Peff.), acid dissociation constant (pKa), blood-brain barrier (BBB) penetration are also important drug-like characteristics to determine the ability of the molecule to permeate into the systemic circulation, present the pharmacological actions and may cause dose-related toxicity[18-19]. Therefore, the consideration of the interactions involving metabolic enzymes and transporters are very important to investigate the therapeutic outcome of the drugs. According to nelfinavir is mediated metabolism by CYP3A4 and CYP2C19 enzymes and P-glycoprotein transporter[7] and cepharanthine is mediated metabolism by CYP3A4, CYP2E1 and CYP2C9 enzymes and P-glycoprotein transporter[16], therefore, the DDIs of this combination would consider the interaction of CYP3A4 and P-glycoprotein transporter intensively. Computer-based simulation or *in silico* modeling software such as GastroPlus™ (Simulations Plus Inc., USA)[20] can be used to predict the drug characterization when extensive preclinical and clinical data are limited. The GastroPlus™ can predict physicochemical properties, pharmacokinetics (PK) parameters including ADME, and potential DDIs properties based on the structural features and dosage regimens. Firstly, the physicochemical properties, metabolic and transporter profiles are derived from the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) predictor module. Secondly, the physiologically based pharmacokinetic (PBPK) module is used for PK simulations. And finally, the drug-drug interaction module is used to simulate the potential drug interaction through GastroPlus™ software. The simulations of DDIs module predict the ability of a pair of drugs to affect the metabolism and disposition of each other. During performing the simulation of DDIs, the "victim" is called for a drug is either inhibited or induced by another drug. The "perpetrator" is called for a drug causes inhibition or induction of metabolic enzymes or transporters of the victim drug. The dynamic simulation is used to calculate baseline and full simulations to investi-gate the PK parameters especially the predicted C_{max}, AUC_{0-t}, AUC_{0-inf}, C_{max} ratio, AUC_{0-t} ratio and AUC_{0-inf} ratio. After that, the perspective dosing regimens is considered.

The ADME and PK properties of nelfinavir and cepharanthine combination is limited information in human. In addition, DDIs of this combination related to COVID-19 regimens especially in an oral route administration and comorbidities remain totally unevaluated. The objective of this study was to conduct the *in silico* simulations using GastroPlus™ software on physicoche-

mical properties and PK parameters. The PBPK models of healthy adults using different dosage regimens were created. The DDIs analysis of nelfinavir and cepharanthine combination in the oral route administration was carried out to optimize the dosage regimens as a potential against COVID-19.

2. MATERIALS AND METHODS

2.1. Structures import of nelfinavir and cepharanthine for DDIs simulation

The Spatial Data File (SDF) format of nelfinavir and cepharanthine structures obtained from PubChem, a National Institutes of Health chemical structure database[21] were used to carry out *in silico* predictions for physicochemical properties, PK parameters and DDIs simulations. Nelfinavir and cepharanthine structures are presented in Figure 1.

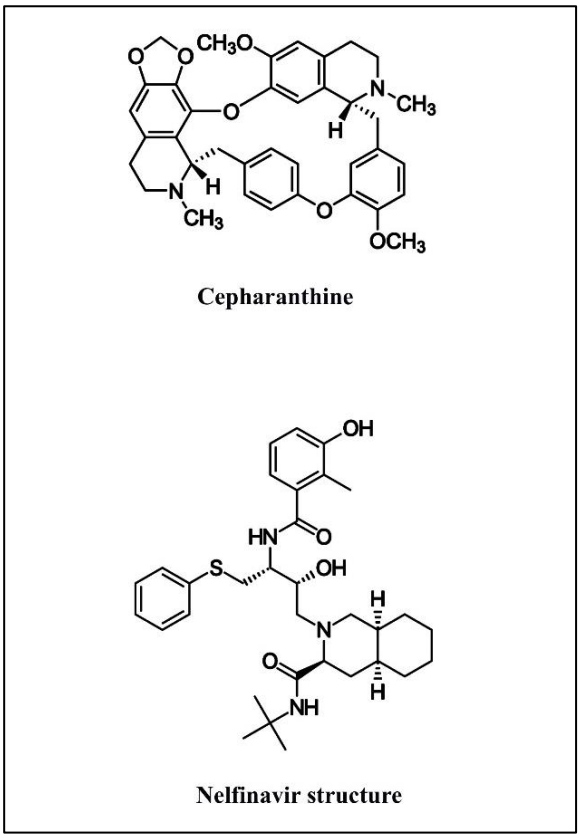

Figure 1. The chemical structures of cepharanthine and nelfinavir.

2.2. GastroPlus™ software simulations

GastroPlus™ software version 9.8 (Simulations Plus Inc., Lancaster, CA, USA) was granted the five years license to the Faculty of Pharmacy, Mahidol University from October 12, 2021 to October 12, 2026 and used to predict physicochemical properties, PK parameters and DDIs by calculating metabolic enzyme and transporter interactions of drugs in human PBPK model.

2.3. Physicochemical properties and pharmacokinetics simulations

The basement model to predict physicochemical properties and PK parameters utilized several aspects of modules such as ADMET Predictor, Metabolism and Transporter, PBPK model in GastroPlus™ database[20]. Since pharmacometrics properties significantly differed based on dosage regimens and route of administration, this study selected the oral route of administration from the workstation features. The simulation process was initiated by navigating through five different Tabs, namely, Compound, Gut Physiology, Pharmacokinetics, Simulation, and Graph. Among these, the prediction in each Tabs were compound properties dependent except Gut Physiology Tab. Firstly, the Compound Tab was used to import the SDF structure by creating a "New Drug Database". Nelfinavir and cepharanthine were imported for simulations before performing the DDIs process. Secondly, the Gut Physiology Tab used the predicted physicochemical properties from the Compound Tab by using the ADMET Predictor module. The Gut Physiology Tab presented several features such as pH, volume, gastrointestinal length and metabolic enzyme expression. The Gut physiology Tab was set for healthy individuals with average population physiological specifications in fasted conditions. Thirdly, the Pharmacokinetics Tab created the important PK parameters especially C_{max}, C_{max} in liver, C_{max} brain, C_{max} in lung, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ systemic clearance (CL_{sys}), steady state volume of distribution (V_{ss}) and elimination half-life (T_{half}) from PBPK model using American healthy young adults having age of 30 years, weight of 85.53 kg based on a body mass index (BMI) scale of 27.48 kg/m^2. In this study, the predicted $AUC_{0\text{-}t}$ was simulated for 120 hours ($AUC_{0\text{-}120}$) to reach the steady state and complied with the simulation antiviral activity of the *in vitro* study[5,16]. Fourthly, the Simulation Tab calculated the drug concentration profiles in every organ in the body using information obtained from the Gut Physiology Tab. Finally, the Graph Tab showed the concentration-time profiles as needed to be initiating information for DDI module of simulation.

2.4. Drug-drug interaction simulations of nelfinavir and cepharanthine

The predicted results of nelfinavir and cepharanthine from the step of physicochemical and pharmacokinetics simulations were used to perform the DDIs simulation. In the previous study, cepharanthine was a weak CYP3A4 inhibitor[16]. Therefore, in this study, nelfinavir and cepharanthine were classified to be the "victim" and the "perpetrator", respectively for metabolic enzymes and transporters interaction in the DDIs simulation module. The DDIs simulation process was initiated

by navigating through two different Tabs, namely, Current Compound and Interacting Compound. Nelfinavir was set in the Current Compound Tab for the "victim" or substrate compound. Cepharanthine was set in the Interacting Compound Tab for the "perpetrator" or interacting compound. The metabolicfraction (f_m) of nelfinavir were 93.21% and 6.79% for CYP3A4 and CYP2C19, respectively. The reversible unbound corrected for nonspecific binding inhibition enzyme kinetics constant ($K_{i\text{-}rev\text{-}in\ vitro,\ U}$) of cepharanthine were 1, 1, 1 µM for CYP3A4, CYP2E1 and CYP2C9, respectively. However, the transporters interaction parameters obtained from the default values of DDIs simulation module in GastroPlus™ database[20]. The dynamic simulation feature was selected to calculate and investigate the PK parameters especially C_{max}, $AUC_{0\text{-}120}$ and $AUC_{0\text{-}inf}$ from Baseline Simulation Tab and Full Simulation Tabs. The DDIs evaluations of all dosage regimen combinations were investigated from the C_{max}, $AUC_{0\text{-}120}$, $AUC_{0\text{-}inf}$, C_{max} ratio, $AUC_{0\text{-}120}$ ratio and $AUC_{0\text{-}inf}$ ratio of nelfinavir-cepharanthine interaction comparing with nelfinavir 500 mg every 8 hours monotherapy using as a reference target. The victim or nelfinavir dosage regimens were used four oral administration regimens, 500 mg every 12 hours, 500 mg every 8 hours, 750 mg every 12 hours and 750 mg every 8 hours for simulations. The perpetrator or cepharanthine dosage regimens were used in several regimens from 10 mg to 120 mg in every 8, 12 and 24 hours of oral administration as presented in DDIs results. From all predicted results, the potential perspective dosing regimen was considered.

3. RESULTS AND DISCUSSION

The predicted results of physicochemical properties, PK parameters and DDIs simulation from GastroPlus™ database using American healthy young adults having age of 30 years, weight of 85.53 kg based on a body mass index (BMI) scale of 27.48 kg/m^2 PBPK model are presented as follows.

3.1. Physicochemical properties and pharmacokinetics simulations

The results of physicochemical properties predicted from ADMET Predictor and Metabolism and Transporter module in GastroPlus™ database are presented in Table 1.

From these results, values of partition coefficient (logP), solubility, diffusion coefficient (Diff. Coeff.), permeation coefficient (Peff.), acid dissociation constant (pKa), blood-brain barrier (BBB) penetration, metabolic fraction (fm), Extended clearance classification system (ECCS), mechanistic clearance, metabolic profile of enzymes, transporter inhibitor and transporter substrate were used to simulate the PK profiles using the PBPK model in GastroPlus™ database. After performing the simulation, the PK parameters especially C_{max}, C_{max} in liver, C_{max} brain, C_{max} in lung, $AUC_{0\text{-}120}$ and $AUC_{0\text{-}inf}$ are presented in Table 2.

From these results, the C_{max} in lung estimated in approximately of 93% of C_{max} (or C_{max} in plasma). In the previous study, the antiviral activity was able to predict from C_{max} in lung or C_{max} in plasma [5]. Therefore, in this study, the predicted C_{max} in plasma was used for DDIs simulation. According to there was a limitation of biopharmaceutical parameters from human to create the PBPK model, therefore the simulation using the human biopharmaceutical parameters created by the PBPK model in GastroPlus™ database was manipulated. This PBPK model simulation using American healthy young adults having age of 30 years, weight of 85.53 kg based on a body mass index (BMI) scale of 27.48 kg/m^2. The systemic clearance (CL_{sys}), steady state volume of distribution (V_{ss}) and elimination half-life (T_{half}) of nelfinavir under the multiple dosing simulation were 45.295 L/hr, 790.315 L and 12.092 hr, respectively in which the clearance of previous study reported in the range of 30 to 51 L/hr and the terminal half-life of 11.3 hr[21]. The systemic clearance (CL_{sys}), steady state volume of distribution (V_{ss}) and elimination half-life (T_{half}) of cepharanthine

Table 1. Physicochemical properties prediction of nelfinavir and cepharanthine from GastroPlus™

	Nelfinavir	Cepharanthine
Log P	4.6	6.47
MW (g/mol)	567.8	606.72
Solubility (µg/mL)	75.4	0.85
Diff. Coeff. (cm^2/s x 10^{-5})	0.50	0.51
Peff. (cm/s x 10^{-4})	0.96	3.27
pKa	Acid (11.32), Acid (10.17), Base (6.21)	Base (6.41), Base (7.28)
Blood-Brain-Barrier Penetration	Low	high
Predicted CYP fm	CYP3A4 (93.21%) CYP2C19 (6.79%)	CYP3A4 (0.25%) CYP2E1 (94.9%), CYP2C9 (6.5%)
ECCS Classification	Class 4 renal	Class 2 metabolism
Mechanistic clearance	Hepatic uptake	metabolism
Transporter: Inhibitor	OATP1B1, OATP1B3, P-gp, BSEP	OATP1B3, OCT1, P-gp, BSEP
Transporter: Substrate	OATP1B1, OATP1B3, P-gp	OATP1B1, OATP1B3, P-gp, BCRP

logP (partition coefficient solubility), Diff. Coeff. (diffusion coefficient), Peff. (permeation coefficient)
pKa (acid dissociation constant), ECCS (Extended clearance classification system), fm (metabolic fraction)

Table 2. Pharmacokinetics parameters of nelfinavir and cepharanthine

	C_{max} (ng/mL) in plasma	C_{max} (ng/mL) in liver	C_{max} (ng/mL) in brain	C_{max} (ng/mL) in lung	$AUC_{0\text{-}inf}$ (ng.h/mL)	$AUC_{0\text{-}120}$ (ng.h/mL)
CEP 10 mg Single dose	0.02841	0.6988	0.2405	0.02631	0.03964	0.03625 ($AUC_{0\text{-}24}$)
CEP 10 mg Q24	0.02854	0.7017	0.2334	0.02641	0.1988	0.1932
CEP 10 mg Q12	0.02893	0.7023	0.2376	0.02673	0.3934	0.3828
CEP 10 mg Q8	0.02941	0.7030	0.2429	0.02731	0.5839	0.5720
CEP 30 mg Single dose	0.07051	1.7400	0.5924	0.06536	0.1105	0.1010 ($AUC_{0\text{-}24}$)
CEP 30 mg Q24	0.07101	1.7320	0.5975	0.06578	0.5541	0.5385
CEP 30 mg Q12	0.07210	1.7340	0.6112	0.06642	1.0960	1.0670
CEP 30 mg Q8	0.07346	1.7360	0.6242	0.06768	1.6270	1.5940
CEP 60 mg Single dose	0.12610	3.2090	0.9954	0.11670	0.1991	0.1820 ($AUC_{0\text{-}24}$)
CEP 60 mg Q24	0.12820	3.1360	0.9491	0.11860	0.9985	0.9703
CEP 60 mg Q12	0.13020	3.1400	0.9628	0.12010	1.9750	1.9220
CEP 60 mg Q8	0.13270	3.1440	0.9899	0.12240	2.9310	2.8720
CEP 120 mg Single dose	0.23650	6.0460	1.1830	0.21880	0.3593	0.3283 ($AUC_{0\text{-}24}$)
CEP 120 mg Q24	0.23970	5.9600	1.6460	0.22240	1.8030	1.7510
CEP 120 mg Q12	0.24340	5.9660	1.6850	0.22520	3.5640	3.4690
CEP 120 mg Q8	0.24800	5.9740	1.7370	0.22880	5.2880	5.1280
NEL 500 mg Single dose	0.50860	11.3300	3.0650	0.44480	43.7900	4.6870 ($AUC_{0\text{-}24}$)
NEL 500 mg Q24	0.69150	13.0000	4.8150	0.60680	49.7000	27.9800
NEL 500 mg Q12	0.75380	13.5900	5.4510	0.65960	67.4600	35.6600
NEL 500 mg Q8	0.77470	13.6000	5.6780	0.67970	53.5100	41.1200
NEL 750 mg Single dose	0.74680	16.6000	4.3850	0.65150	25.4600	5.1340 ($AUC_{0\text{-}24}$)
NEL 750 mg Q24	0.93320	18.3100	6.1580	0.81920	45.4700	30.5600
NEL 750 mg Q12	0.99590	18.8900	6.8180	0.87170	64.9200	39.1100
NEL 750 mg Q8	1.02300	18.9100	7.0760	0.89570	56.2300	46.2500

Note: CEP = Cepharanthine, NEL = Nelfinavir, Q8 = every 8 hours, Q12 = every 12 hours, Q24 = every 24 hours

under the multiple dosing simulation were 114.651 L/hr, 1,306.184 L and 7.895 hr, respectively in which the half-life of previous study presented in the range of 4.1 to 9.2 hr. In addition, the elimination rate constant of the PBPK model was 0.0729 hr^{-1} whereas the previous study showed in the range of 0.052 to 0.170 hr^{-1}. However, there was not report the clearance or volume of distribution[22]. When considering to the C_{max} and $AUC_{0\text{-}24}$ of predicted and the observe data, the underestimate results were presented. The further should improve the PBPK model using the existing PK parameters especially CL and V_d obtained from human.

3.2. Drug-drug interaction simulations of nelfinavir and cepharanthine

The predicted results of nelfinavir and cepharanthine calculated and investigated from the dynamic simulation feature using the victim dosage regimens of 500 mg every 12 hours, 500 mg every 8 hours, 750 mg every 12 hours and 750 mg every 8 hours for simulations. The perpetrator dosage regimens were used in several regimens from 10 mg to 120 mg in every 8, 12, and 24 hours of oral administration are presented in Figure 2.

The DDIs classification was defined from the GastroPlus™ simulated monitoring of AUC ratio comparing between presence and absence of perpetrator in the simulation. The perpetrator or inhibitor was classified as weak, moderate and strong by considering to the predicted AUC ratio based on the FDA draft guidance for drug interaction studies. Weak inhibitor had the predicted AUC ratio in the range of 1.25 to 2. Moderate inhibitor was defined the predicted AUC ratio in the range of 2 to 5. The predicted AUC ratio over 5 was classified to be strong inhibitor. In the opposite direction, weak inducer had the predicted AUC ratio in the range of 0.5 to 0.8. Moderate inducer was defined the predicted AUC ratio in the range of 0.2 to 0.5. The predicted AUC ratio less than 0.2 was classified to be strong inducer[23]. In these results, the predicted AUC ratio presented cepharanthine to be weak and moderate inhibitors depend on the dosage regimens of simulation.

From the predicted AUC ratio results of nelfinavir in all four dosage regimens combining with cepharanthine in the regimes of 10 mg to 90 mg daily, there were no inhibition activity of cepharanthine. However, when combining cepharanthine 120 mg daily with nelfinavir in all four dosage regimens, cepharanthine showed a weak inhibitor activity. Moreover, cepharanthine presented a moderate inhibition for all four dosage regimens of nelfinavir when using the dose of 360 mg daily. The inhibition activity of cepharanthine can change from weak inhibitor to be moderate inhibitor because of a dose-dependent manner[5-6]. However, to consider the predicted C_{max} ratio of nelfinavir in all four dosage regimens combining with cepharanthine using the same criteria of DDI classification as the predicted AUC ratio, cepharanthine in the range of 10 mg to 90 mg daily, there

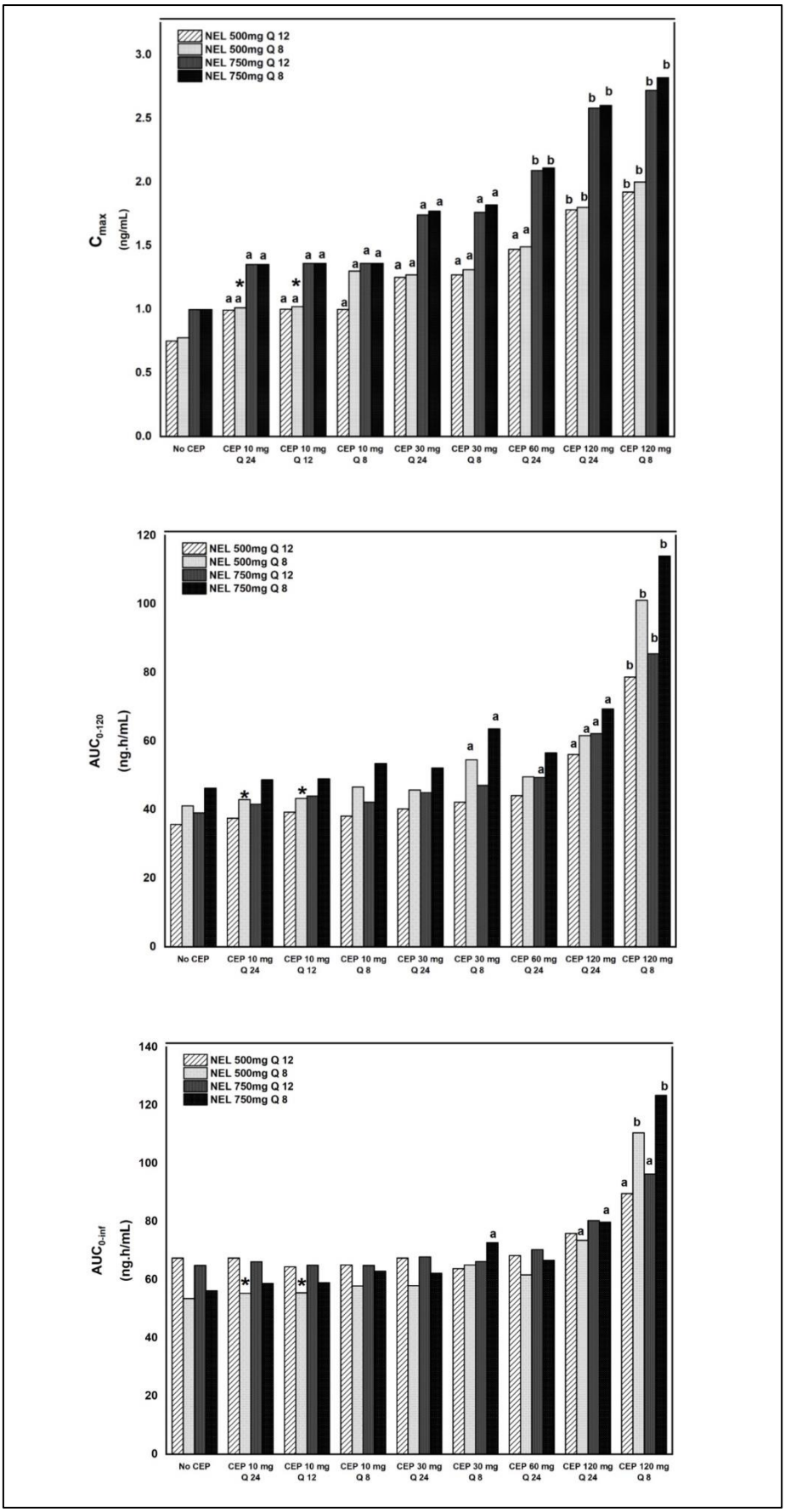

Figure 2. Simulation of pharmacokinetic drug-drug interaction parameters (C_{max}, AUC_{0-120} and AUC_{0-inf}) of nelfinavir with or without cepharanthine in several combination dosage regimens
Note: CEP = Cepharanthine, NEL = Nelfinavir, Q8 = every 8 hours, Q12 = every 12 hours, Q24 = every 24 hours
a = weak inhibitor, b = moderate inhibitor, * = dosage regimen represents PK parameters to target regimen (NEL 750 mg Q8)

were weak inhibition activity of cepharanthine. However, when combining cepharanthine 120 mg daily and 360 mg daily with nelfinavir in all four dosage regimens, cepharanthine showed a moderate inhibitor activity. In the previous studies, nelfinavir inhibited SARS-CoV-2 spike-mediated membrane fusion and related with C_{max} level closely to nelfinavir dosage regimen of 750 mg every 8 hours orally as AUC[5-7,21]. Therefore, in this study, the predicted C_{max} $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}120}$ were used to investigate DDIs simulation. The predicted C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}120}$ of nelfinavir 750 mg every 8 hours orally was the reference targets. From the simulation results, there were two dosage regimens achieve target parameters. The combination of nelfinavir 500 mg every 8 hours orally and cepharanthine 10 mg every 24 hours orally showed the predicted C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}120}$ of nelfinavir in approximately of 98.73%, 98.38% and 92.93%, respectively comparing with the values of the reference targets. However, the other showed the predicted C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}120}$ values more closely to the references targets when using the combination of nelfinavir 500 mg every 8 hours orally and cepharanthine 10 mg every 12 hours orally presenting the predicted C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}120}$ values of nelfinavir were in approximately of 99.71%, 98.70% and 93.64%, respectively. Previous studies, the combination dosage regimen of nelfinavir and cepharanthine provided a synergistic activity by predicting from the mathematical modeling concerning the anitiviral activity. The proposed combination was nelfinavir 500 mg every 8 hours orally and cepharanthine 25 mg or 100 mg intravenous weekly[5-6]. However, this study conducted *in silico* simulations based on the PK parameters and considered only the oral route administration of the combination different from the previous targets. In addition, when considering to the dose of cepharantine used in PBPK model from 10 to 120 mg, the higher dose especially 120 mg daily or more amount presented the stronger inhibition activity[6]. Therefore, the high dose of cepharanthine might inappropriate to use in combination with nelfinavir because of its moderate inhibitor action. Moreover, side effects such as diarrhea and headache were more commonly reported when nelfinavir was used in the high plasma level especially from multiple dosing[23]. Furthermore, in this study, the PBPK model using American healthy young adults having age of 30 years, weight of 70 kg based on a body mass index (BMI) scale of 22.56 kg/m^2 was also performed in which the systemic clearance (CL_{sys}), steady state volume of distribution (V_{ss}) and elimination half-life (T_{half}) of nelfinavir under the multiple dosing simulation were 37.647 L/hr, 625.240 L and 11.509 hr, respectively. The systemic clearance (CL_{sys}), steady state volume of distribution (V_{ss}) and elimination half-life (T_{half}) of cepharanthine under the multiple dosing simulation were 74.669 L/hr, 1,024.041 L and 9.504 hr, respectively. Results revealed that the combination of nelfinavir 500 mg every 8 hours orally and cepharanthine 10 mg every 12 hours orally showed the predicted C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}120}$ of nelfinavir in approximately of 97.62%, 97.42% and 92.95%, respectively comparing with the values of the reference targets. However, the predicted C_{max}, $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}120}$ of nelfinavir using the PBPK model of American healthy young adults having age of 30 years, weight of 70 kg based on BMI scale of 22.56 kg/m^2 presented more higher level in approximately of 16, 22 and 22 times comparing with values predicted from American healthy young adults having age of 30 years, weight of 85.53 kg based on BMI scale of 27.48 kg/m^2. Unfortunately, the PBPK model of Asian population was not create because the model needs several physiological parameters and PK parameters to optimize the final model before performing the DDIs simulation. However, the information of this study might be applied to Asian population as PBPK model using American healthy young adults having age of 30 years, weight of 70 kg based on BMI scale of 22.56 kg/m^2. Therefore, in this study, the potential dosage regimens would be nelfinavir 500 mg every 8 hours orally and cepharanthine 10 mg every 12 hours orally. Nevertheless, the further study of this combination in human would provide valuable information for pharmacokinetic DDIs and improve benefits of the PBPK model.

4. CONCLUSION

There is a limitation of human biopharmaceutical information of a combination of nelfinavir and cepharanthine against COVID-19. This study performed the simulation to predicted pharmacokinetic drug-drug interactions using PBPK model and optimized the potential perspective dosing regimens based on the predicted C_{max}, $AUC_{0\text{-}120}$ and $AUC_{0\text{-}inf}$, C_{max} ratio, $AUC_{0\text{-}120}$ ratio and $AUC_{0\text{-}inf}$ ratio. The results showed that dosage regimens as a potential combination against COVID-19 was nelfinavir 500 mg every 8 hours and cepharanthine 10 mg every 12 hours.

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Ethics approval
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Author contribution

WN: study conceptualization and design, methodology, data analysis and interpretation, article structuring, writing, revision, approval and supervision.

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