Population Pharmacokinetic Analysis of Diurnal and Seasonal Variations of Plasma Concentrations of Cilostazol in Healthy Volunteers

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Background: The background of this study was (1) to examine factors influencing cilostazol pharmacokinetics by developing a population model incorporating diurnal variation and other covariate effects and (2) to assess the feasibility of applying the developed model to determine the optimal dosing times.

Methods: Data obtained from a cilostazol pharmacokinetic study consisting of 2 clinical trials (a single twice-a-day (BID) dosing trial in winter and a multiple BID dosing trial in summer) conducted in healthy Korean subjects were used for model building. A basic model was built, followed by a diurnal variation model, and then a final model was built incorporating covariates, including a seasonal difference. The optimal morning and evening dosing times were determined from simulations.

Results: Diurnal variation in cilostazol pharmacokinetics was explained by the morning absorption rate constant being faster than in the evening, yielding values of 0.278 versus 0.234/h in summer, when 24- and 12-hour circadian rhythms were included in the model. The seasonal variation was explained by a 26.9% and a 31.8% decrease in the absorption rate constant and clearance, respectively, in winter compared with summer. Based on twice-a-day (BID) dosing, dosing times of 9 AM and 5 PM in summer and 10 AM and 7 PM in winter were expected to produce the smallest peak-to-peak fluctuations in cilostazol concentration, possibly minimizing unwanted effects of the drug.

Conclusions: This study demonstrated the intraday and interseasonal time-varying nature of cilostazol pharmacokinetics using a population modeling approach and developed a strategy for optimizing dosing times. It is suggested that these methods can be similarly applied to analyses and controls of other drugs that exhibit characteristics of time-varying pharmacokinetics.

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INTRODUCTION

Although pharmacokinetics and pharmacodynamics are generally assumed to be time invariant, some drugs have been reported to exhibit time-varying pharmacokinetics or pharmacodynamics, also called chronokinetics or chronodynamics.[1–3] Chronokinetics is observed as variations in absorption, distribution, metabolism, or excretion and chronodynamics as variations in drug effects or adverse drug reactions. For example, propranolol, a lipophilic antihypertensive drug, has been reported to be circadian-phase dependent, showing a higher maximum plasma concentration (C_{max}) and an earlier time to maximum plasma concentration (t_{max}) after the morning dose than after the evening dose,[4] and heparin has been reported to exhibit higher anticoagulant effects at night than in the morning.[5]

We observed diurnal variations similar to propranolol in a pharmacokinetic study of cilostazol,[6] which is also a lipophilic drug. The drug has been used in the treatment of intermittent claudication and in the prevention of coronary artery diseases and stroke.[7–11] In our pharmacokinetic study, we observed that the C_{max} values for cilostazol and its metabolite (OPC-13015) after the morning dose were 3.53% and 5.11% higher than after the evening dose, respectively, with a shorter t_{max} after the morning dose, and their trough concentrations before the morning dose were 14.1% and 13.9% higher than before the evening dose, respectively (Fig. 1, upper panels). The latter finding is consistent with results published in the literature[12,13] and demonstrates similar characteristics of many lipophilic drugs, known to have higher C_{max} and shorter t_{max} in the morning than in the evening, owing to higher gastrointestinal perfusion rates and faster gastric emptying times in the morning.[1,14,15] In addition to diurnal variations, our previous study found a potential seasonal variation in cilostazol pharmacokinetics, another source of variation in chronopharmacokinetics.[6] The area under the concentration versus time curve and C_{max} observed in winter were higher than in summer, with relative increases of 21.57% and 21.67% for cilostazol and 25.02% and 20.60% for OPC-13015, respectively (Fig. 1, lower panels). The differences in day length and

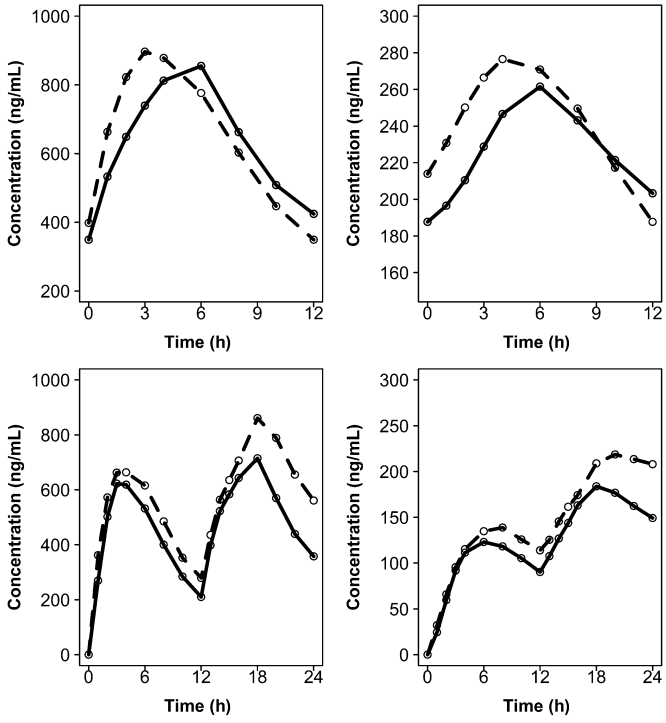

FIGURE 1. Mean observed concentrations of cilostazol (left) and its metabolite (OPC-13015) (right), reflecting diurnal variation (upper panels; morning dose, dashed line, versus evening dose, solid line) and seasonal variation (lower panels; part A, dashed lines, versus day 1 of part B, solid line). In the upper panels, the concentration curves for the evening dose (solid line) have been shifted to the left by 12 hours to be superimposed on those for the morning dose. In this and subsequent figures, except for Figures 2 and 5, *x* axes denote the time after the morning dose. Part A represents a single BID dosing trial conducted in winter and part B a multiple BID dosing trial in summer. See text for details.

temperature between the 2 seasons are approximately 5 hours and 35°C (30°C in summer versus −5°C in winter), respectively, in Korea, and such differences have been conjectured to affect the pharmacokinetics of cilostazol.

In our previous study, we found headache to be the most frequently observed adverse drug reaction to cilostazol.[6] The headaches occurred near t_{max}, indicating that the adverse drug reaction might be related to the high blood concentration of cilostazol. Safety data obtained from 8 cilostazol phase 3 trials reported that treatment discontinuation because of headache occurred in 3.7% of patients who received a commonly used dose of 100 mg cilostazol BID, which was more than 10-fold compared with the occurrence rate of 0.3% in placebo-treated patients.[16] Selection of appropriate dosing times may help to reduce the incidence of high blood concentrations of cilostazol.

In this study, we aimed (1) to examine factors influencing cilostazol pharmacokinetics in the Korean population by developing a population model incorporating diurnal and seasonal variations and other covariate effects and (2) to assess the feasibility of applying the developed model to determine the optimal dosing times.

METHODS

Cilostazol Plasma Concentrations

The pharmacokinetic data analyzed in this work comprised the plasma concentration–time profiles for the parent drug (cilostazol) and its metabolite (OPC-13015) from the immediate-release formulation of cilostazol (Pletal; Otsuka Pharmaceutical Co, Ltd, Tokyo, Japan), obtained from a previous pharmacokinetic study conducted in healthy volunteers.[6] In that study, 26 subjects received 100 mg of cilostazol orally twice a day (8 AM and 8 PM) for 1 day (part A, conducted in winter 2009) and 40 subjects received 100 mg of cilostazol orally twice a day (7 AM and 7 PM) for 8 consecutive days (part B, conducted in summer 2009). In part A and on days 1 and 8 of part B, the subjects were administered the morning dose after an overnight fast of 10 hours. To minimize food effect on drug absorption, lunch and sport drinks were provided at 4 and 8 hours after the dose, respectively, and the evening dose was given at 12 hours after the morning dose, followed by liquid food 4 hours later. On day 2 through day 7 of part B, subjects were administered the dose after a 4-hour fast, taking the meal at 1.5 hours after the administration, to minimize food effect. Blood samples were collected at 0 (predose), 1, 2, 3, 4, 6, 8, 10, 12, 13, 14, 15, 16, 18, 20, 22, 24, 36, 48, and 72 hours after the dose for part A and for day 1 and 8 of part B, in which the last 3 samples at 36, 48, and 72 hours were not collected for day 1 of part B.

Among the 66 subjects who participated in the study, 63 (26 for part A and 37 for part B) completed the study, and a total of 3712 cilostazol and OPC-13015 plasma concentrations were included in the analysis. Given that OPC-13015 (the first metabolite) is 3 times more potent than cilostazol and that OPC-13213 (the second metabolite) is only 0.33 times as potent as cilostazol, OPC-13213 was not included in the analysis.[17] Study participants gave written informed consent, and the study was approved by the institutional review board of Yonsei University Severance Hospital (Seoul, Korea). The study was performed in accordance with the Declaration of Helsinki[18] and Korean Good Clinical Practice.[19] Demographic characteristics of the 63 subjects who completed the study can be found in the previous work.[6]

Population Pharmacokinetic Model

Model building for the parent drug (cilostazol) and its metabolite (OPC-13015) was performed sequentially, under the assumption that OPC-13015 does not affect the pharmacokinetics of cilostazol. After the final pharmacokinetic model for the parent drug was determined, pharmacokinetic model building for the metabolite was performed using the posterior Bayes estimates of the model parameters for the parent drug. For each of the parent drug and its metabolite, the model-building process was performed in 3 steps. First, a basic structural model was built; second, a diurnal variation model; and finally, a model incorporating seasonal variation and other important covariates was developed. Inter-occasion variability was not included in the model because each subject participated in only one part of the study, either part A or B.

Basic Model for Cilostazol

A compartment model with first-order absorption was used to build a basic structural model, with an exponential error model for random interindividual variability as defined below:

$$P = \theta_p \cdot \exp\left(\eta_p\right),$$

where P is the individual pharmacokinetic parameter, θ_P the fixed-effect parameter, and η_P the interindividual random effect, distributed as a multivariate normal distribution with mean zero and variance–covariance matrix Ω.

Model Incorporating Circadian Variability

The time-varying features of C_{max} and t_{max} of cilostazol between the morning and evening doses were assumed to originate from the diurnal variation that might exist in the bioavailability fraction (F), the absorption rate constant (k_a), and/or the elimination rate constant (CL), as these are directly related to C_{max} and t_{max}.

In modeling the diurnal variation, F was assumed to have 2 distinct values between the morning and the evening doses as follows:

$$F_{AM} = 1,$$

$$F_{PM} = \theta_{F,PM} \cdot \exp\left(\eta_{F,PM}\right) / \left(1 + \theta_{F,PM} \cdot \exp\left(\eta_{F,PM}\right)\right),$$

where F_{AM} and F_{PM} represent the bioavailability of the morning and evening doses, respectively. The idea was to model the relative bioavailability of the evening dose assuming that the morning dose was fully available to the system ($F_{AM} = 1$).

On the other hand, the diurnal variation of k_a and CL was modeled using the circadian rhythm, which was assumed to be composed of a sum of cosine functions with different periods, each being formulated as follows:

$$CR_T = A_T \cdot COS(2\pi / T(t - \varnothing_T)),$$

$$A_T = \theta_{A_T} \cdot \exp\left(\eta_{A_T}\right),$$

$$\varnothing_T = \theta_{\varnothing_T} \cdot \exp\left(\eta_{\varnothing_T}\right),$$

where CR_T is the circadian rhythm pertaining to a cosine function with period T, amplitude A_T, and acrophase $\varnothing_T$, where A_T and $\varnothing_T$ are log normally distributed around the fixed-effect values θ_{A_T} and $\theta_{\varnothing_T}$ via interindividual random errors η_{A_T} and $\eta_{\varnothing_T}$ distributed with mean zero and variances $\omega^2_{A_T}$ and $\omega^2_{\varnothing_T}$, respectively. Various linear combinations of cosine functions with different periods were tested, and the selected combination according to the likelihood ratio test was included in the model as follows:

$$CR_P = CR_{P,T_1} + CR_{P,T_2} + \ldots + CR_{P,T_m},$$

$$P = \theta_P \cdot \exp(CR_P) \exp(\eta_P),$$

where CR_P is the overall circadian rhythm for the individual pharmacokinetic parameter P and CR_{P,T_j}, j = 1, …, m, the circadian rhythms comprising CR_P, each with period T_j.

Selection of Covariates

After a visual inspection of the relationship between individual post hoc parameter estimates, obtained from the diurnal variation model and covariates, a regression analysis using a generalized additive model approach was performed to initially screen the potential covariates influencing each parameter. Based on the model structure of the diurnal variation model selected in the previous stage, covariate selection was performed using these potential covariates and stepwise covariate modeling using the PsN (Perl-speaks-NONMEM) software (version 3.4.2, http://psn.sourceforge.net).[20,21] Likelihood ratio test was used to select significant covariates, with a significance levels of $P < 0.05$ for forward selection and $P < 0.005$ for backward elimination. The following covariates were tested: age (AGE), body mass index (BMI), weight (WT), and height (HT) for continuous covariates and caffeine (XAN), smoking (SMK), alcohol (ALC), gender (SEX), and season of the conducted study (SEA) for dichotomous covariates, where SEA reflects seasonal variation.

Model for OPC-13015

Model building for OPC-13015 was conducted sequentially by fixing the parent drug model parameters at their estimates obtained at the previous step of covariate model building for cilostazol. As for cilostazol, model building proceeded in the order of basic model, diurnal variation model, and covariate model building.

Software and Model Evaluation

All analyses described in the Methods were performed using NONMEM (version 7.2; ICON Development Solutions, Ellicott City, MD), and ADVAN5 and 6 and the First-Order Conditional Estimation method with INTERACTION between interindividual and residual variability were used. All the plots were created using R (http://www.r-project.org). The final model was evaluated by the visual predictive check (VPC) for 1000 simulations performed using the PsN tool (version 3.4.2).

TABLE 1. Parameter Estimates for the Final Model With Diurnal Variation and Covariates Included

Parameter	Model Equation	Θ (RSE%)	ω^2 (coefficient of variation, CV%) (RSE%)	*P*
Cilostazol				
k_a (/h)	$k_a = \theta_{k_a}(1 + \theta_{k_aSEASON} \cdot SEASON)\exp(CR_{k_a})\exp(\eta_{k_a})$	$\theta_{k_a} = 0.229$ (11.7%)	$\omega_{k_a}\% = 32.7\%$ (13.8%)	0.0018
	$CR_{k_a} = A_{24} \cdot COS(2\pi/24(t-\varnothing_{24})) + A_{12} \cdot COS(2\pi/12(t-\varnothing_{12}))$	$\theta_{k_aSEASON} = -0.269$ (28.5%)		
A_{24}	$AMP_{24} = \theta_{AMP_{24}} \cdot \exp(\eta_{AMP_{24}})$	$\theta_{AMP_{24}} = 0.291$ (18.8%)	$\omega_{AMP_{24}}\% = 78.8\%$ (16.0%)	
$Ø_{24}$ (h)	$\varnothing_{24} = \theta_{\varnothing_{24}}$	$\theta_{\varnothing_{24}} = 7.44$ (4.24%)		
A_{12}	$AMP_{12} = \theta_{AMP_{12}}$	$\theta_{AMP_{12}} = 0.404$ (9.05%)		
$Ø_{12}$ (h)	$\varnothing_{24} = \theta_{\varnothing_{24}}$	$\theta_{\varnothing_{24}} = 1.38$ (23.1%)		
CL (L/h)	$CL = \Theta_{CL}(1 + \Theta_{CL\_SEASON} \cdot SEASON) \cdot \exp(\eta_{CL})$	$\Theta_{CL} = 13.9$ (5.21%)	ω_{CL} % = 28.3% (10.5%)	
		$\Theta_{CL\_SEASON} = -0.318$ (19.2%)		0.0000
V_2 (L)	$V_2 = \theta_{V_2}$	$\theta_{V_2} = 45.6$ (14.4%)		
Q_P (L/h)	$Q_P = \theta_{Q_P}$	$\theta_{Q_P} = 11.2$ (11.1%)		
V_3 (L)	$V_3 = \theta_V \cdot \exp(\eta_{V_3})$	$\theta_{V_3} = 68.1$ (10.2%)	$\omega_{V_3}\% = 65.6\%$ (15.7%)	
$\sigma_{cilostazol}$ (%)		$\sigma_{cilostazol}$ = 31.9 (4.73%)		
OPC-13015				
Q_{PM} (L/h)	$Q_{PM} = \theta_{Q_{PM}}$	$\theta_{Q_{PM}} = 16.3$ (12.0%)		
CL_M (L/h)	$CL_M = \theta_{CL_M} \cdot \exp(\eta_{CL_M})$	$\theta_{CL_M} = 47.7$ (13.3%)	$\omega_{CL_M}\% = 19.0\%$ (9.67%)	
Q_M (L/h)	$Q_M = \theta_{Q_M} \cdot \exp(\eta_{Q_M})$	$\theta_{Q_M} = 113$ (23.6%)	$\omega_{Q_M}\% = 68.3\%$ (14.6%)	
V_5 (L)	$V_5 = \theta_{V_5} \cdot \exp(\eta_{V_5})$	$\theta_{V_5} = 190$ (15.2%)	$\omega_{V_5}\% = 26.6\%$ (18.6%)	
$\sigma_{OPC\text{-}13015}$ (%)		$\sigma_{OPC\text{-}13015}$ = 26.3 (2.21%)		

A_{24} and $Ø_{24}$ denote the amplitude and acrophase for a 24-hour rhythm of circadian variation and A_{12} and $Ø_{12}$ for a 12-hour rhythm, which comprises the overall circadian variation in the absorption rate constant for cilostazol, CR_{k_a}. Residual errors are represented in the unit of coefficient of variation, CV%. *P* values were obtained by the likelihood ratio test between the models including and excluding a covariate to be tested. *P* value denotes the relative importance of the covariate.

The other parameters are defined according to ADVAN6: k_a, the absorption rate constant from absorption compartment to central compartment for cilostazol; CL, the clearance for cilostazol; V_2, the central volume of distribution for cilostazol; Q_p, the intercompartment clearance between central and peripheral compartments for cilostazol; V_3, the peripheral volume of distribution for cilostazol; Q_{PM}, the formation clearance from cilostazol to OPC-13015; Q_M, the intercompartment clearance between central and peripheral compartments for OPC-13015; CL_M, the clearance for OPC-13015; V_5, the peripheral volume of distribution for OPC-13015; RSE, the relative standard error (= standard error of parameter estimate/parameter estimate); SEASON, 0 for summer, 1 for winter.

Simulation of Optimal Dosing Scenarios

A set of optimal dosing times, $T^* = (T^*_{AM}, T^*_{PM})$, to be applied in multiple dosing, with T^*_{AM} and T^*_{PM} denoting optimal dosing times in the morning and evening, respectively, was chosen as the one that minimizes the overall relative error ERR defined as below, where (T^*_{AM}, T^*_{PM}) was searched over T_{AM} = (6, 7, …, 11 AM, noon) and T_{PM} = (5, 6, …, 11 PM), with a search interval of 1 hour:

$$ERR = (ERR_1 + 3ERR_2)/4,$$

where ERR_1 and ERR_2 are the relative errors for cilostazol and OPC-13015, respectively, with the weighted average being used considering that OPC-13015 is 3 times more potent than cilostazol, as reported in the literature.[22] ERR_1 and ERR_2 were defined as follows:

$$ERR_i = \left(ERR_{i,1} + ERR_{i,2}\right)/2, \text{ for } i = 1, 2,$$

$$ERR_{i,j} = abs\left(C_{max\ i,j} - Target_i\right)/Target_i \times 100,$$

where $C_{max\ i,j}$ and $ERR_{i,j}$ denote the population predicted value of C_{max} given a multiple BID dosing for 8 days and the associated error for cilostazol (i = 1) or OPC-13015 (i = 2) for the morning (j = 1) or the evening dose (j = 2). $Target_i$ denotes the population predicted value corresponding to the smaller of $C_{max\ i,1}$ and $C_{max\ i,2}$ evaluated at the original dosing times of 8 AM and 8 PM. Given that $C_{max\ i,1} > C_{max\ i,2}$ for the original dosing times, $target_i$ was chosen as $C_{max\ i,2}$, which was 756.3 and 226.9 ng/mL in summer and 1090.2 and 336.1 ng/mL in winter for cilostazol and OPC-13015, respectively.

For the computation details, ERR was obtained as follows. First, for each of the original dosing times and an optimal dosing time candidate, 1000 datasets were simulated from the original model. Second, for each simulated dataset k (k = 1, …, 1000), ERR_{ijkl}, ERR for individual l (l = 1, …, 63) of simulated dataset k for cilostazol (i = 1) or OPC-13015 (i = 2) for the morning (j = 1) or the evening dose (j = 2), was computed, followed by ERR_{ikl}, the mean of ERR_{ijkl} over j. Third, ERR_{ik}, the mean of ERR_{ikl} over l, was computed. Fourth, ERR_k, the mean of ERR_{ik} over i, was obtained as $(ERR_{1k} + 3ERR_{2k})/4$ and, finally, ERR, the mean of ERR_k over k.

The significance test for the performance difference between the original and the optimal sampling time approaches, as measured by ERR, was conducted using a paired *t* test between the 2 sets of 1000 ERR_k values obtained from the 2 approaches.

RESULTS

Population Pharmacokinetic Model

Basic Model for Cilostazol

The basic model for cilostazol was best described by a 2-compartment model with first-order absorption; Akaike

information criterion values were 19,052 for the 1-compartment model, 18,837 for the 2-compartment model, and 18,841 for the 3-compartment model. The pharmacokinetic parameter estimates (percentage of relative standard error%) for the selected 2-compartment model were 0.321/h (10.4%), 80.1 L (10.1%), 11.9 L/h (4.40%), 9.6 L/h (7.70%), and 58.3 L (15.2%) for k_a, V_2, CL, Q, and V_3, respectively. The interindividual variabilities (coefficient of variation, CV%) for these 5 parameters were 21.8%, 26.0%, 34.6%, 27.4%, and 85.1%, respectively. Covariance components of the interindividual variability of pharmacokinetic parameters were not significant, so the variance–covariance matrix Ω was assumed to be diagonal for the rest of the model-building process. Residual variability was best explained by a proportional error model (35.6%).

Model Incorporating Circadian Variability

When the diurnal variation of F was included in the basic model, it was not significant as judged by Akaike information criterion values (18,846 versus 18,844), so was neglected.

The diurnal variation of k_a was best described by a sum of 24- and 12-hour rhythms. The amplitude (in log scale) and acrophase were 0.291 and 7.44 hours for a 24-hour rhythm and were 0.404 and 1.38 hours for a 12-hour rhythm, respectively. When these circadian rhythms were included in k_a, the model improved significantly ($P < 0.005$, $\Delta OBJ = -353$). The degrees of shrinkage in the model parameters of the diurnal variation model were all less than 23%. Interindividual variability was found significant for A_{24} only. The diurnal variation of CL was less significant than that of k_a and was not included in the model.

Selection of Covariates

The final model reported in Table 1 shows that there exists a significant seasonal variation in k_a and CL ($P < 0.005$), resulting in a 26.9% decrease in the circadian rhythm of k_a and a 31.8% decrease in CL in part A, as compared with part B. The estimated precision of the model parameters for cilostazol was within 30% in relative standard error for the fixed-effect parameters. The estimated interindividual variability was larger in absorption and distribution phases, reaching up to 78.8% in coefficient of variation of A_{24}, as compared with that of the elimination phase, which was less than 30%. Q_{PM} was estimated to be 16.3 L/h. The interindividual variability of CL_M was smaller than that of CL.

Model for OPC-13015

As was found for cilostazol, the 2-compartment model best described OPC-13015 data as judged by Akaike information criterion values of 13,722 for the 1-compartment model, 13,148 for the 2-compartment model, and 13,150 for the 3-compartment model. As the clearance of the metabolite and its volume of distribution were not separately identifiable, the central volume of distribution of metabolite (V_4) was fixed to be equal to V_2. During the model-building process, without loss of generality, we assumed that if diurnal variations in metabolite kinetics were present, it would be caused by the parent drug; thus, no additional circadian component was included in the metabolite model (Table 1). No covariate was found significant during the stepwise covariate modeling process. Figure 2 depicts the model structure for cilostazol and OPC-13015 finally selected in this work.

Model Evaluation

Figure 3A depicts the goodness-of-fit plot for cilostazol obtained from the basic model, where the distributions of residuals show fluctuating trends over time. Figure 3B shows the goodness-of-fit plot for the final model incorporating circadian rhythms and covariate effects, including seasonal variations for cilostazol and OPC-13015, indicating that there is a good agreement between model predictions and the data overall, which supports evidence of the presence of a diurnal variation in cilostazol pharmacokinetics. In Figure 3A, the goodness-of-fit plot for OPC-13015 for the basic model was not obtainable as the metabolite model was built sequentially after the parent model, including diurnal variation and covariates, was determined.

When evaluated by a VPC, more than 90% of observed concentrations were included within the 90% prediction intervals. In the case of cilostazol, the percentages of observed concentrations below the fifth and above the 95th percentile prediction curves were 1.96% and 5.88% for part A, 3.02% and 6.36% for day 1 of part B, and 1.47% and 4.71% for day 8 of part B, respectively. For OPC-13015, they were 0.975% and 1.75% for part A, 3.51% and 2.23% for day 1 of part B, and 0.978% and 2.51% for day 8 of part B. VPC results are plotted in Figure 4, showing a good overall agreement between the prediction and the data, although for OPC-

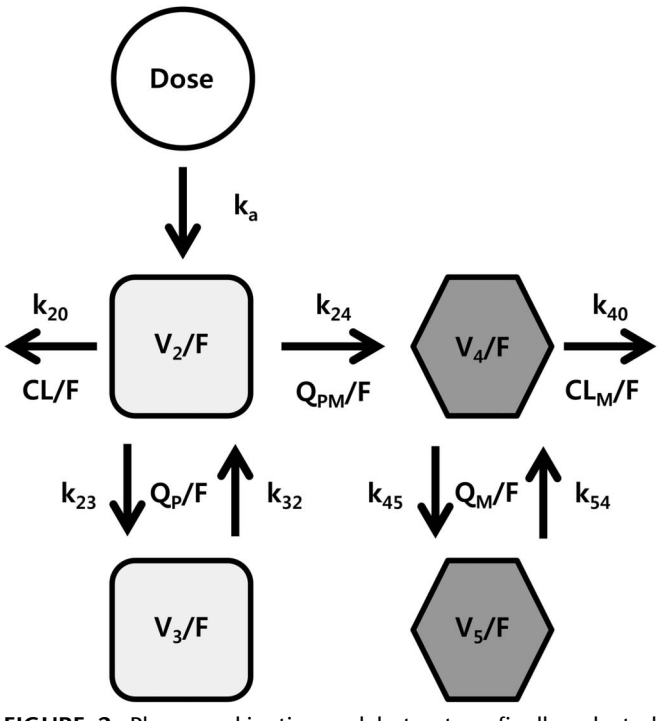

FIGURE 2. Pharmacokinetic model structure finally selected for cilostazol and OPC-13015. The central volume of distribution of metabolite (V_4) was fixed to be equal to V_2. See footnotes to Table 1 for symbols.

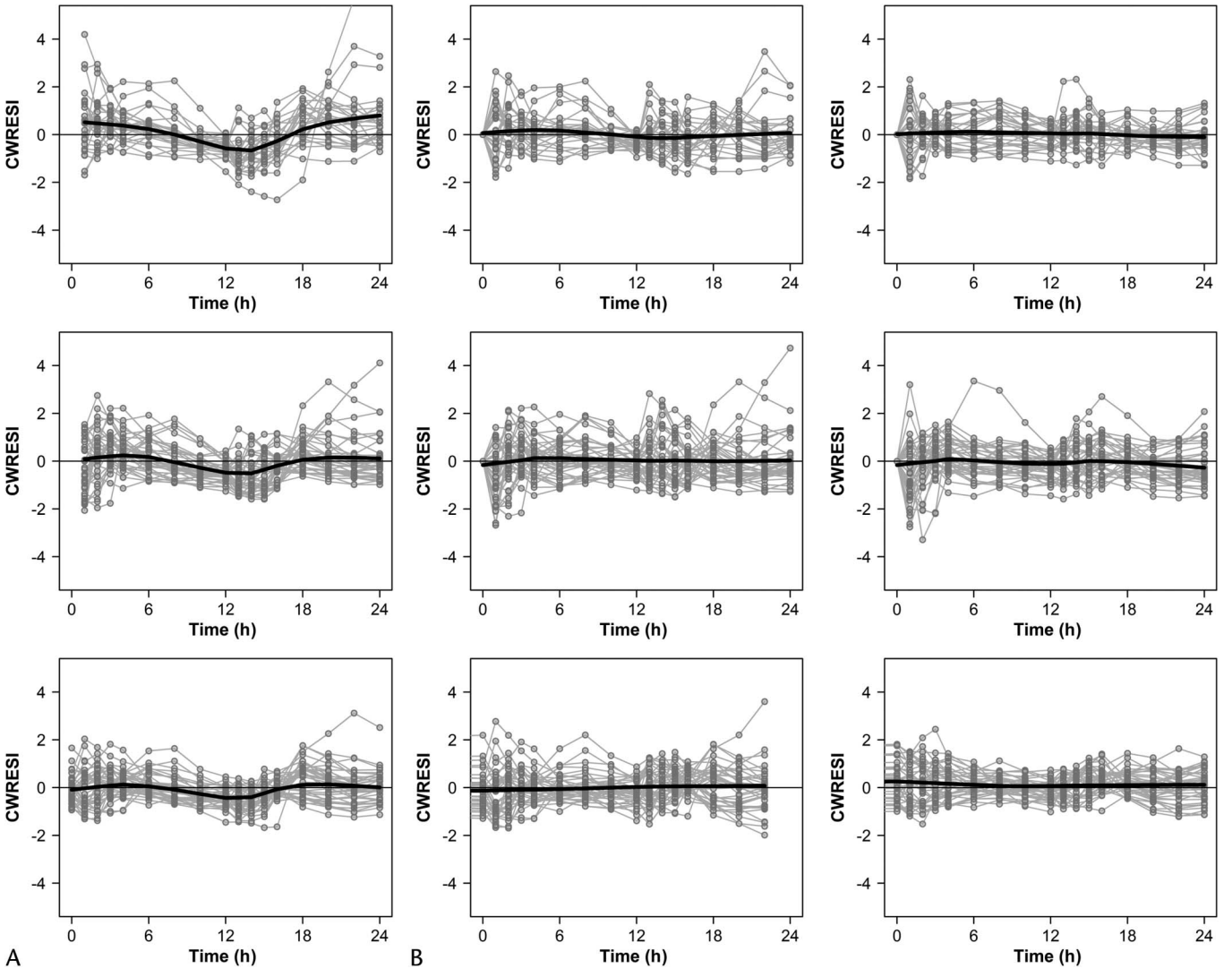

FIGURE 3. Goodness-of-fit plots for part A (upper panels), day 1 of part B (middle panels), and day 8 of part B (lower panels) obtained from (A) the basic model for cilostazol and (B) the final model for cilostazol (left) and OPC-13015 (right). The circles are conditional weighted residuals with eta–epsilon interaction included, and the solid lines are smooth curves.

13015 in part B one subject's data were far above the prediction interval.

The influence of circadian rhythm on k_a predicted by the final model is shown in Figure 5, which shows that, in summer (part B), the predicted value of k_a in the morning varied between 0.205 and 0.351/h with an average of 0.278/h, which was faster than that in the evening, varying between 0.114 and 0.354/h with an average of 0.234/h. A similar trend of a higher rate of k_a in the morning than in the evening was predicted in winter (part A).

Simulation of Optimal Dosing Scenarios

The optimal dosing times were determined to be 9 AM and 5 PM in summer and 10 AM and 7 PM in winter, yielding ERRs of 3.00% and 0.89%, respectively, whereas the original dosing times yielded ERRs of 6.49% and 5.06%, respectively (Table 2), indicating that the optimal dosing schedule performs significantly better than the original dosing schedule ($P < 0.0001$) in minimizing the concentration deviation from the target concentration.

The predicted concentrations for optimal dosing times superimposed on the predicted concentrations for the original dosing times are shown in Figure 6, indicating a lower peak concentration after the morning dose and a smaller peak-to-peak fluctuation achieved by the optimal dosing time. Specifically, in the case of summer dosing, the predicted population values of C_{max} for cilostazol obtained using optimal dosing times, as measured by the median value of 1000 simulations, were 866.9 ng/mL after the morning dose and 708.3 ng/mL after the evening dose, which were, respectively, 19.35% higher and 6.34% lower than the target concentration of 756.3 ng/mL, whereas those for OPC-13015 obtained using optimal dosing times were 228.9 ng/mL after the morning dose and 227.1 ng/mL after the evening dose, which were,

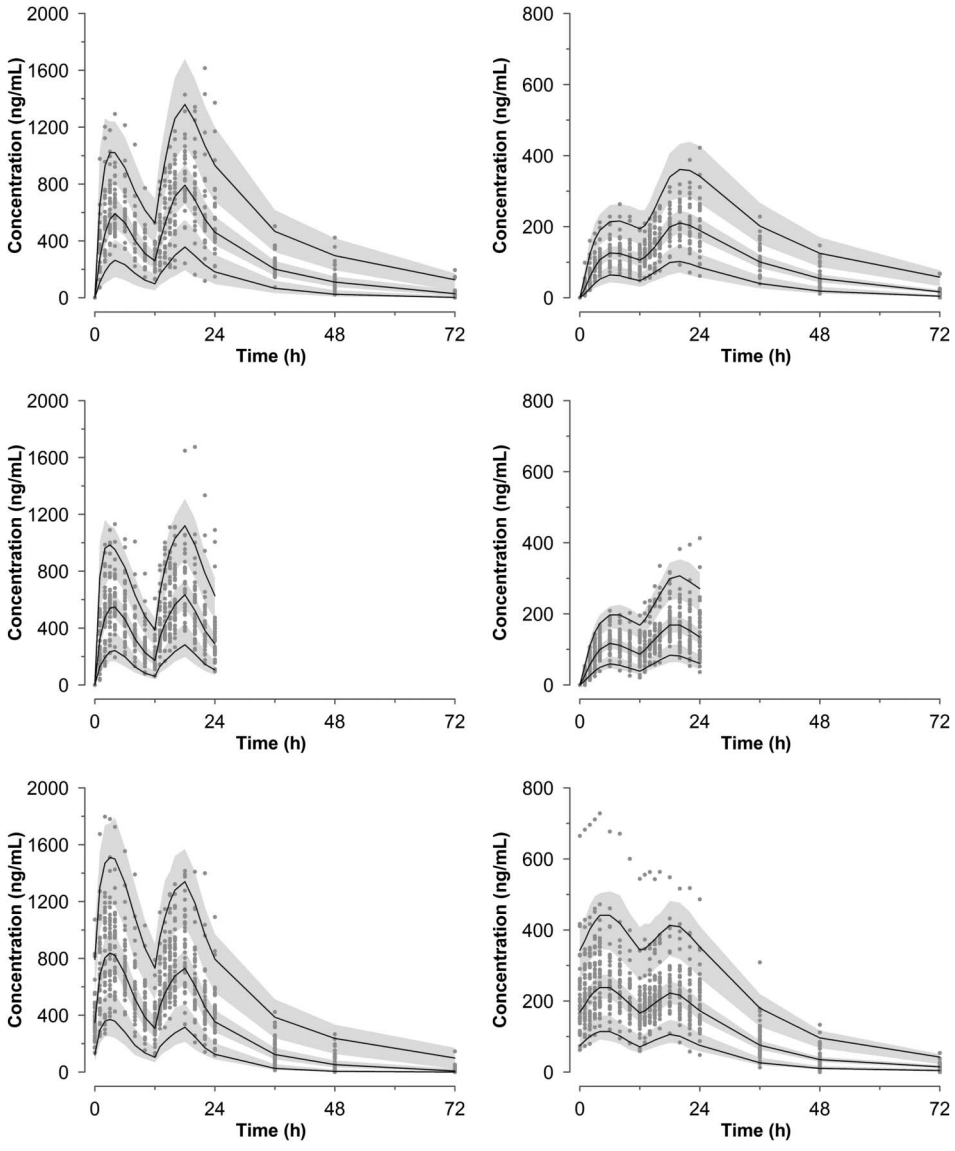

FIGURE 4. VPC for the predicted concentration of cilostazol (left) and OPC-13015 (right) for part A (upper panels), day 1 of part B (middle panels), and day 8 of part B (lower panels). The gray areas represent the 90% prediction intervals obtained from 1000 datasets simulated from the final model where the thick curve in the middle denotes the 50th percentile.

respectively, 0.91% higher and 0.11% higher than the target concentration of 226.9 ng/mL. On the other hand, in the case of winter dosing, the predicted values of C_{max} for cilostazol obtained using optimal dosing times were 1147.0 ng/mL after the morning dose and 1083.4 ng/mL after the evening dose, which were, respectively, 5.21% higher and 0.62% lower than the target concentration of 1090.2 ng/mL, whereas those for OPC-13015 were 335.9 ng/mL after the morning dose and 334.8 ng/mL after the evening dose, which were, respectively, 0.05% lower and 0.38% lower than the target concentration of 336.1 ng/mL. Each 90% prediction band for the optimal and original dosing times consists of 1000 simulations, based on a set of 63 simulated subjects.

DISCUSSION

The diurnal variation of cilostazol has already been reported in other pharmacokinetic studies. For example, it was reported that the steady-state concentration–time profiles showed a trend for the trough concentrations of cilostazol and its metabolites to be higher before the morning dose than before the evening dose.[12,13] However, none of the previous studies used a modeling approach to analyze formally the sources of diurnal variation in cilostazol pharmacokinetics.

In this study, diurnal variations in C_{max} and t_{max} of cilostazol were explained by the circadian rhythms in k_a only because allowing for diurnal variation in CL or F did not significantly improve the model. From the biological point of view, this was considered to be acceptable in that k_a can be affected by changes in posture, blood flow, tissue perfusion, gastric pH, gastric emptying, and gastrointestinal motility over a 24-hour period.[2] The result using the final model shows that the estimates of the pharmacokinetic parameters of cilostazol changed considerably when diurnal variation was included in k_a, as compared with the basic

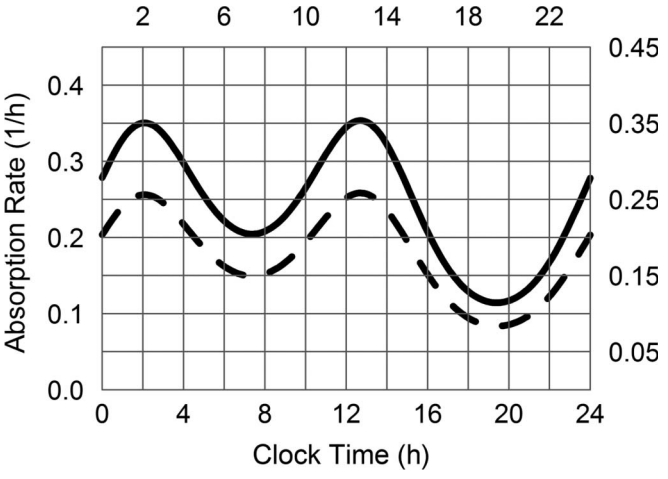

FIGURE 5. Circadian variation in the absorption rate constant (k_a) predicted from the final model. The dashed line denotes part A and the solid line part B.

model; the central volume of distribution decreased from 80.1 to 45.6 L, CL increased from 11.9 to 13.9 L/h, Q increased from 9.60 to 11.2 L/h, and V_3 increased from 58.3 to 68.1 L (Table 1). This reflects the importance of considering the influence of diurnal variation on pharmacokinetics, indicating the possibility of inaccurately assessing pharmacokinetics, thereby inappropriately suggesting the treatment guideline when the influence of diurnal variation was not taken into account. Interestingly, the influence of bioavailability on diurnal variation in cilostazol pharmacokinetics was insignificant.

When modeling the circadian rhythm in k_a, we used an exponential form rather than an additive form. However, given that the amplitude of circadian rhythm was much smaller than 1.0 as seen in Figure 5, an additive form, if used, would give approximately the same result, based on the property of Taylor series expansion that allows for exp(x) to be approximated by 1 + x when x is small as in this case.

Consistent with the observation in Figure 1, season was found to affect cilostazol pharmacokinetics significantly when incorporated into k_a and CL, which are pharmacokinetic parameters associated with trough and peak plasma concentration values of a drug. One seasonal trend consistently observed in this study was that the mean plasma concentrations of cilostazol and OPC-13015 measured in summer were lower than in winter.

The value of Q_{PM} for OPC-13015 was estimated to be 16.3 L/h, much smaller than that of CL_M, which was estimated to be 47.7 L/h. This indicates a possibility of "flip-flop" phenomenon for OPC-13015, which was also observed in the previous work done using a noncompartment analysis.[6] The consistent result between the current and the cited works supports the appropriateness of our model.

In the predicted concentration plots for the optimal dosing times illustrated in Figure 6, an interesting result was obtained; the predicted concentration curve did not show a distinct peak after the evening dose such as was seen after the morning dose. Instead, it shows a curve that is almost flat over a period between approximately 8 and 12 hours after the morning dose (or between 4 PM and 8 PM in clock time) and very slowly increasing thereafter. We conjecture that this would be because the predicted absorption rate approaches its minimum near the evening dosing time of 8 PM (Fig. 5), which might play the role of inhibiting the concentration increase, resulting in almost a flat concentration curve over this period.

As OPC-13015 is known to have an antiplatelet aggregation effect 3 times more potent than cilostazol, it was important to understand its pharmacokinetics and influencing factors. We analyzed OPC-13015 data together with its parent drug data in our model. Among the pharmacokinetic parameters of OPC-13015 obtained in this study, V_5 was not an actual value but a ratio of k_{45}/k_{54} with V_4 being fixed to V_2.

Although the most commonly observed adverse drug reaction in cilostazol is headache and severe adverse drug reactions rarely occur with a common dose, this study was conducted in an effort to characterize diurnal variations of a drug using a pharmacometric approach and to illustrate how to apply the acquired knowledge to design an optimal dosing time so as to reduce the incidence of adverse drug reactions and increase patient compliance. In doing so, we did not consider changing the dose amount, as the intention was to find the optimal dosing time given the fixed amount of dose and given the practical difficulty with changing the amount of dose per tablet. In this regard, the control of seasonal variation, which was unavoidable without dose adjustment, was not considered for optimizing dosing scenarios.

Because this work was conducted in healthy volunteers using the drug concentration as the outcome measure, the significance of the results obtained was tested statistically only, indicating that diurnal and seasonal variations found to

TABLE 2. Comparison of Relative Errors Obtained From the Original and the Optimal Dosing Times

Season	Regimen	Dosing Time	ERR_1 (%)	ERR_2 (%)	ERR (%)	*P**
Summer	Original regimen	7 AM, 7 PM	7.96	6.01	6.49	<0.0001
	Optimal regimen	9 AM, 5 PM	10.48	0.51	3.00	
Winter	Original regimen	8 AM, 8 PM	6.52	4.57	5.06	<0.0001
	Optimal regimen	9 AM, 6 PM	2.92	0.22	0.89	

*Paired *t* test for ERR between the original and the optimal regimens in each season. See text for the mathematical definition of ERR_1, ERR_2, and ERR.
ERR_1, the relative error for cilostazol; ERR_2, the relative error for OPC-13015; ERR, the overall relative error for cilostazol and OPC-13015.

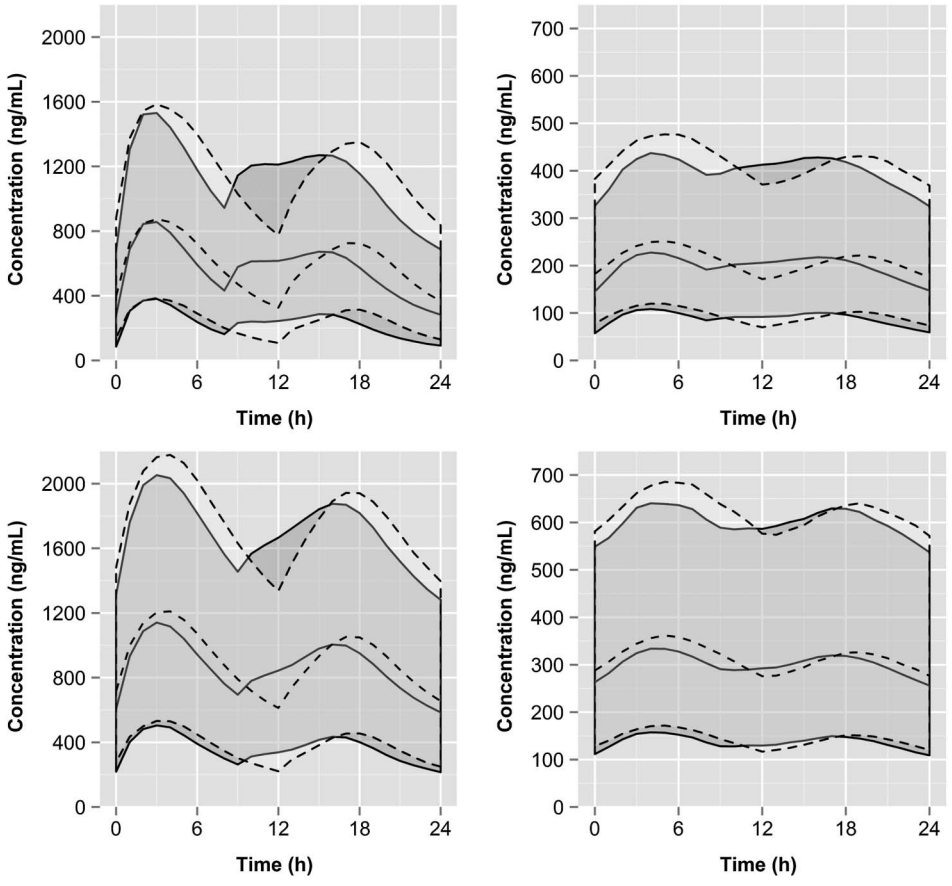

FIGURE 6. Comparison of the 90% confidence interval of the predicted concentration between the optimal dosing time (solid line) and the original dosing time (dashed line) for summer (upper panels) and winter (lower panels) for cilostazol (left) and OPC-13015 (right) obtained from 1000 datasets simulated from the final model where the curve in the middle denotes the 50th percentile and the abscissa is time after the morning dose at steady state.

influence cilostazol pharmacokinetics significantly may be within the daily variability that a clinician would expect from their patients and not clinically relevant. Nevertheless, we consider that the concept and methodology developed here can be applied similarly to other drugs that have characteristics of chronokinetics.

In this study, the meal was carefully controlled to minimize food effects, as the bioavailability of cilostazol is known to be considerably affected by a high-fat meal.[6,23] However, to investigate how the food effect relates to the diurnal and seasonal variations in cilostazol pharmacokinetics, studies that formally incorporate the food effect into the model might be needed.

CONCLUSIONS

Using a model-based approach, this study demonstrated that the time-varying nature of cilostazol pharmacokinetics is attributed to diurnal variation, explained by the increased morning absorption rate, and seasonal variation, explained by the delayed time to the peak absorption rate and the decreased drug elimination rate in winter, and developed a strategy for optimizing dosing times. We suggest that our methods can be applied similarly to analyses of other drugs that exhibit characteristics of time-varying pharmacokinetics.

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