Cardiovascular effects and simultaneous pharmacokinetic and pharmacodynamic modeling of cilostazol in healthy subjects

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Abstract

Aim To relate cardiovascular effects of cilostazol and plasma drug concentration using simultaneous pharmacokinetic-pharmacodynamic (PK-PD) modeling techniques. Methods A single oral dose of 100 mg cilostazol was administered to 20 male healthy volunteers. Blood samples and pharmacodynamic measurements (HR, systolic blood pressure (SBP) and diastolic blood pressure (DBP)) were performed prior to dosing and over to 48 h thereafter. The plasma concentration (\(C_p\)) of cilostazol was determined by the reversed-phase HPLC method. Results The plasma concentration-time course followed a two-compartment open model. Mean peak plasma concentration (\(C_{\text{max}}\)) was 749.2 ± 348.7 \(\mu\text{g} \cdot \text{L}^{-1}\) approximately 3.7 h after administration. The maximal increase in HR was 14.9 % at 6 h after administration. There was no significant effect on SBP, but the maximal decrease in DBP was 28.4 %. An important delay was observed between \(C_p\) of cilostazol and its cardiovascular effects. Plots of effects versus \(C_p\) showed counterclockwise hysteresis loops. After PK-PD simulating, the relationship between effect compartment concentration (\(C_e\)) and the effect was successfully characterized by an effect-link sigmoidal \(E_{\text{max}}\) model. Conclusion A simultaneous PK-PD modeling was successful established to describe the relation between plasma concentration of cilostazol and its effects on HR and DBP.

Key words cilostazol; pharmacokinetics; pharmacodynamics; pharmacokinetic-pharmacodynamic modeling; antiplatelet agent

Introduction

Cilostazol (6-[4-(1-cyclohexyl-1H-tetrazol-5-yl) butoxy]-3, 4-dihydro-2(1H)-quinolinone; OPC-13013), a 2-oxo-quinoline derivative, was approved for marketing in Japan in 1988, as an antiplatelet agent to improve ischemic symptoms, such as pain and cold sensation\cite{1}. In 1999, the agent was approved by the US Food and Drug Administration for the treatment of intermittent claudication (IC)\cite{2, 3}. Cilostazol is a selective inhibitor of phosphodiesterase (PDE)\textsubscript{3A}, an enzyme that breaks down cyclic (cAMP), with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation\cite{4, 5}.

It has been found that cilostazol exerts beneficial positive chronotropic and dromotropic effects resulting in increased HR in patients with sick sinus syndrome, bradycardiac atrial fibrillation, indicating that cilostazol has therapeutic utility for the treatment of cardiovascular diseases, such as chronic atrial fibrillation\cite{6-8}. The increase of HR may be mediated
by improvement of conductivity in the atrioventricular node and increase of coronary blood supply caused by dilation of vessels.\[^9\]

The pharmacokinetics, pharmacodynamics, safety, and side effects of single dose, short- and long-term administration of cilostazol have been extensively studied\[^10-12\]. However, little is known about the precise relationship between the cardiovascular effects of cilostazol and its plasma concentrations in human bodies. The purpose of this study was to characterize the relationship between plasma concentrations and the cardiovascular effects of cilostazol after oral administration to healthy volunteers using simultaneous pharmacokinetic-pharmacodynamic (PK-PD) modeling techniques.

**Materials and methods**

**Subjects**

20 male healthy volunteers (mean ± SD) (23 ±1.1 years, range: 21-25 years; weight 60 ±5.3 kg, range: 50-69 kg; height 168 ±5.8 cm, range: 158-175 cm) were investigated. All subjects were in good health as determined by medical history, complete physical examination, vital signs, 12-leads electrocardiograms (ECG) recording, and routine biochemical and hematological test results. No concomitant drug therapy was allowed 2 weeks before and during the study period. The subjects were also asked not to consume alcoholic or caffeine-containing beverages 10 hours prior to and throughout the whole study. The study was conducted in accordance with good clinical practice (GCP) procedures, State Food and Drug Administration regulation, P.R.China, and the Declaration of Helsinki (as revised in Edinburgh 2000). Each subject gave written informed consent before taking part in the study, which was approved by the independent ethical committee of the Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China).

**Protocol**

After an overnight fast, all subjects were given a single oral dose of 100 mg cilostazol tablets (Guilin Pharmaceutical Company, Guangxi, China, Lot No 030402). Blood samples were taken before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 h after drug administration. The blood samples were collected in heparin-treated tubes, immediately subjected to centrifugation at 3000 r·min\(^{-1}\) for 10 min and then stored at –80 °C until HPLC analysis. Recordings of HR and BP variables before taking medication were taken as baseline measurements. And HR and BP were recorded just before the blood samples for 48 h. During the study period, all subjects remained under closely medical supervision and were supplied uniform diets.

**Drug analysis**

Five mL of acetonitrile was added to 1mL aliquot of plasma. The mixture was vortex mixed for 5 min, then centrifuged at 3000 r·min\(^{-1}\) for 10 min. The supernatant was transferred to another centrifuging tube and evaporated under a gentle nitrogen stream. To the residue, 1 mL of 0.2 mol·L\(^{-1}\) NaOH and 4 mL of ethyl ether were added, and the mixture was shaken and centrifuged at 3000 r·min\(^{-1}\) for 10 min. The ethyl ether layer was transferred to another centrifuging tube and evaporated to dryness at 40 °C under a gentle nitrogen stream. The residue was reconstituted in 100 µL of mobile phase, and then 20 µL of the solution was injected into the HPLC system for analysis.

The chromatographic system used to analyze cilostazol in plasma samples consisted of a Waters 510 pump and Waters 484 UV-detector operated at 254 nm and an EChrom98 chromatographic workstation (Dalian Elite Instrument Company). The analytical column (hypersil-ODS2, 150 mm×4.6 mm, 5 µm) was protected with a guard column packed with the same material. The mobile phase was composed of 45 % acetonitrile and 55 % distilled water running at a flow rate of 1.0 mL·min\(^{-1}\). The limit of quantitation (LOQ) was 10 µg·L\(^{-1}\) of plasma, and the intra- and inter-assay coefficient of variation was < 5.05 % and 5.50 %, respectively. The retention time (RT) of cilostazol was equal to 6.01 min. The HPLC chromatograms of cilostazol in plasma were showed in Fig 1.
Simultaneous PK-PD Modeling

To establish a simultaneous PK-PD modeling, the first step is to obtain PK parameters, which were determined by noncompartmental and compartmental approaches according to standard procedures. The elimination rate constant ($k_e$) and its corresponding half-life ($T_{1/2\beta}$) were estimated by ordinary least-square fit of data points (time, log plasma drug concentration) in the terminal phase of the decline. The area under the plasma concentration-time curve (AUC) was calculated with the trapezoidal rule and extrapolated to infinity. Total body clearance (CL) was calculated as $\text{CL}=\text{dose}/\text{AUC}$. The AUMC was calculated by the linear trapezoidal method.

A two-compartment open model was used to characterize the plasma concentration-time profiles of cilostazol after administration. The selected model gave the best fit for the data as assessing by the Akaike information criterion \([13]\). Then, the second step is to apply the PK parameters in the link PK model. The $C_p$ and $C_e$ can be expressed as follow:

\[
C_p = \frac{X_0 (\alpha - K_{21})}{V_C (\alpha - \beta)} \cdot e^{-\alpha t} + \frac{X_0 (K_{21} - \beta)}{V_C (\alpha - \beta)} \cdot e^{-\beta t}
\]

\[
C_e = \frac{X_0 K_{e0}}{V_C} \left[ \frac{K_{21} - \alpha}{\beta - \alpha (K_{e0} - \alpha)} + \frac{K_{21} - \beta}{\alpha - \beta (K_{e0} - \beta)} + \frac{K_{21} - K_{e0}}{\alpha - K_{e0}} \right] \frac{e^{-K_{e0} t}}{e^{-K_{e0} t}}
\]

Where $\alpha$ is distribution rate constant, $\beta$ is elimination rate constant, and $K_{e0}$ is rate constant for drug removal from effect compartment.

Plots of plasma concentration versus percent changes in HR and DBP in time sequence showed counterclockwise hysteresis loops. We postulated a hypothetical effect compartment linked to the plasma compartment by a first order process (link PK model;
Fig. 2). It is assumed that drug enters and leaves the effect compartment with no appreciable reflux of drug back into the PK system. In the link PK model, the concentration and time course of drug at the effect compartment are determined by the elimination rate constant of the effect-compartment ($k_{e0}$). The greater the $k_{e0}$, the less is the time lag between the central and effect compartments. To determine an appropriate $k_{e0}$, a parametric approach with sigmoidal $E_{\text{max}}$ model was included in the simultaneous PK-PD modeling \cite{14}.

\[ K_{21} \]
\[ K_{10} \]
\[ K_{1e} \]
\[ GI \]
\[ E \]
\[ C \]
\[ P \]

Fig 2. The link PK model defined as the link of the hypothetical compartment to the PK model. GI: gastrointestinal compartment; C: central compartment; P: peripheral compartment; E: effect compartment.

The last step is to integrate the link PK model into a PD model, which becomes a simultaneous PK-PD model. The relationship between the cardiovascular effects of cilostazol and effect compartment concentration can be represented by the sigmoidal $E_{\text{max}}$ model.

\[ E = \frac{E_{\text{max}} \cdot C_{e}^\gamma}{EC_{50}^\gamma + C_{e}^\gamma} \quad (3) \]

Where $E$ is the observed effect; $C_e$ is the effect compartment concentrations; $E_{\text{max}}$ is the maximal effect in predicted response that can be produced by the drug; $EC_{50}$ is the effect compartment concentration required to achieve 50% of $E_{\text{max}}$ and $\gamma$ is the Hill coefficient (or slope factor), which determines the sigmoidicity of the concentration-effect relation.

**Statistical analysis**

The pharmacokinetic parameters were calculated with 3P97 program edited by Chinese Pharmacological Society. PK-PD modeling was undertaken by the PK-PD Parameters Estimate Program (Nanjing Military General Hospital, China). All data were expressed as mean ± SD. Statistical difference was determined by a paired t-test. A value of $P<0.05$ was considered as significant.

**Results**

**Pharmacokinetic analysis**

The mean plasma concentration-time curves after oral administration of 100 mg cilostazol in 20 healthy subjects were shown in Fig 3. A two-compartment open model was chosen to describe the data and corresponding main pharmacokinetic parameters were listed in Table 1. Peak plasma concentrations of cilostazol occurred about 3.7 h after drug administration and then declined biexponentially with concentrations detectable (>10 µg·L$^{-1}$) in the plasma for at least 48 h post-dose. The apparent elimination half-life ($T_{1/2b}$) of cilostazol was approximately 17 h.

**Pharmacodynamic measurements**

The percentage changes in HR, SBP and DBP compared with baseline were used as pharmacodynamic measurements. Cilostazol induced a significant reduction in HR and DBP ($P<0.05$), whereas SBP was not changed significantly from baseline. HR was increased maximally 14.9% at 6 h after dosing, and then returned to baseline by 24-36 h. DBP started
to decrease after a short lag time of approximately 2 h from doing, and reached its maximum changes about 28.4% at 6 h after dosing, and returned to baseline by 36-48 h (Fig 3).

Cilostazol was very well tolerated. Only one subject experienced light headache while voiding at approximately 2 h after the oral dose.

Fig 3. Mean plasma concentration, HR and DBP (percent change from baseline) versus time curves after a single oral dose of 100 mg cilostazol in healthy subjects. n=20. Mean ± SD.

Table 1. Pharmacokinetic parameters of cilostazol after a single oral dose of 100 mg in healthy subjects. (n=20, Mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (µg·L⁻¹)</td>
<td>749.2 ± 348.7</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>$AUC_0-4$ (µg·h·L⁻¹)</td>
<td>10088.5 ± 4606.1</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (µg·h·L⁻¹)</td>
<td>10926.3 ± 4713.6</td>
</tr>
<tr>
<td>$AUMC_{0-4}$</td>
<td>135885.6 ± 77403.5</td>
</tr>
<tr>
<td>$AUMC_{0-\infty}$</td>
<td>189161.6 ± 108139.2</td>
</tr>
<tr>
<td>$MRT_{0-4}$ (h)</td>
<td>13.1 ± 3.4</td>
</tr>
<tr>
<td>$V_d/F$ (L·kg⁻¹)</td>
<td>3.7 ± 3.3</td>
</tr>
<tr>
<td>$CL/F$ (L·h⁻¹·kg⁻¹)</td>
<td>0.13 ± 0.09</td>
</tr>
<tr>
<td>$T_{1/2\alpha}$ (h)</td>
<td>3.4 ± 1.4</td>
</tr>
<tr>
<td>$T_{1/2\beta}$ (h)</td>
<td>17.3 ± 7.2</td>
</tr>
</tbody>
</table>
Simultaneous PK-PD modeling

There was an important delay between the peaking time of $C_p$ and the cardiovascular $E_{\text{max}}$ (changes in HR and DBP). When the observed effects were plotted against $C_p$ in time sequence, large counterclockwise hysteresis loops were observed (Fig. 4, A and B). After applying the simultaneous PK-PD modeling, plasma concentration of cilostazol was linked, by means of an effect compartment model with a sigmoidal $E_{\text{max}}$ model, to the observed effects. When the effects were plotted against the $C_e$, the hysteresis loop collapsed (Fig. 4, C and D). There was good agreement between the predicted and observed effects. This demonstrated that concentration-effect relationship or pharmacodynamics of cilostazol could be found by using the simultaneous PK-PD modeling.

The estimated pharmacodynamic parameters of the simultaneous PK-PD modeling by fitting the effect with plasma concentration of cilostazol were calculated. The hysteresis collapsed for optimal values of $K_{e0}$ was $0.51 \pm 0.12$ and $0.69 \pm 0.18$ h$^{-1}$ for HR and DBP, respectively. No significant differences of $K_{e0}$, $EC_{50}$, $E_{\text{max}}$, and $\gamma$ on HR and DBP were found ($P>0.05$) (Table 2).

### Table 2. Pharmacodynamic parameters of simultaneous PK-PD modeling of cilostazol after a single oral dose of 100 mg in healthy subjects, $n=20$. Mean ± SD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HR</th>
<th>DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{e0}$/h$^{-1}$</td>
<td>0.51 ± 0.12</td>
<td>0.69 ± 0.18</td>
</tr>
<tr>
<td>$E_{\text{max}}$/%</td>
<td>23.7 ± 9.5</td>
<td>38.2 ± 12.2</td>
</tr>
<tr>
<td>$EC_{50}$/µg·L$^{-1}$</td>
<td>152.4 ± 25.3</td>
<td>184.7 ± 48.6</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>6.5 ± 1.1</td>
<td>4.6 ± 0.8</td>
</tr>
</tbody>
</table>

Fig 4. Mean plasma concentrations of cilostazol versus mean cardiovascular effects (A. HR; B. DBP) showed counterclockwise hysteresis loops; When effects were plotted against the hypothetical effect compartment concentrations (C, D), the hysteresis loops collapsed. Arrow: Direction of time course.
Discussion

The objective of this study was to investigate the relationship between plasma concentration of cilostazol and its cardiovascular effects after oral administration to healthy male volunteers. To our knowledge, this is the first study to directly correlate plasma concentrations of cilostazol and changes in HR and DBP using the simultaneous PK-PD modeling techniques. Our study may help elucidate the relation between plasma concentration of cilostazol and its effects on HR and DBP.

The pharmacokinetics of cilostazol was described by a two-compartment open model with first-order absorption. No statistical differences were observed between PK parameters estimated by compartmental and noncompartmental approaches. The parameters obtained indicated that the absorption rate of cilostazol was relatively rapid compared to elimination rates. Furthermore, the apparent volume of distribution ($V_d/F=3.7 \text{ L.kg}^{-1}$) suggested extensive distribution of cilostazol in the tissues. The clearance of cilostazol ($CL/F=0.13 \text{ L.h}^{-1}.\text{kg}^{-1}$) was much lower than liver blood flow, and hence low probability of a significant first-pass effect.

In most cases, the concentration of drug in the systemic circulation will be parallel to that at the sites of action. So there is a direct link between PK and PD models. But for some drugs, there is no clear relation between pharmacological effect and plasma concentration. Many factors, such as formation of active metabolites, enzyme induction or inhibition, or delayed equilibrium in concentration between plasma and sites of action may account for the lack of correlation. Simultaneous PK-PD modeling is currently considered the preferred method of analysis, which is first elaborated by Sheiner et al. The potential applications in drug development of the modeling techniques are numerous. And we have successfully applied this modeling to another cardiovascular drug.

In present study, the maximal changes in HR and DBP were detected approximately 6 h after administration of the drug. The results showed that there existed an important time delay between plasma concentration of cilostazol and its cardiovascular effects, which can be explained by postulating that the drug moved from the plasma to the effect compartment in a first-order process. Another factor that may also contribute to the formation of a counterclockwise hysteresis loop was the two active metabolites, 3, 4-dehydro-cilostazol (4-7 times as active as cilostazol), and 4'-trans-hydroxy-cilostazol (one fifth as active as cilostazol). The effects of cilostazol on HR and DBP were satisfactorily described with a compartment model with sigmoidal $E_{max}$. Furthermore, the hysteresis loops observed in the plots of cilostazol effect versus plasma drug concentration collapsed in the effect versus effect compartment concentration plots.

In conclusion, the present study demonstrated the cardiovascular effects of cilostazol after oral administration in healthy subjects. A simultaneous PK-PD model was developed to collapse the hysteresis loop and to predict the pharmacological effect. The effect-link sigmoidal $E_{max}$ model can describe the relation between the effect compartment concentrations and the cardiovascular effects of cilostazol. It can provide valuable information on dose-effect relationships and on a choice of optimal dosing intervals in drug development.

References


