A Pharmacokinetic Model of Lopinavir in Combination with Ritonavir in Human

Khanita Duangchaemkarn, Pharm.D.-*EMBS Member*, Brad Reisfeld, Ph.D., and Manupat Lohitnavy, Ph.D.

Abstract— **Ritonavir-boosted lopinavir (LPV/r) has been recommended as an alternative regimen for HIV-naive patients who cannot tolerate nevirapine (NVP) and/or efavirenz (EFV). Although combinations of ritonavir and lopinavir have shown higher plasma concentration level of LPV in clinical settings, dosage adjustment is still required to maintain an adequate therapeutic efficacy and reduce side effects. A compartmental pharmacokinetic (PK) model of LPV/r was developed, including a mechanistic description of competitive inhibition. Systematic simulations were performed and predicted plasma drug concentration levels were compared with those from the literature. In particular, the simulated and experimental area under the curve (AUC) based on oral dosing were 76.10 µMol/L, and 76.25 µMol/L, respectively Results from the mathematical model support the hypothesis that the mechanism of LPV/r interaction is due to the competitive inhibition of CYP3A4 in the liver by ritonavir, resulting in an increasing LPV plasma concentration levels. The simulated plasma concentration-time courses were consistent with those from the literature with the goodness of fit (R^2) of 0.9025 (0.8269-0.9862 95%CI).**

I. Introduction

Protease inhibitors (PI) are a group of the antiretroviral drugs recommended by the US Food and Drug Administration (US FDA) for treatment of human immunodeficiency viral (HIV) infection in humans. Recently, clinical studies of combinations of protease inhibitors (PIs) with low-dose ritonavir (RTV) showed not only increased clinical therapeutic efficacy, but also decreased morbidity and mortality in HIV infected patients [1], [2]. Among protease inhibitors, lopinavir (LPV) has the highest potency for inhibiting the HIV protease enzymes; however, it has a very low oral bioavailability due to rapid metabolism by cytochrome P450 3A4 (CYP3A4) in the gastrointestinal tract and liver. However, by co-administering with ritonavir, the most potent reversible CYP3A4 inhibitor [3], the plasma concentration of lopinavir is maintained at a much higher level over time [4].

To better understand and characterize the pharmacokinetic interactions and the mechanistic between lopinavir and ritonavir, a mathematical model was developed with a capability to predict the concentration time-course for both drugs following oral dosing. We anticipate that predictions from this model will help inform the optimization of dosing regimens for these important drugs in HIV patients.

K. Duangchaemkarn and M. Lohitnavy are with the Center of Excellence for Environmental Health and Toxicology, Pharmacokinetic Research Unit, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, 65000 Thailand. (corresponding author: M. Lohitnavy, Tel. +6683-956-5065; e-mail: manupatl@gmail.com).

B. Reisfeld, was with Center of Excellence for Environmental Health and Toxicology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, 65000 Thailand, on leave from the Department of Chemical & Biological Engineering, College of Engineering, Colorado State University, Fort Collins, CO 80523 USA (e-mail: brad.reisfeld@colostate.edu).

II. Methodology

A. Subjects and Study Design

Data from two published studies were used for model calibration (determination of model parameters) and validation (verification of model accuracy):

1) *Sham et al. [5] (R1):* 14 healthy human volunteers were given single dose 400 mg capsules of lopinavir with a single 50 mg capsule of the semisolid formulation of ritonavir. Lopinavir plasma concentration levels were sampling and plotted in the published study.

2) *Jackson et al. [6] (R2):* 22 human volunteers were given combination tablet of lopinavir and ritonavir (LPV/r), twice daily for 7 days in the different 3 regimens; LPV/r 400/100 mg, LPV/r 200/50 mg, and LPV/r 200/150 mg, sequentially, separated by a 7 days wash-out period. Lopinavir and ritonavir plasma concentration levels were sampling and plotted in the published study.

Time-course plasma concentration values were obtained by digitizing figures from these references. In this study, these digitized data will be call reference data.

In silico experiments were conducted with the PK model using the PK parameters involving with the process of absorption, distribution, metabolism and elimination in human body or ADME parameters of lopinavir and ritonavir obtained from published papers [3], [7–10]. Unknown parameter values were estimated using numerical optimization, utlizing a selected portion of the available experimental data. Administration of lopinavir alone or in combination with ritonavir was simulated for the range of conditions used in the experimental studies cited, and comparisons were made to assess the validity of the model.

B. Mathematical Modeling

The pharmacokinetic interaction model was developed by linking individual models of lopinavir and ritonavir through mathematical relationships describing joint competitive inhibition of CYP3A4 by each of the drugs (see Fig. 1). The models for the individual drugs were based in large part on previous one-compartment PK models for lopinavir [11] and ritonavir in human.

The PK interactions were assumed to follow reversible competitive inhibition with a saturable Michaelis-Menten-like mechanism [12]. The species mass balance equations

978-1-4244-7929-0/14/$26.00 ©2014 IEEE

consistent with Fig. 1. and the assumed nature of the drug-drug interactions areas follow:
For lopinavir (LPV);

$$\frac{dX_{CLPV}}{dt} = X_{GILPV} \cdot K_{aLPV} - \frac{X_{CLPV} \cdot V_{mLPV}}{K_{mLPV} \cdot \left(1 + \frac{X_{CRTV}}{K_{iRTV}}\right)} - K_{eLPV} \cdot X_{CLPV} \quad (1)$$

For ritonavir (RTV);

$$\frac{dX_{CRTV}}{dt} = X_{GIRTV} \cdot K_{aRTV} - \frac{X_{CRTV} \cdot V_{mRTV}}{K_{mRTV} \cdot \left(1 + \frac{X_{CLPV}}{K_{iLPV}}\right)} - K_{eRTV} \cdot X_{CRTV} \quad (2)$$

Where K_i is the inhibition rate constant, X_C is the amount of drug in the central compartment, X_{GI} is the amount of drug in the gastrointestinal depot, K_a is the absorption rate, K_e is the elimination rate, K_m is a Michealis-Menten constant, K_i is enzyme inhibition rate, and V_m is the maximum velocity of the drug metabolism.

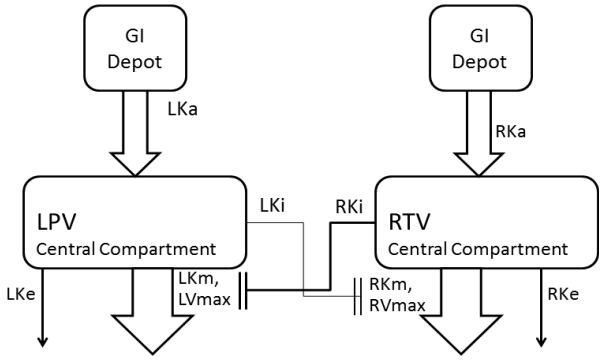

Figure 1. Structure of the pharmacokinetic model

A number of parameters values are required to complete the specification of the PK model. When available, these values were taken from the literature (Table 1). In cases where parameter values could not be found, they were estimated through numerical optimization using the calibration portion of the data.

TABLE I. PHARMACOKINETIC PARAMETERS USED IN THE SIMULATION PROCESSES.

PARAMETERS	VALUE	%CV	REFERENCES
Lopinavir			
K_a (h^{-1})	0.26	6.9	[7]
V/*F* (L)	15.9	8.7	[7]
K_e (h^{-1})	0.08	-	[9]
K_m (µMol/L)	6.80	-	[10]
K_i (µMol/L)	130.0	-	[8]
Ritonavir			
K_a (h^{-1})	0.18	6.1	[7]
V/*F* (L)	13.7	17.4	[7]
K_e (h^{-1})	0.6	-	[13]
V_m (µMol/L)	65.05	-	[14]
K_m (µMol/L)	4.7576	-	[14]
K_i (µMol/L)	0.013	-	[8]

Following a series of systematic simulations, predicted concentration time-course values were compared to those from the literature, and the goodness of fit was assessed. In addition, classical pharmacokinetic parameters were estimated and compared to the reference data. These parameters included the area under the curve (AUC), maximum plasma concentration (C_{MAX}), minimum plasma concentration (C_{MIN}), time to trough concentration (T_{MIN} at C_{LPV} < 0.0159 µMol/L), and time to peak concentration (T_{MAX}).

C. Modeling and analysis software

Plot data were digitized using PlotDigitizer v.2.6.3 (http://plotdigitizer.sourceforge.net).. Simulations and statistical analyses were performed with Python v. 2.7.3 (https://www.python.org/), NumPy v.1.8.0 (http://www.numpy.org/), and SciPy v.0.13.2 (http://www.scipy.org/).

III. RESULTS

As shown in Table 1, many of the values for the model parameters were available from the literature; however, the relative bioavailability (F), and V_m, and K_m, were not and were determined using numerical optimization via a Nelder-Mead algorithm. Table II contains the initial estimates and final optimized parameter values.

TABLE II. PHARMACOKINETIC PARAMETER ESTIMATION FROM THE MODEL USING OPTIMIZATION TECHNIQUE

PHARMACOKINETIC PARAMETERS	INITIAL VALUE	OPTIMIZED VALUE
Lopinavir		
Relative bioavailability (*F*)	0.77	0.46
Vmax (µMol/L)	160	132
Ritonavir		
Relative bioavailability (*F*)	0.15	0.30
K_i (µMol/L)	0.013	0.014

A. Oral Single Dosing Modeling

As illustrated in Fig. 2., plasma lopinavir concentrations following oral administration of combination 400 mg lopinavir and 50 mg ritonavir are in reasonable agreement with corresponding experimental data.

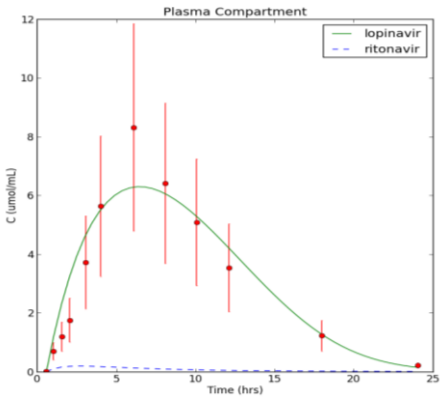

Figure 2. The concentration of lopinavir and ritonavir in the plasma after oral administration of a combination tablet 400mg/50mg single dose. The solid line shows plasma concentration of lopinavir plotted against the reference data, solid dotted, digitized from Sham et al. 1998 [5]. Dashed line shows plasma concentration of ritonavir.

These and other time-course concentration values from the simulations were utilized to estimate the classical

pharmacokinetic parameters described earlier. Table III contains a summary comparison of these results to those from the literature. The overall goodness of fit of plasma lopinavir concentrations following single dosing oral administration of combination 400 mg lopinavir and 50 mg ritonavir (R^2) was 0.90 (0.83-0.99 95% CI).

B. Oral Repeated Dosing Modeling

Lopinavir and ritonavir plasma concentration levels were well described by the PK model utilizing a saturable metabolism model with competitive inhibition mechanism. Examples comparing simulated time-course plasma concentrations with values from the literature for different dosage regimens are depicted in Fig. 3-5.

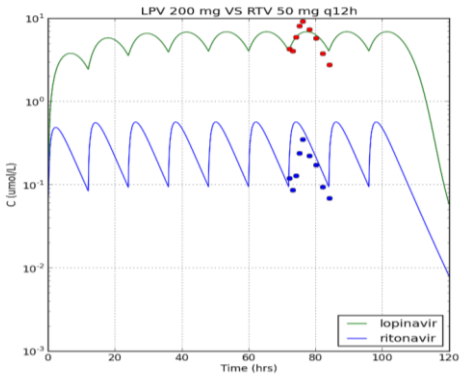

Figure 3. Plasma concentration time-course of oral lopinavir 200 mg with ritonavir 50 mg every 12 hours

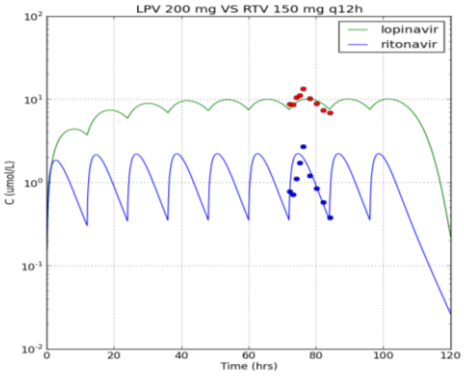

Figure 4. Plasma concentration time-course of oral lopinavir 200 mg with ritonavir 150 mg every 12 hours

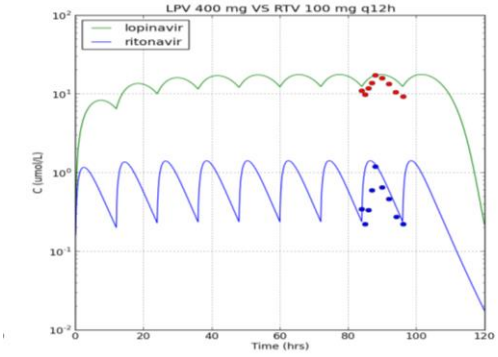

Figure 5. Plasma concentration time-course of oral lopinavir 400 mg with ritonavir 100 mg every 12 hours

TABLE III. PHARMACOKINETIC PARAMETER OF LOPINAVIR WHEN GIVEN COMBINATION WITH OR WITHOUT RITONAVIR

PARAMETERS	SIMULATION VALUE		LITERATURE VALUE [5]	
Lopinavir	**LPV/r**	**LPV** [11]	**LPV/r**	**LPV**
AUC (µMol/L.h)	76.11	1.30	76.25	1.12
C_{MAX} (µMol/L)	6.29	0.43	8.31	0.70
T_{MAX} (hours)	6.34	1.74	6.11	3.00
T_{MIN} (hours)	24.13	4.28	n/a	4.19

IV. DISCUSSION

Both single-dose and repeated-dose simulations led to lower values of C_{MAX} compared to those found in the literature. However, the values of AUC from the single-dose modeling were in reasonable agreement with those from the literature.

Single oral dosing pharmacokinetic studies in rats and human without ritonavir were available and revealed low levels of the drug plasma concentration below 0.01 µg/ml (0.0159 µMol/L), while a recommendation require through plasma concentration level of lopinavir in both children and adult naïve patients to be above 1 µg/ml [14–16]. These results may be due to its poor oral bioavailability and extensively metabolized before entering the systemic circulation [18].

The model developed in this study adequately described many aspects of the pharmacokinetic interaction of lopinavir and ritonavir. This is important because ritonavir plasma concentration has played the major role in the mechanism of the interaction by inhibit the function of CYP3A4, resulted in reduced overall metabolism process of lopinavir. Effect of ritonavir inhibition can increase the lopinavir AUC level to 58 fold compared to non-ritonavir regimen. Lopinavir C_{MAX} was also increasing from 0.43 µMol/L to 6.29 µMol/L, predicted from the single dosing model simulation.

For repeated dosing regimen, the predicted plasma concentration for both drugs was well described by the proposed pharmacokinetic interaction model. Hence, some pharmacokinetic parameters need to be optimized to produce more fitted during the first absorption phase. Although, the repeated dosing model was able to replicate the data from humans, further internal and external validation will be required, as well as parameter optimization to make the simulation fit to the real experimental data. Specifically, to account for parameter, model, and data variability and uncertainty, Bayesian inference will be employed to estimate parameter distributions that will be used to generate families of time-course results using Monte Carlo simulations. In addition, the complicated mechanism during the absorption process should be discussed. Finally, because physiologically based pharmacokinetic (PBPK) modeling has been shown to be is a powerful computational approach in incorporating any biological process into the developed models [19]–[22], such a model should be considered for lopinavir and ritonavir.

Although there were some pharmacokinetic studies reported on the interaction between lopinavir and ritonavir in various dosage regimens [1], [2], [23], [24], with or without others antiretroviral agents. However, these studies reported the pharmacokinetic effects of the others protease inhibitors on lopinavir plasma concentration only, but the mechanism of such interaction is still question. The pharmacokinetic interaction model is considered to be a quantitative tool in

aiding dosage adjustment of the combination drugs in the treatment of HIV infection.

ACKNOWLEDGEMENT

This research was supported by the Fulbright Foundation; the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education; the Opa Tangpitukkul's Scholarship; and the Center of Excellence for Environmental Health & Toxicology, Naresuan University.

REFERENCES

[1] J. T. Ramos, "Boosted protease inhibitors as a therapeutic option in the treatment of HIV-infected children," pp. 536–547, 2009.

[2] J. van der Lugt, R. S. Autar, S. Ubolyam, et al., "Pharmacokinetics and short-term efficacy of a double-boosted protease inhibitor regimen in treatment-naive HIV-1-infected adults.," *J. Antimicrob. Chemother.*, vol. 61, no. 5, pp. 1145–53, May 2008.

[3] J. Smith, K. Erdman, N. Lyons, et al., "Multiple-Dose Pharmacokinetics of Ritonavir in Human Immunodeficiency Virus-Infected Subjects," *Antimicrob. Agents Chemother.*, vol. 41, no. 5, pp. 898–905, 1997.

[4] S. S. Kaplan and C. B. Hicks, "Safety and antiviral activity of lopinavir/ritonavir-based therapy in human immunodeficiency virus type 1 (HIV-1) infection.," *J. Antimicrob. Chemother.*, vol. 56, no. 2, pp. 273–6, Aug. 2005.

[5] H. L. Sham, D. J. Kempf, a Molla, et al., "ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease.," *Antimicrob. Agents Chemother.*, vol. 42, no. 12, pp. 3218–24, Dec. 1998.

[6] A. Jackson, A. Hill, R. Puls, et al., "Pharmacokinetics of plasma lopinavir/ritonavir following the administration of 400/100 mg, 200/150 mg and 200/50 mg twice daily in HIV-negative volunteers.," *J. Antimicrob. Chemother.*, vol. 66, no. 3, pp. 635–40, Mar. 2011.

[7] L. Dickinson, M. Boffito, D. Back, et al., "Sequential Population Pharmacokinetic Modeling of Lopinavir and Ritonavir in Healthy Volunteers and Assessment of Different Dosing Strategies," *Antimicrob. Agents Chemother.*, vol. 55, no. 6, pp. 2775–2782, 2011.

[8] EMEA, "Initial scientific discussion for the approval of Kaletra," *European Medicine Agency*, 2005. [Online]. Available: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000368/WC500039040.pdf. [Accessed: 14-Nov-2013].

[9] R. Ter Heine, R. Van Waterschoot, R. J. Keizer, et al., "An Integrated Pharmacokinetic Model for the Influence of CYP3A4 Expression on the In Vivo Disposition of Lopinavir and Its Modulation by Ritonavir," *J. Pharm. Sci.*, vol. 100, no. 6, pp. 2508–2515, 2011.

[10] J. T. Randolph, P. Huang, C. Flentge, et al., "A-681799 , a Novel HIV Protease Inhibitor," in *the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)*, 2004, p. F–485.

[11] K. Duangchaemkarn and M. Lohitnavy, "Compartmental pharmacokinetic modeling of lopinavir in humans.," *Conf. Proc. IEEE Eng. Med. Biol. Soc.*, vol. 2013, pp. 3523–6, Jul. 2013.

[12] R. a B. van Waterschoot, R. ter Heine, E. Wagenaar, et al., "Effects of cytochrome P450 3A (CYP3A) and the drug transporters P-glycoprotein (MDR1/ABCB1) and MRP2 (ABCC2) on the pharmacokinetics of lopinavir.," *Br. J. Pharmacol.*, vol. 160, no. 5, pp. 1224–33, Jul. 2010.

[13] C. Zhang, P. Denti, E. Decloedt, et al., "Model-based approach to dose optimization of lopinavir/ritonavir when co-administered with rifampicin.," *Br. J. Clin. Pharmacol.*, vol. 73, no. 5, pp. 758–67, May 2012.

[14] a Hsu, G. R. Granneman, G. Witt, et al., "Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects.," *Antimicrob. Agents Chemother.*, vol. 41, no. 5, pp. 898–905, May 1997.

[15] Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children, "Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection The Health Resources and Services Administration," *National Institute of Health*, 2011. [Online]. Available: http://aidsinfo.nih.gov/ContentFiles/lvguidelines/PediatricGuidelines.pdf. [Accessed: 02-Jan-2013].

[16] Panel on Antiretroviral Guidelines for Adults and Adolescents, "Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents," *Department of Health and Human Services*, 2012. [Online]. Available: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. [Accessed: 02-Feb-2013].

[17] S. Sungkanuparpha, W. Techasathit, C. Utaipiboon, et al., "Thai national guidelines for antiretroviral therapy in HIV-1 infected adults and adolescents 2010," *Asian Biomed.*, vol. 4, no. 4, pp. 515–528, 2010.

[18] G. N. Kumar, V. K. Jayanti, M. K. Johnson, et al., "Metabolism and disposition of the HIV-1 protease inhibitor lopinavir (ABT-378) given in combination with ritonavir in rats, dogs, and humans.," *Pharm. Res.*, vol. 21, no. 9, pp. 1622–30, Sep. 2004.

[19] Y. Lu, M. Lohitnavy, M. B. Reddy, et al., "An updated physiologically based pharmacokinetic model for hexachlorobenzene: incorporation of pathophysiological states following partial hepatectomy and hexachlorobenzene treatment.," *Toxicol. Sci.*, vol. 91, no. 1, pp. 29–41, May 2006.

[20] M. Lohitnavy, Y. Lu, O. Lohitnavy, et al., "A possible role of multidrug resistance-associated protein 2 (Mrp2) in hepatic excretion of PCB126, an environmental contaminant: PBPK/PD modeling.," *Toxicol. Sci.*, vol. 104, no. 1, pp. 27–39, Jul. 2008.

[21] Y. Lu, S. Rieth, M. Lohitnavy, et al., "Application of PBPK modeling in support of the derivation of toxicity reference values for 1,1,1-trichloroethane.," *Regul. Toxicol. Pharmacol.*, vol. 50, no. 2, pp. 249–60, Mar. 2008.

[22] C. J. Belfiore, R. S. H. Yang, L. S. Chubb, et al., "Hepatic sequestration of chlordecone and hexafluoroacetone evaluated by pharmacokinetic modeling.," *Toxicology*, vol. 234, no. 1–2, pp. 59–72, May 2007.

[23] E. Ribera, R. M. Lopez, M. Diaz, et al., "Steady-state pharmacokinetics of a double-boosting regimen of saquinavir soft gel plus lopinavir plus minidose ritonavir in human immunodeficiency virus-infected adults.," *Antimicrob. Agents Chemother.*, vol. 48, no. 11, pp. 4256–62, Nov. 2004.

[24] B. L. Robbins, E. V Capparelli, E. G. Chadwick, et al., "Pharmacokinetics of high-dose lopinavir-ritonavir with and without saquinavir or nonnucleoside reverse transcriptase inhibitors in human immunodeficiency virus-infected pediatric and adolescent patients previously treated with protease inhibitors.," *Antimicrob. Agents Chemother.*, vol. 52, no. 9, pp. 3276–83, Sep. 2008.