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IN SILICO PREDICTION AND SIMULATION FOR OF ORAL PIPERINE (1-PIPEROYL PIPERIDINE) SOFTWARE

Chatsiri Jesadakultavee

Korbthum Sathirakul

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IN SILICO PREDICTION AND SIMULATION FOR PHARMACOKINETICS OF ORAL PIPERINE (1-PIPEROYL PIPERIDINE) USING GASTROPLUS™ SOFTWARE

Chatsiri Jesadakultavee and Korbthum Sathirakul

Department of Pharmacy, Faculty of pharmacy, Mahidol university

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INTRODUCTION

Piperine is a major alkaloid component of *Piper longum Lin.* and *Piper nigrum Lin.* Several beneficial physiological and pharmacological properties of piperine have been reported [1]. The experiments to determine the transport and absorption of new chemical entities (NCEs) are time-consuming since they require the studies in cell culture, animal and human. *In silico* simulation of absorption is powerful tool to investigate the possible mechanism of absorption and it is recommended by regulatory bodies in all stages of drug development. If sufficiently reliable, such simulations could also help to select the most promising candidates for development or reject those unacceptable candidates in early stage [2].

GastroPlus™ software from Simulation Plus, Inc., which is an advanced technology computer program that simulates absorption, pharmacokinetics, and pharmacodynamic in human and animals. The underlying model in GastroPlus™ is the advanced compartmental absorption and transit (ACAT). The physiologically based ACAT model consists of nine compartments corresponding to different segments of the digestive tract and is based on the original compartment absorption and transit model described by Yu and Amidon [3]. The unique numerical integration process governing the absorption in GastroPlus™ make it an intelligence software for predict and simulation of drugs.

MATERIALS AND METHODS

Computer hardware and software: The simulations were performed on a Toshiba laptop with Intel core i5 CPU 2450 M (2.5 GHz) using GastroPlus™ version 8.0.0002 software and ADMET Predictor™ version 6.0.0007 software (Simulation Plus Inc., Lancaster, CA). It was used to model the absorption, distribution, and elimination of piperine. *In silico* models developed are based on physicochemical properties of the compound determined experimentally or taken from the literature. Rat and human plasma concentration versus time data were used to define the gastrointestinal absorption parameters, as well as the distribution and elimination parameters.

Gastrointestinal Absorption Model. GastroPlus™ utilizes the ACAT model to predict the rate and extent of drug absorption from the gastrointestinal tract. It is also incorporates intestinal drug efflux and metabolism in its predictions of pharmacokinetics parameters. The ACAT model was used to simulate and predict the absorption of piperine. The theoretical basis and mathematical description of the ACAT model have been described in several studies [4, 5]. The program has three input tabs, compound, physiology, and Pharmacokinetics, comprising three sets of factors influencing oral absorption. The required input parameters related to piperine physicochemical and pharmacokinetic parameters were *in silico* predicted or taken from the literature.

Input parameters: Physicochemical properties such as molecular weight, pKa, logD, pH solubility, and particle size were *in silico* predicted using a neural network model in ADMET predictor™ based on the structure properties of compound. For simulate absorption of piperine in rat model, apparent MDCK permeability (S+MDCK) was predicted by ADMET Predictor™ and caco2 permeability value obtained from literature [6]. S+MDCK and caco2 permeability value were used to convert into human jejunal effective permeability (P_{eff}) using a built in model in GastroPlus™. The absorption of piperine in human was utilized S+MDCK, caco2 permeability, and predict human jejunal effective permeability ($S+P_{eff}$) parameter predicted by ADMET predictor™ program to simulate absorption. To investigated metabolism, enzyme substrate was predicted by ADMET predictor™. K_m and V_{max} of interested enzyme were obtained and clearance was replaced. Standard rat and human physiology (rat-human Opt-log D model) default values were used. The factor such as changes in the pH, surface area and transit time are also considered by adding an absorption scaling factor for each compartment. Table 1 describes the ACAT physiological model parameters used in the simulations.

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compartment	ASF	pH	Transit time (h)	Volume (mL)	Length (cm)	Radius (cm)
Stomach	0.000	1.30	0.25	46.56	28.29	9.67
Duodenum	2.795	6.00	0.26	41.56	14.13	1.53
Jejunum 1	2.751	6.20	0.93	154.2	58.40	1.45
Jejunum 2	2.729	6.40	0.74	122.3	58.40	1.29
Ileum 1	2.698	6.60	0.58	94.29	58.40	1.13
Ileum 2	2.646	6.90	0.42	70.53	58.40	0.98
Ileum 3	2.584	7.40	0.29	49.83	58.40	0.82
cecum	4.491	6.40	4.19	47.49	13.19	3.39
Ascending colon	8.749	6.80	12.57	50.33	27.65	2.41

RESULTS

Parameter sensitivity analysis: A parameter sensitivity analysis for piperine is shown in Figure 1. This is a convenient way to assess the sensitivity of the uncertainties in inputs. The most sensitive parameter is solubility. It can be seen that simulated $AUC_{(0-t)}$ much effected by solubility (3-fold) while small changes of simulated $AUC_{(0-t)}$ by P_{eff} (0.5 fold) within the range of value tested. The 3D parameter sensitivity analysis explored the influence of solubility and permeability as show in figure 2.

Simulation of Oral Absorption in Rat: PK Plus module estimated pharmacokinetic (PK) parameter based on a compartmental model fit to piperine 13.525 mg. oral dose data obtained from literature [7].The observed data is best fit with two compartmental linear pharmacokinetic with the lowest Akiake information criteria (AIC) (-22.0805). The simulation inputs for P_{eff} conversion were compared between caco-2 permeability, data from literature, and S+MDCK from ADMET Predictor™. C_{max} and T_{max} (5181 ng/mL, 2.3h) obtained from simulation based on the P_{eff} conversion from caco-2 P_{app} are better fitted to the observed data than those obtained from simulation based on the S+MDCK (C_{max}=4142 ng/mL and t_{max}=2.8h). The simulation rat plasma concentration time profile compared to observed data as show in figure 3.

Simulation of Oral Absorption in Human: The observed data of 20 mg. piperine oral administration was linked to a model of the disposition in human. The disposition model was constructed by fitting a compartmental model to observed plasma concentration after oral dosing to 1 subject. The best fit model was linear pharmacokinetic 2 compartment model suggested by Akiake information criteria (- 63.1623). The total plasma clearance obtained from the fitting was 2.9164 L/h. Subsequently, the match of the simulation to observed data were investigated by three source of permeability value (S+MDCK, caco2 Papp, and predict P_{eff}). The S+MDCK value scaled to a human permeability by GastroPlus™ converter delivered a good fit to observe data as show in Table 2 and as show in figure 4.

Explorations of enzyme metabolism: Clearance of piperine which obtained from fitting compartment model to observed data was replaced by predicted clearance obtained from V_{max} and K_m. Based on ADMET predictor™ , piperine was predicted to be CYP 1A2, 2C9, 2C19, 2D6, and 3A4 substrate. The hypothesis for nonlinear enzyme metabolism was investigated. The linear clearances will not be used in this case, because enzyme metabolism clearance from metabolism module in GasrtoPlus™ will be replaced by V_{max} and K_m of each enzyme. These values will be converted to in vivo conditions and units for GastroPlus™ using the Metabolism and Transporter Units Converter. C_{max}, T_{max} and $AUC_{(o-t)}$ of the simulation of 20 mg of piperine oral administration were 264.98 ng/mL , 1.4 h, and 5258 ng h/mL, respectively. As show in figure 5, it was consistent to observed C_{max}, T_{max} and $AUC_{(o-t)}$ data (255 ng/mL, 1.87 h, 5660.2 ng h/mL), respectively. In order to assess combined effect of variation in the predicted disposition parameters, a simulation was performed using the virtual trial feature in GastroPlus™. This virtual trial was simulated for 9 subjects which were same number of volunteers used in the vivo study[8]. The coefficient of variation (CV%) values of V_{max} and K_m were set to 50%. Simulation results for virtual trial as show in Figure 6.

DISCUSSION

Parameter sensitivity analysis enhanced understanding of uncertainties which affect to absorption of piperine and guiding initial experimental work. Solubility of piperine is the most sensitive parameter. To simulate absorption of piperine, the model of absorption in rat and human was fit with linear 2 compartment pharmacokinetic. Based on predicting by ADMET predictor™ , P_{eff} scaled from Caco2 permeability delivered a good fit to rat observe data whereas S+MDCK gave a better fit to human observed data than those. ADMET predictor suggested that piperine is CYP 1A2, 2C9, 2C19, 2D6, and

3A4 substrate. Clearance was replaced by enzyme kinetic parameter and it exhibited a well predict plasma concentration time profile compared with observed data. Since, there were no transporter parameter inputs in the model, plasma concentration time profile is fitted with observed data by only change the cell permeability values and enzyme kinetic constant. This simulation may confirm the absorption of piperine is solely via passive diffusion which is consistent with the study by Khajuria et al [9].

Table 2 Rat and human pharmacokinetic parameter of observed values and simulated after scaled from difference source.

Pharmacokinetic parameter	Rat			Human			
	Observed	Source of permeability value		Observed	Source of permeability value		
		S+MDCK	Caco2		S+MDCK	Caco2	Predict P_{eff}
C_{max} (ng/ml)	4190	4142.3	5181	255	276.93	284.39	239.58
T_{max} (h)	2.03	2.8	2.3	1.87	1.4	1.1	4.7
AUC_{0-t} (ng h/mL)	2.325E+4	2.95E+4	2.621E+4	5660.2	5556	5557	5522.8

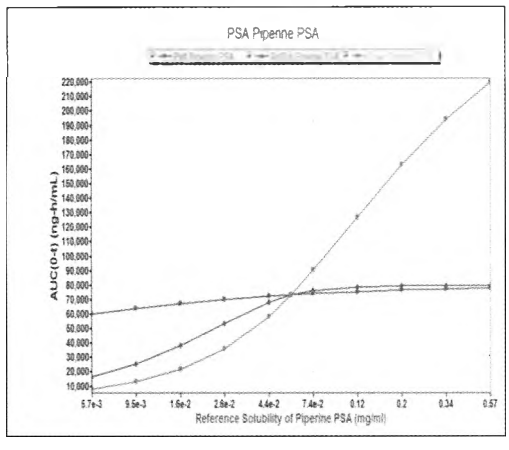

Figure 1. show parameter sensitivity analysis of inputs parameters on simulated fraction absorbed

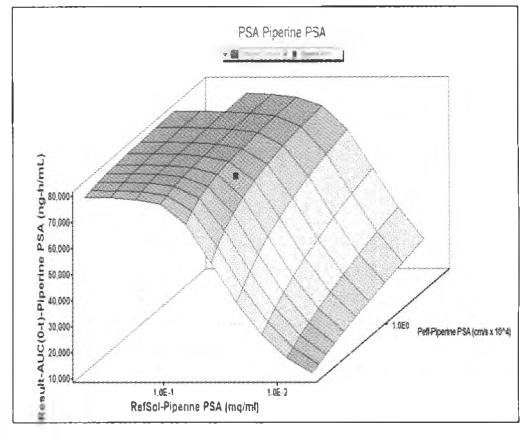

Figure 2. show surface plot parameter sensitivity analysis to explore the influenced of solubility and permeability. The dot shows the current base point.

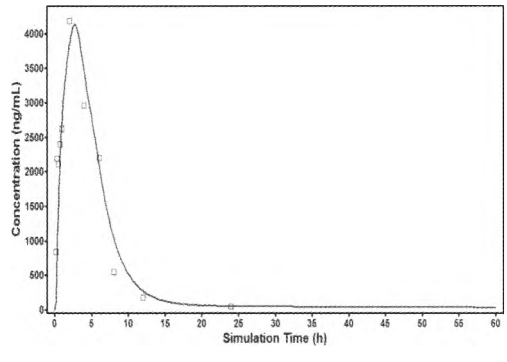

Figure 3. Rat plasma concentration time profile after oral admistration piperine 13.525 mg. observed values (⊏) and predicted (solid line). Scaled P_{eff} from caco2 apparent permeability (P_{app}) in literature.

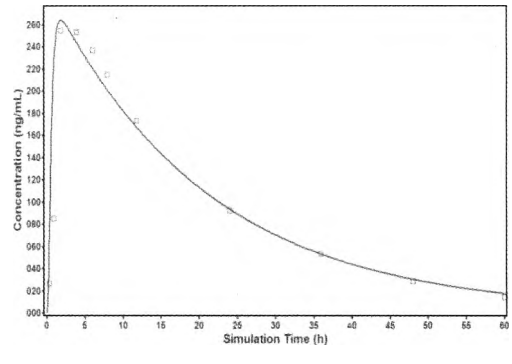

Figure 4. Human plasma concentration time profile after oral admistration piperine 20 mg. observed values (⊏) and predicted (solid line) scaled P_{eff} from S+MDCK

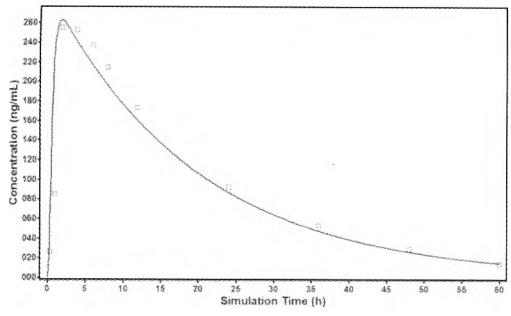

Figure 5. Human plasma concentration time profile of 20 mg. piperine after replaced clearance by K_m and V_{max} of CYP enzyme.

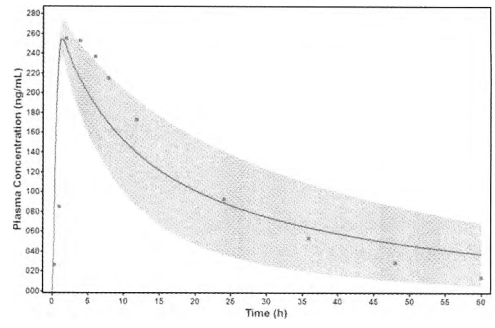

Figure 6. Virtual trial simulation for 9 subjects following an oral dose of 20 mg. piperine. Solid line represents the mean of 9 simulations. Squares represent the observed plasma concentration time profile. The green area represents the 90% confidence interval for the simulated data.

CONCLUSION

GastroPlus™ is a useful tool to demonstrate that piperine absorption. The absorption is possible via passive diffusion in both rat and human. Additional studies are required for better simulation model in any situation of absorption such as solubility, lipophilicity at define pH, and in vitro metabolism data.

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