Pharm Res (2013) 30:1110–1122
DOI 10.1007/s11095-012-0947-6

RESEARCH PAPER

Joint Population Pharmacokinetic/Pharmacodynamic Model for the Heart Rate Effects at Rest and at the End of Exercise for Cilobradine

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Received: 2 August 2012 / Accepted: 20 November 2012 / Published online: 11 December 2012

ABSTRACT

Purpose To develop a semi-mechanistic population pharmacokinetic/pharmacodynamic (PKPD) model for the selective bradycardic agent cilobradine describing simultaneously the heart rate (HR) measured at rest and just after the end of exercise sharing the same set of PKPD parameters.

Methods Healthy subjects received cilobradine orally once daily over 2 weeks at 0.25–5 mg doses or placebo. Plasma drug concentrations and HR were measured at rest and following 3 min of exercise over the entire study period. PK and HR data were analyzed using the population approach with NONMEM VII.

Results Plasma disposition of cilobradine was described with a three compartment model. Cilobradine showed dose proportional and time independent pharmacokinetics. HR response was drug concentration dependent and appeared with a significant delay with respect to PK profiles, a phenomenon modeled using two transit compartments. Perturbation in HR at rest as a consequence of exercise was described assuming that physiological processes controlling cardiac frequency were constantly increased over the period of exercise only.

Conclusions The selected model provides a useful modeling tool for cases where the PD response measured is the result of a temporal experimental induced perturbation.

KEY WORDS bi-variate response modeling · cilobradine · heart rate · NONMEM · population PKPD

ABBREVIATIONS

CIRC	circadian variation
HR	heart rate
k_{S_HR} and k_{D_HR}	zero order and first order rate constants representing heart rate generation and dissipation processes, respectively
k_{TR}	rate constant controlling the turnover kinetics of the signal transit compartments
NPC	numerical predictive check
PKPD	pharmacokinetic/pharmacodynamic
PLCB	placebo effect
SBA	selective bradycardic agent
TR_1 and TR_2	signal transit compartments
VPC	visual predictive check
θ_{wkl}	parameter scaling the workload effect on k_{S_HR}

Electronic supplementary material The online version of this article (doi:10.1007/s11095-012-0947-6) contains supplementary material, which is available to authorized users.

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INTRODUCTION

Chronically increased heart rate (HR) is a risk factor for mortality and morbidity with adverse consequences on vascular function, atherogenesis, myocardial ischemia, myocardial energetics and left ventricular function (1).

Selective bradycardic agents (SBAs) are direct sinus node inhibitors. In contrast to β-blockers and verapamil-type calcium antagonists, they have demonstrated negative chronotropic but not negative inotropic effects. These substances, therefore, may possess desirable therapeutic properties for the treatment of various cardiac disease states e.g. heart failure. SBAs act through inhibition of the hyperpolarization activated, cyclic nucleotide dependent channel (HCN). By blocking the HCN, the hyperpolarization-activated current I_f (in sino-atrial tissue) is inhibited. As a consequence, the frequency of spontaneous depolarizations of the sino-atrial pacemaker cell is slowed (2,3). SBAs represent a promising therapy option when the most common used agents such as β-blockers or calcium channel blockers are contraindicated (4,5).

During early clinical development of bradycardic agents, such as I_f channel blockers, HR measured at rest conditions and at the end of a short period of induced exercise are the surrogate endpoints to characterize the pharmacodynamics of the new compounds (4). So far, the relationship between drug concentrations in plasma and HR has been described using an effect compartment model (6), and HR at rest and HR at the end of exercise were fitted separately, thus providing two different sets of pharmacodynamic (PD) parameters for the two situations (7,8).

In the current work, a population pharmacokinetic/pharmacodynamic (PKPD) model has been developed for the HR effects of cilobradine, an I_f blocker, for which only limited information about its PKPD characteristics in humans is available. The population PK characteristics of cilobradine have been previously published after single and multiple dosing where cilobradine was administered as solution, capsule or intravenous infusion (9).

The aim of the current work was to characterize the relationship between cilobradine plasma concentrations and HR by integrating drug related and exercise effects on HR into one model. This approach allowed the estimation of a joint set of system- and drug-dependent parameters for both types of responses (HR measured at rest and HR measured just at the end of the exercise). The selected model provides a useful modeling tool for cases where the PD response measured is the result of a temporal experimental induced perturbation.

MATERIALS AND METHODS

Study Design and Subject Population

This study was a randomized, partly double-blind (see below), placebo-controlled, parallel-group phase I study performed in healthy volunteers. All participants provided written informed consent consistent with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use—Good Clinical Practice (ICH-GCP) and local legislation, once the nature and the intention of the investigation were fully explained. The studies were performed in accordance with the Declaration of Helsinki and were approved by the independent IEC committee. *Inclusion criteria*: healthy volunteers (n=88 males, n=8 females) with an age between 21 and 55 years, a body mass index (BMI) ranging from 19.9 to 29.9 kg/m^2 and a resting HR (after 10 min. in the supine position) of more than 55 beats per minute (bpm). *Main exclusion criteria*: Any finding of the medical examination (including blood pressure [BP], HR and electrocardiogram [ECG]) deviating from normal and of clinical relevance; gastrointestinal, hepatic, renal, respiratory, cardiovascular, metabolic, immunological, hormonal, psychiatric disorders, neurological disorders; relevant ophthalmological disease; history of relevant orthostatic hypotension, fainting spells or blackouts; history of asthma or obstructive pulmonary disease; smoker and inability to refrain from smoking on trial days; alcohol abuse and/or drug abuse; consumption of more than 2 cups of coffee or black tea, or cola drinks, per day during the last 6 weeks; blood donation (≥100 ml within 4 weeks prior to administration or during the trial); excessive physical activities (within the last week before the study); and participation in another trial with an investigational drug (≤2 months prior to administration or during the trial).

Drug Administration

Subjects were randomized to receive either a placebo solution (n=20), or a cilobradine solution with doses of 0.25 (n=12), 0.5 (n=12), 1 (n=18), 2 (n=16), and 5 mg (n=18) given once daily orally for a period of 14 days. Placebo and cilobradine treatments were administered in a double-blind fashion (0.25 mg, 0.5 mg, 1.0 mg or 2.0 mg cilobradine, or placebo once daily). The dose group cilobradine 5.0 mg once daily, which was introduced per amendment, was blinded against placebo but volunteers and investigators knew that it was the 5.0 mg cohort. Placebo and cilobradine were administered as a 100 mL aqueous solution 30 min before breakfast. Immediately following cilobradine administration, subjects were asked to drink 50 mL of water.

Sample Collection and Determination of Cilobradine Concentrations in Plasma

Blood samples (9 mL) were taken from a forearm vein at the following times: pre-dose (1 h before administration), 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after drug administration during the first day of treatment; pre-dose on days 2, 3, 4, 6, and 9 of treatment, and pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, and 144 h after drug administration during the last day of treatment. Blood samples for PK analyses were centrifuged immediately after collection for 10 min at 3000 g at room temperature. Two aliquots of plasma samples were obtained. One of those aliquots contained at least 2.0 mL of plasma. Concentrations of cilobradine in plasma were quantified by high performance liquid chromatography using reversed phase and fluorescence detection (excitation at 280 nm, emission at 320 nm). Due to the lower limit of quantification (LLOQ) of 1.5 ng/mL, plasma samples taken at late points after administration were quantified by a competitive enzyme linked-immunosorbent-assay (ELISA) with a LLOQ of 0.1 ng/mL (9).

Heart Rate at Rest

HR at rest measurements were measured at the following times: pre-dose (1 h before administration), 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after drug administration during the first day of treatment; pre-dose on days 2 to 13 of treatment, and pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, and 144 h after drug administration at the last day of treatment. Resting HR was measured after 10 min in the supine position by pulse palpation and counting for 1 min before start of the exercise. Resting pulse was considered equal to HR.

Heart Rate at Exercise

HR at exercise was monitored immediately at the end of exercise at the following times: pre-dose (1 h before administration), 3, 7, 10, and 24 h after drug administration during the first day of treatment; pre-dose on days 2, 3, 6, and 9 of treatment, and pre-dose, 3, 7, 10, 24, 48, 72, and 144 h after drug administration during the last day of treatment.

Screening Day

Volunteers performed a bicycle exercise test for 3 min using a ERG 900 Fahrrad-Ergometer (GE Medical Systems; Information Technologies (Germany)) to determine the individual workload. The bicycle exercise test system was connected to a 12-lead ECG monitoring system (MAC 5000 Ruhe-EKG; GE Medical Systems; Information Technologies (Germany)). The initial workload was chosen according to the individual muscular and training status. Subsequently, the workload was adapted in steps of 25 or 50 W in order to approach the individual target HR of $0.8 \times [200 - \text{age (years)}]$ bpm. Fine-tuning of the workload required for this HR was achieved through adaptation of the duration of the exercise. Volunteers were at rest for approximately 1 h between each exercise test. At the end of the screening day, exact workload and duration of exercise (between 1 and 3 min) necessary to reach the target HR ($\pm$ 10 bpm) of each individual was documented as screening workload and duration.

Treatment Days

At baseline of day 1, volunteers performed the exercise at the screening workload. However, the bicycle exercise was stopped at exactly the time-point where the target HR ($\pm$ 10 bpm) was reached (which might be somewhat longer, or shorter, than that defined in the screening workload and duration). The new exercise workload and duration was documented as baseline workload and duration. All exercise tests during the course of the study were performed exactly with the same workload and duration as the baseline exercise.

Data Analysis

The population approach using the First Order Conditional Estimation method with the INTERACTION option implemented in the software NONMEM version VII (Icon Development Solutions, Hanover, Maryland) was used to analyze the plasma drug concentration and HR data. The population analysis was performed sequentially, first the PK model was developed, the response data were described afterwards using the individual predicted concentration *versus* time profiles. Inter-subject and inter-occasion variability, ISV and IOV (10), respectively, were modeled using exponential functions. A combined model was first used to describe residual variability, then if one of the elements of the model (the additive or the concentration/response dependent parts) was found to be negligible it was removed from the residual error model.

Model Selection

Selection between models was based mainly on the visual inspection of goodness of fit plots. The minimum value of the objective function provided by NONMEM which is approximately equal to -2xlog likelihood (-2LL), served as a guide during model building. For two nested models a decrease in -2LL of 6.63 or 10.83 points for one additional parameter was regarded as a significant improvement of the model corresponding to p-values of 0.01 and 0.001, respectively. For non-nested models the Akaike information criteria (AIC) was used (11). Initial parameter estimates for the different models explored were approximated using the System Dynamics framework that

combines the power of differential equations with a graphical design and representation of the variables. The VENSIM software (Ventana Systems, Inc., MA, United States.) was used for seeking plausible initial parameter estimates. This software automatically performs a visual sensitivity analysis by simulations instead of a time consuming approach involving changing and testing of different initial estimates.

Pharmacokinetic Modeling

Disposition of cilobradine in plasma was described with compartmental models parameterized in apparent volumes of distribution, inter-compartmental clearances, and total elimination clearance. Selection was made between one-, two-, and three-compartment models. To best characterize the absorption process, first and zero order rate processes, and transit compartment models (12) were investigated. Dose and time dependent pharmacokinetics were explored testing dose and time (day 1 and day 14) as covariates on the PK parameters.

Pharmacokinetic/Pharmacodynamic Modeling of the HR Effects

A model in which HR measurements obtained at rest and at the end of exercise were described simultaneously, sharing the same set of parameters, was developed in the following steps.

Step 1: Baseline and Placebo Models. To develop the baseline and placebo models the data obtained from the placebo group were used. Equation 1 represents an indirect response model (13) where the rate of change of HR at rest is described as a balance between processes that promote HR, represented by the zero order rate constant k_{S_HR}, and processes that degrade HR, represented by the first order rate constant k_{D_HR}. At steady state dHR/dt=0 and $k_{S\_HR}=k_{D\_HR} \times HR_0$, where HR_0 represents HR at baseline.

$$\frac{dHR}{dt} = k_{S\_HR} - k_{D\_HR} \times HR \quad (1)$$

HR measured just at the end of exercise was modeled as a transient increase in k_{S_HR} as follows (Eq. 2):

$$\frac{dHR}{dt} = k_{S\_HR} \times [1 + \theta_{WKL} \times WKL] - k_{D\_HR} \times HR \quad (2)$$

where WKL has a value equal to the individual documented baseline workload during exercise [mean=140 W, standard deviation=34.5], and 0 otherwise. Duration of exercise for each subject was also available. θ_{WKL} is a model parameter scaling the WKL effects on k_{s_HR}.

Different models were explored to evaluate the presence of placebo effects. Placebo effect models (PLCB(t) in Eq. 3) consisted of linear or non-linear (i.e, E_{MAX} type, biexponential) functions of the time after start of treatment or the time after the last administration before a measurement.

$$\frac{dHR}{dt} = k_{S\_HR} \times [1 + \theta_{WKL} \times WKL] \times PLCB(t) - k_{D\_HR} \times HR \quad (3)$$

The presence of circadian variations in the HR response was studied incorporating a periodic function CIRC(t) in the model as it is represented in Eq. 4.

$$\frac{dHR}{dt} = k_{S\_HR} \times [1 + \theta_{WKL} \times WKL] \times PLCB(t) \times CIRC(t) - k_{D\_HR} \times HR \quad (4)$$

Step 2: Drug Effects Model. Data obtained under placebo and active treatments were used to develop the PKPD model including drug effects. The model established in step 1 was used at this stage but its parameters were re-estimated.

Based on results published previously in the literature where the effects of ivabradine were described with the use of an effect compartment (7), the same approach was initially followed. During the current analysis, the equilibration half-life between the plasma and the effect site compartment was determined to be 47.14 h, a value that was considered too large to truly represent a delay in distribution between these compartments. Therefore, despite good model performance and precise parameter estimates, it was assumed that cilobradine exerted its effects indirectly through a set of signal transit compartments (TR_1 and TR_2) as represented by the following set of ordinary differential equations:

$$\frac{dTR_1}{dt} = k_{TR} \times \left[1 - I_{max} \times \frac{C_P^n}{IC_{50}^n + C_P^n}\right] - k_{TR} \times TR_1 \quad (5)$$

$$\frac{dTR_2}{dt} = k_{TR} \times TR_1 - k_{TR} \times TR_2 \quad (6)$$

where k_{TR} represents the rate constant controlling the turnover kinetics of the signal transit compartments, I_{MAX} is the maximum attainable drug effect constrained between 0 and 1, IC_{50} is the value of C_P, the predicted drug concentration in plasma, that elicits an effect equal to half of I_{MAX}, and n is the hill coefficient. Before drug administration (initial condition) $TR_1(t=0)=TR_2(t=0)=1$. During model building different numbers of signal transit compartments were tested as well as different models for C_P effects (e.g. linear or E_{MAX}).

Finally, the induced drug effects were integrated in the HR model as represented by Eq. 7.

$$\frac{dHR}{dt} = k_{S\_HR} \times [1 + \theta_{WKL} \times WKL] \times PLCB(t) \times CIRC(t) \times TR_2 - k_{D\_HR} \times HR \quad (7)$$

Figure 1 shows the schematic representation of the PKPD model developed for the HR effects of cilobradine in healthy volunteers.

Covariate Selection

Once the model was developed describing the PK and PKPD characteristics of cilobradine, selected covariates were tested for their impact on the model parameters. Covariates listed in Table I and additionally dose and time were first tested individually on each model parameter with inter-subject variability. A full model was developed by combining the covariates individually identified as significant ($p<0.01$). Once the full model was established, the significance of the potential covariates was evaluated using a backward selection (model reduction) technique. Beginning with the full model, each covariate was individually removed and its effect on the -2LL was evaluated. The least significant covariate not resulting in an increase ≥10.83 points ($p<0.001$) was dropped. The reduced model was then subjected to the same reduction technique and criterion. This was repeated until all remaining covariates were statistically significant. It has to be taken into account that this study was performed in healthy volunteers and therefore the range of the covariate values was narrower than in a patient population.

Model Evaluation

Precision of parameter estimates expressed as 2.5th to 97.5th percentiles were computed from the analysis of 100 bootstrap data sets. The bootstrap analysis was performed using Perl-speaks-NONMEM (14). Model performance was evaluated with visual and numerical predictive checks (referred to as VPC and NPC). For the VPCs and NPCs, 200 simulated studies with the same design characteristics as the original study were generated. VPC: At each time point with a measurement the 2.5, 50 and 97.5th percentiles were calculated in every simulated study. The 95% prediction interval from the resultant 50th percentiles was computed and represented over time together with the raw data. The 50th percentiles of the resultant 2.5 and 97.5th percentiles were also plotted. NPC: For each simulated dataset and dose group the median of the following descriptors were calculated: maximum concentration of cilobradine in plasma after the first and last dose, C_{MAX1st}, $C_{MAX14th}$, respectively, the area under the concentration *versus* time curve for 0–24 h after the first and last dose of treatment, AUC_{1st}, AUC_{14th}, respectively, HR_0, minimum HR (HR_{min}) and maximum HR (HR_{max}) achieved after treatment was stopped. Those three last descriptors were calculated both at rest and at the end of the exercise. Then, the 2.5, 50, and 97.5th percentiles of the overall 50th percentile distribution were computed, and compared with the 50th percentiles obtained from the raw data. Predictive checks were performed using MATLAB environment (The Mathworks, MA, United States).

Evaluation of the Covariate Effects

The impact of BMI on the cilobradine plasma concentration profiles and on HR dynamics was explored simulating 1,000 subjects receiving 5 mg of cilobradine orally once daily for 14 days. Individual BMI values were randomly generated from a distribution with the same mean and variance as the original data. The impact of the AGE covariate on HR dynamics was

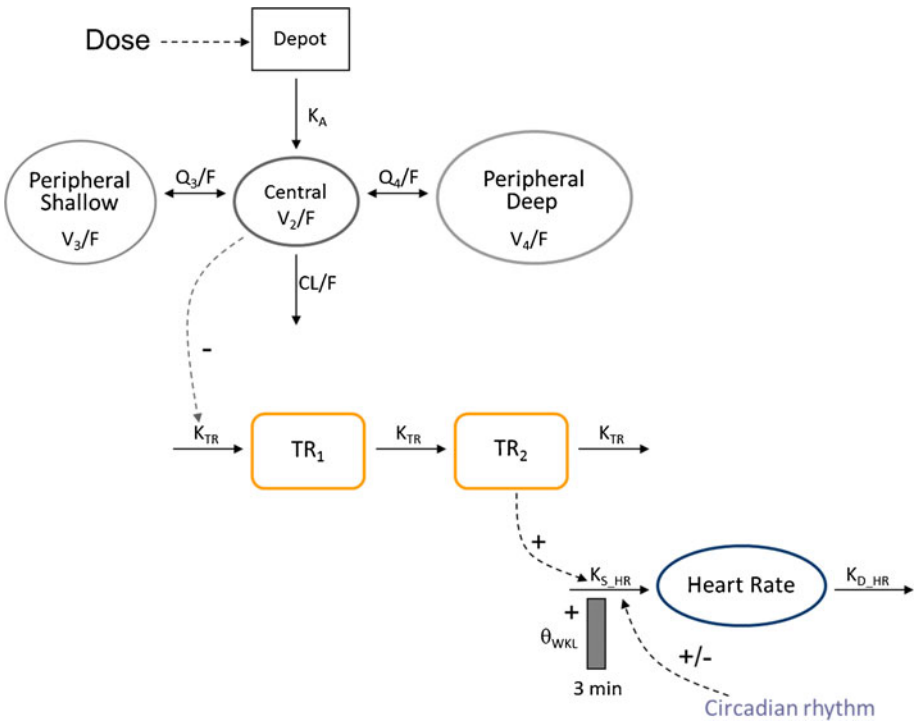

Fig. 1 Schematic representation of the selected PKPD model. PK: K_A first order rate constant of absorption; V_2/F, V_3/F, and V_4/F apparent volumes of distribution of the central and the peripheral compartments, respectively; CL/F apparent total plasma clearance; Q_3/F and Q_4/F inter-compartmental distribution clearances. PKPD: k_{S_HR} and k_{D_HR}, zero order and first order rate constants representing heart rate generation and dissipation processes, respectively; θ_{WKL} scales the workload effect on k_{S_HR}. TR_1 and TR_2, transit compartments , k_{TR} represents the rate constant controlling the turnover kinetics of the signal transit compartments; C_P, predicted cilobradine plasma concentration.

Table I Summary of Subject Characteristics

Covariate		
Continuous	Mean (CV(%))	Range
Age (year)	34 (26)	22–55
Height (cm)	179 (4.3)	157–196
Weight (kg)	80.6 (12)	58–104
Body Mass Index (kg/m^2)	25.2 (9)	21–29.4
Body Surface Area (m^2)	1.97 (7.4)	1.6–2.3
Serum Creatinine (mg/dl)	0.97 (11)	0.8–1.2
Non-continuous	Category	Percentage
Sex	Male	92
	Female	8
Smoking status	Non smoker	48
	Former smoker	11
	Current smoker	41
Alcohol consumption	Never	6
	Average	94

CV coefficient of variation

explored simulating 1,000 subjects receiving placebo orally once daily for 14 days. AGE values were randomly generated from a distribution with the same mean and variance as the original data.

RESULTS

Pharmacokinetic Modeling

Disposition of cilobradine in plasma was best described by a three compartment open model. The use of simpler models (i.e., one or two compartments models) resulted in significantly worse fits ($p<0.01$). Drug absorption was described by a first order rate constant. Latency in the absorption process was not supported by the data. Inter-subject variability was found to be significant ($p<0.01$) for the apparent total plasma clearance (CL/F), apparent volume of distribution in the central compartment (V_2/F), first order rate constant of absorption (K_A), and bioavailability (F) for which the typical value was fixed to 1. Covariance between random effects associated to K_A and F, and between CL/F and F were significant ($p<0.01$). Data did not support to account for significant inter-occasion variability in drug absorption and disposition ($p>0.05$). BMI was the only covariate that showed statistical significant effects on CL/F ($p<0.01$). None of the PK parameter was time or dose dependent ($p>0.05$). It has to be taken into account that only a few females were included in the study, and therefore the effect of sex could not be studied in detail (Table I).

Table II lists the parameter estimates obtained from the final population PK model together with the corresponding 95% confidence intervals (CI) which did not include the zero value for any parameter. η-shrinkage (%) was 6.8 (CL/F), 30.3 (V_2/F), 16.9 (K_A), and 2.0 (F), ε-shrinkage was 5.9%. Figure 2 shows the results from the visual predictive check corresponding to the first and last day of the treatment. Both typical profiles and data dispersion were captured very well. Supplementary Material Table SI shows the result from the numerical predictive check. The 50th percentiles of the four descriptors (C_{MAX} and AUC for the first and last day of treatment) from the raw data are similar to the 50th percentiles of the simulated 50th percentiles and are well contained within the 2.5th–97.5th intervals of the simulated 50th percentiles.

For healthy volunteers with BMI equal to the median value of the studied population (25.5 kg/m^2; range 21–29.4 kg/m^2), cilobradine showed a typical value of CL/F of 54.5 Lxh^{-1}. Figure 3 shows the impact of the covariate effect of BMI on the cilobradine plasma concentration profiles. C_{MAX} and AUC decreased by 17.5 and 27.2% between the high (BMI ≥80th) and the low (BMI ≤20th) intervals.

Pharmacokinetic/Pharmacodynamic Modeling

During the development of the PKPD model it was found that (i) a placebo effect [PLCB(t) in Eqs. 3 and 4] was negligible ($p>0.05$), (ii) the Hill coefficient (referred as n, Eq. 5) in the drug effect relationship was not significantly different from one ($p>0.05$), and (iii) increasing the number of transit (TR) compartments did not lead to better fits. As expected, the I_{MAX} value (Eq. 5) was found to be significantly smaller than 1 ($p<0.01$) which indicates that cilobradine cannot reduce HR to zero.

Age was the only subject characteristic that showed a significant effect in the PD model. Age was incorporated in the model exerting a negative linear effect on HR_0 ($p<0.01$). The use of the periodic function shown in Eq. 8, as representation of CIRC(t) in Eq. 4, significantly decreased −2LL by 18.76 points ($p<0.01$), however as will be shown latter, the relevance of the predicted diurnal variation resulted negligible.

$$\mathrm{CIRC}(t) = e^{A\times\sin\left(\frac{2\Pi}{T}\times t\right)}$$

A corresponds to the amplitude and T to the period. Inter-subject variability was found significant only for HR_0, θ_{WKL} and IC_{50} ($p<0.01$). Table III lists the parameter estimates corresponding to the selected PKPD model, together with the corresponding 95% confidence interval obtained from the nonparametric bootstrap analysis. None of the confidence intervals included zero. η-shrinkage (%) was 0.22 (HR_0), 0.16 (θ_{WKL}), and 29.3 (IC_{50}), ε-shrinkage was 2.2%.

Figure 4a and b show the results from the visual predictive check corresponding to the first 24 h after the first dose, and

Table II Population Pharmacokinetic Parameter Estimates

Parameters	Estimate (2.5-97.5th)	ISV (CV%)	Estimate (2.5-97.5th)
$CL/F = \theta_{CL} * [1 + \theta_{BMI} * (BMI - 25.5)] (Lxh^{-1})$	θ_{CL} = 54.5(50.46 57.95) θ_{BMI} = -0.0432 (-0.0709 -0.017)	ISV_CL	28.58 (23.03 33.93)
V_2/F (L)	26.4 (22.49 27.94)	ISV_V_2	35.91 (13.32 48.10)
K_A (h^{-1})	0.398 (0.360 0.417)	ISV_K_A	14.52 (10.22 19.31)
Q_3 / F (Lxh^{-1})	22.5 (19.06 25.17)		
V_3 / F (L)	155 (136.42 171.28)		
Q_4 / F (Lxh^{-1})	2.94 (2.32 3.72)		
V_4 / F (L)	257 (204.60 326.10)		
F	1 (not estimated)	ISV_F	43.24 (37.73 49.30)
Covariance(ω^2_{KA}, ω^2_F)	-0.0228^{*} (−0.0392 −0.0069)		
Covariance (ω^2_F, ω^2_{CL})	0.076^{**} (0.049 0.113)		
Proportional residual error (%)	19.5 (17.6 20.9)		

Parameters are listed as estimates with 95% confidence intervals from 100 bootstrap datasets in parenthesis. CL/F, total apparent plasma clearance; BMI, body mass index; V_2, V_3 and V_4, apparent volumes of distribution of the central, shallow peripheral, and deep peripheral compartments, respectively; K_A, first order rate constant of absorption; Q_2/F and Q_3/F inter-compartmental distribution clearances between the central and shallow, and between the central and deep compartments, respectively; F, oral bioavailability; ω^2, variance. Correlation coefficients of -0.36^{*}, and 0.61^{**}. Estimates of inter-subject variability (ISV) are shown as coefficient of variation.

the 0–144 h period after the last administration on day 14. Typical HR profiles and the data dispersion were captured very well for every dose group and for both situations at rest and at the end of exercise. HR values through over the 2 weeks period were also well described (data not shown). Supplementary Material Table SII shows the result from the numerical predictive check. For all doses the 50th percentiles of the three descriptors (HR_0, HR_{min}, and HR_{max} calculated at rest and at the end of exercise) from the raw data are well within the 2.5th–97.5th intervals of the 50th percentiles obtained from the simulated studies.

Figure 5 shows the impact of the selected covariates, BMI and AGE, on HR dynamics. The impact of the two selected covariates on HR is negligible.

Figure 6 describes the main features of the model developed in the current study. Figure 6a shows the pharmacokinetic profiles for the 2 mg and 5 mg dose groups, during the whole study period. Figure 6b shows the relationship between C_P and the drug effect on k_{S_HR}, identifying the typical maximum effect reached at the end of the study at the doses of 2 mg and 5 mg. Figure 6c shows the dynamics within the TR_1 and TR_2 compartments during the complete study for the dose of 5 mg. The (marginal) impact of the estimated circadian rhythm on the HR dynamics at rest is represented in Fig. 6d. Figure 6e shows the HR dynamics measured at rest during the entire study period for the doses of 2 mg and 5 mg. The value of the parameter representing the median signal transition time and calculated as $n+1/K_{TR}$, where n is the number of transit compartments, is 83.3 h which predicts that steady-state response HR conditions will be achieved after 21 days of continuous treatment. Figure 6f shows the predicted increase in HR during 3 min of constant workload, and how the individual returns to HR_0 in approximately 3 min after the exercise. The simulation was performed at 12 h after the last 5 mg administration. When a constant workload is induced during 3 min, the maximum HR is achieved during this period. The upper limit does not depend on the duration of the exercise period, i.e. if the exercise period lasted more than 3 min but with the same intensity, the HR would not exceed the maximum HR (Supplementary Material Fig. S1a). However, as Supplementary Material Fig. S1b) shows, if the exercise intensity does not remain constant, the maximum will be increased as the intensity of the exercise increases. The upper limit in HR also depends on the plasma concentrations of cilobradine, being lower at higher plasma concentrations (Supplementary Material Fig. S1c).

A strong negative covariance between random effects associated to HR_0 and θ_{WKL} indicated by a decrease in −2LL of 210.11 points was also identified during the analysis ($p<0.01$).

DISCUSSION

Conventional HR control agents, such as β-blockers or calcium channel blockers, have contributed greatly to the management of various diseases where HR reduction was required. However, there are occasions when these compounds are not indicated, i.e. β-blocker intolerance (4) or weak effect (6). At the end of the eighties, a new class of potential pharmaceuticals compounds emerged which blocks the I_f transmembrane current. These selective bradycardic agents reduce dose dependent the amplitude of the I_f, without modifying either the voltage dependence or the kinetics of channel activation (2).

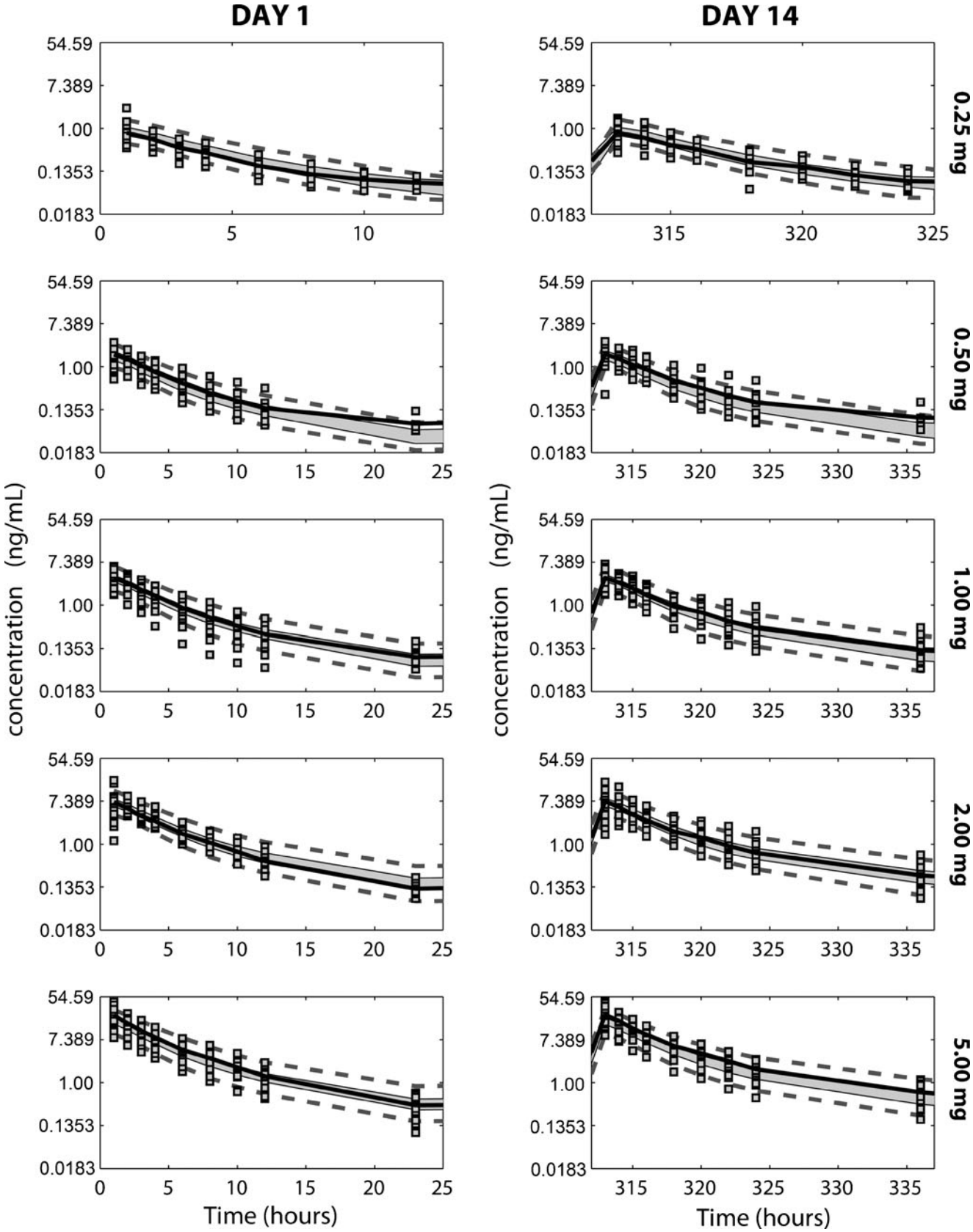

Fig. 2 PK model. Visual Predictive Check. Results from 200 simulated studies. *Grey areas* correspond to the 95% prediction interval of the median. *Dashed lines* represent the median predictions of the 2.5th and 97.5th percentiles, respectively. *Solid lines* represent the median raw data profiles. Points, raw data.

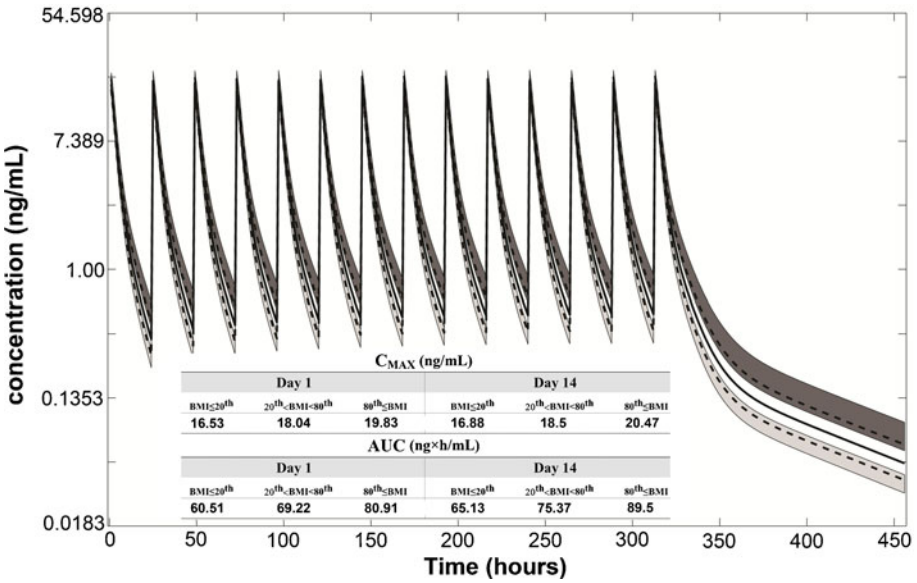

Fig. 3 Evaluation of the body mass index (BMI) covariate effects on the PK profiles. *Colored areas* describe the PK profiles when the simulated individuals have BMI values smaller than the 20th (*light grey*) and above the 80th (*dark grey*) percentiles of the studied population. *Dashed lines* correspond to the median profiles in each of the mentioned percentiles. *Solid line in black* represents the overall median simulated PK profile. The figure also reports the 50th percentiles of each of the four descriptors calculated for each range of BMI.

In our study, a population PKPD model has been developed for the HR effects of cilobradine, an I_f channel blocker, after multiple oral administrations at doses ranging from 0.25 to 5 mg. The model is not only able to accurately predict the typical response observations obtained from every dose group, but also to reproduce the data dispersion in the data as it is shown in Figs. 2 and 4. Its main contribution resides in the way the two response variables, HR measured at rest and just after the end of exercise, were analysed simultaneously sharing the same model and set of parameters. Moreover, given the fact that the available information regarding PK and PD characteristics of cilobradine is very sparse, the results from the developed model increase the understanding of the *in vivo* effects of this drug and others I_f channel blockers.

The population PK characteristics of cilobradine have been recently published after single or multiple doses of cilobradine administered as solution, capsule or infusion (9). The current model and its parameter estimates are in accordance to the previous work. For example the typical values of K_A, CL/F and V/F, the total apparent volume of distribution computed as the sum of V_2/F, V_3/F, and V_4/F are very similar: 0.398 h^{-1}, 54.5 Lxh^{-1}, and 438 L (current) *versus* 0.408 h^{-1}, 55 Lxh^{-1} and 431.55 L (9). Magnitudes of the inter-subject variability were also similar between the two analyses.

Table III Population Pharmacokinetic/Pharmacodynamic Parameter Estimates

Parameters	Estimate (2.5-97.5th)	ISV (CV%)	Estimate (2.5-97.5th)
HR_0 (bpm)	65.4 (65.1 - 65.6)	ISV_HR_{basal}	11.1 (9.5 - 12.7)
K_{TR} (h^{-1})	0.036 (0.033 - 0.38)		
k_{D_HR} (h^{-1})	168 (165.4 -169.0)		
θ_{WKL} ($Watts^{-1}$)	6.9×10^{-3} (6.7×10^{-3}–7×10^{-3})	ISV_k_{WKL}	23.5 (19.5 - 26.4)
I_{max} (unitless)	0.84 (0.83 - 0.85)		
IC_{50} (ng/mL)	11.1 (10.4 - 11.4)	ISV_IC_{50}	57.8 (43.6 - 69.9)
A (unitless)	0.0055 (0.0050 - 0.0059)		
T (h)	14.9 (13.1 - 15.0)		
$HR = HR_0 \times (1 + \theta_{AGE} \times (AGE - 34))$	$\theta_{AGE} = -4.4 \times 10^{-3}\left(-5.1 \times 10^{-3} - 3.5 \times 10^{-3}\right)$		
Covariance (ω^2_{HR0}, $\omega^2_{\theta wk1}$)	-0.025* (-0.032 − −0.017)		
Additive residual error (bpm)	4.6 (4.4 - 4.9)		

Parameters are listed as estimates with 95% confidence intervals from 100 bootstrap datasets in parenthesis. K_{TR} rate constant controlling the turnover kinetics of the signal transit compartments; HR_0, heart rate at baseline; k_{D_HR}, first order rate constants representing heart rate dissipation processes; θ_{WKL} parameter scaling the workload effect on k_{S_HR}; I_{MAX}, maximum attainable drug effect constrained between 0 and 1; IC_{50}, value of C_P the predicted drug concentration in plasma, that elicits an effect equal to half of I_{MAX}; A, amplitude; T, period; bpm, beats per minute. ω^2, variance. Correlation coefficients of -0.96^*. Estimates of inter-subject variability (ISV) are shown as coefficient of variation.

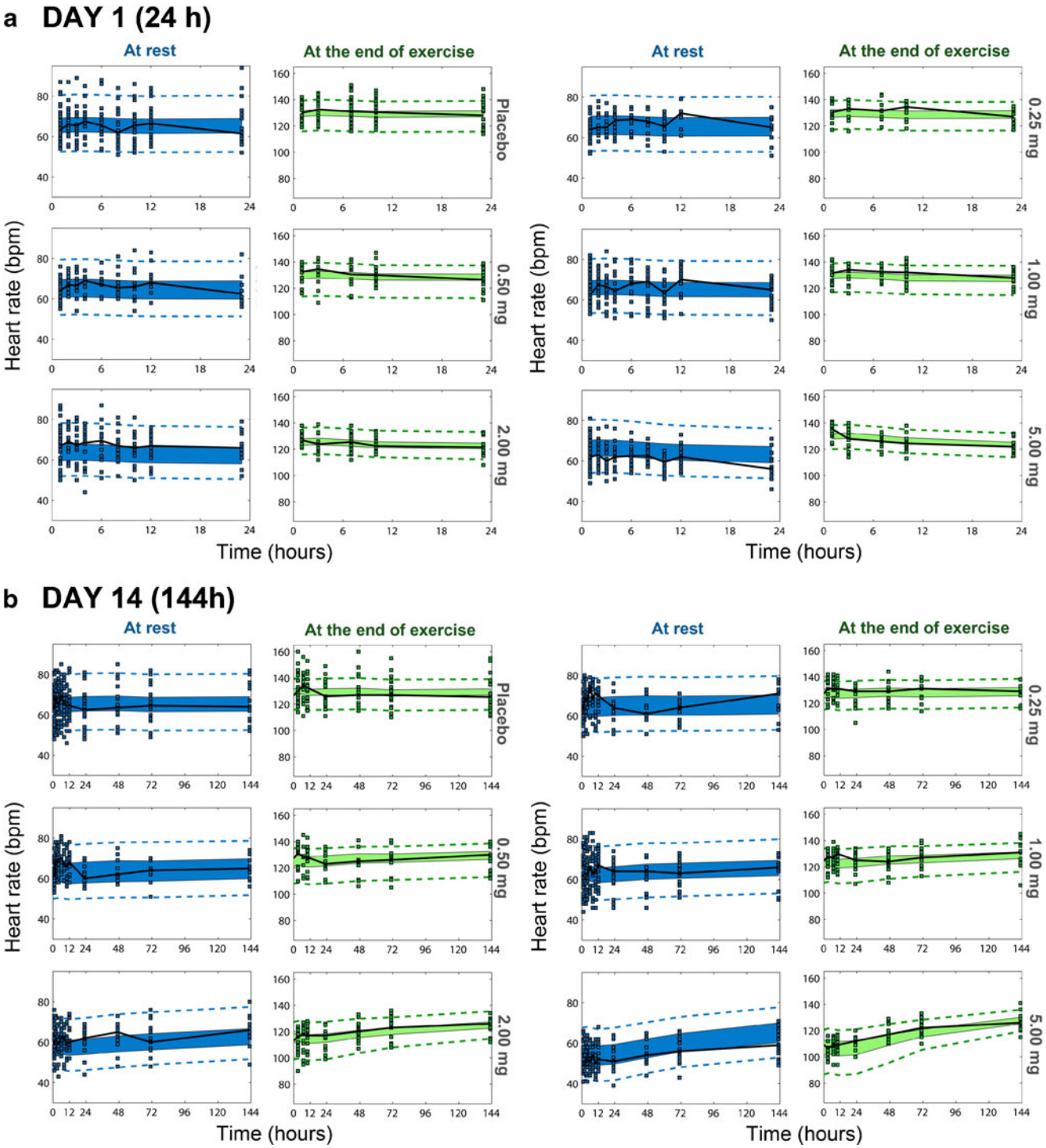

Fig. 4 PKPD model. Visual Predictive Check. Results from 200 simulated studies. *Colored areas* represent the 95% prediction interval for the median. *Dashed lines* represent the median prediction of the 2.5th and 97.5th percentiles respectively. Solid lines represent the median raw data profiles. Points, raw data. HR at rest (*blue*) and HR just at the end of exercise (*green*) are shown during the 24 h after drug administration during the first day of treatment (**a**) and during the 0–144 h after drug administration at the last day of treatment (**b**).

The main innovative features of the PKPD model are the delayed drug-induced response described with a system of two transit compartments and the perturbation model accounting for increase in HR induced by the workload, which can be considered as a general approach in analyzing data during and after experimental induced perturbation. Ragueneau and colleagues used an effect compartment to describe the contribution of ivabradine and its metabolite to the reduction in HR (7). The estimates of k_{e0} for the parent drug and the metabolite were 0.066 h^{-1} and 0.44 h^{-1}, respectively after oral administration.

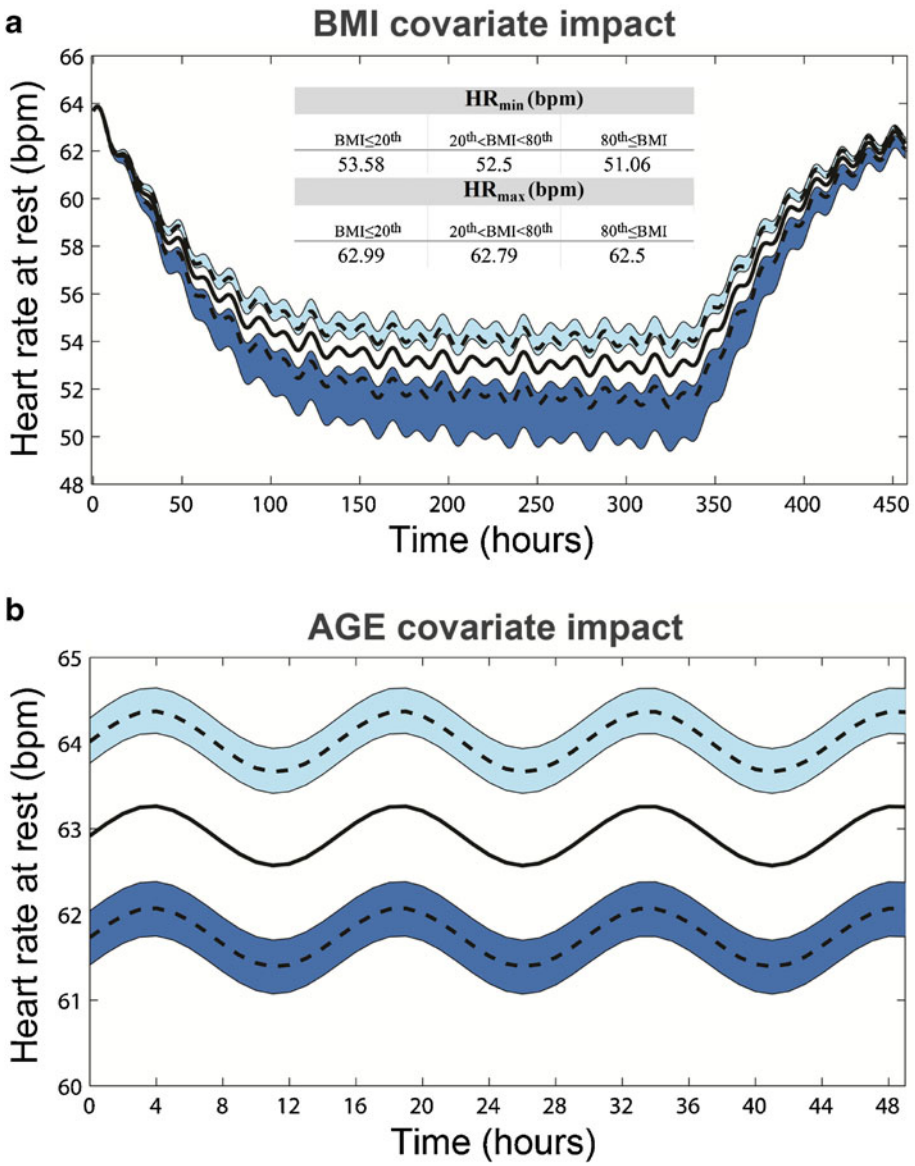

Fig. 5 Evaluation of Body Mass Index (BMI) and AGE covariate effects on the PD profiles. (**a**) BMI covariate effects. Effect of BMI on HR dynamic using 1,000 simulated subjects receiving 5 mg of cilobradine orally once daily for 14 days. BMIs were randomly generated from a normal distribution with same mean and variance as the original data, and using the typical population PKPD estimates. *Shadowed areas* represent the PD profiles at rest when the simulated individuals have BMI values smaller than the 20th percentile (*dark blue*) and higher than the 80th percentile (*light blue*). *Dashed lines* correspond to the median in each of the mentioned percentiles. *Solid black line* is the overall median simulated PD profile. (**b**) AGE covariate effects. Effect of AGE covariate on HR dynamic using 1,000 simulated subjects receiving placebo orally once daily for 14 days. AGE values were randomly generated from a normal distribution with same mean and variance as the original data, and using the typical population PKPD estimates. Shadowed areas represent the PD profiles at rest when the simulated individuals have AGE values smaller than the 20th percentile (*dark blue*) and higher than the 80th percentile (*light blue*). *Dashed lines* correspond to the median in each of the mentioned percentiles. *Solid black line* is the overall median simulated PD profile.

The corresponding values of $t_{1/2ke0}$ were 10.50 h and 1.57 h, although shorter than the one estimated for cilobradine (41.7 h), they are still long for a drug acting in a well-perfused organ such as the heart. Despite the effect compartment model can be used empirically to describe a delayed response regardless of the true mechanism of such a delay, the use of the indirect response framework based on a chain of transit (delay) compartments was considered more physiologic for the current analysis.

Perturbation in baseline HR as a consequence of the workload was included in the model assuming that physiological processes dealing with the generation of cardiac frequency are increased constantly over the period of the exercise only. This implies that the addition of just one extra parameter in the model (with inter-subject variability) is sufficient and there was no need to add extra compartments with the corresponding ordinary differential equations. Duval and Laveille 2005 (8)

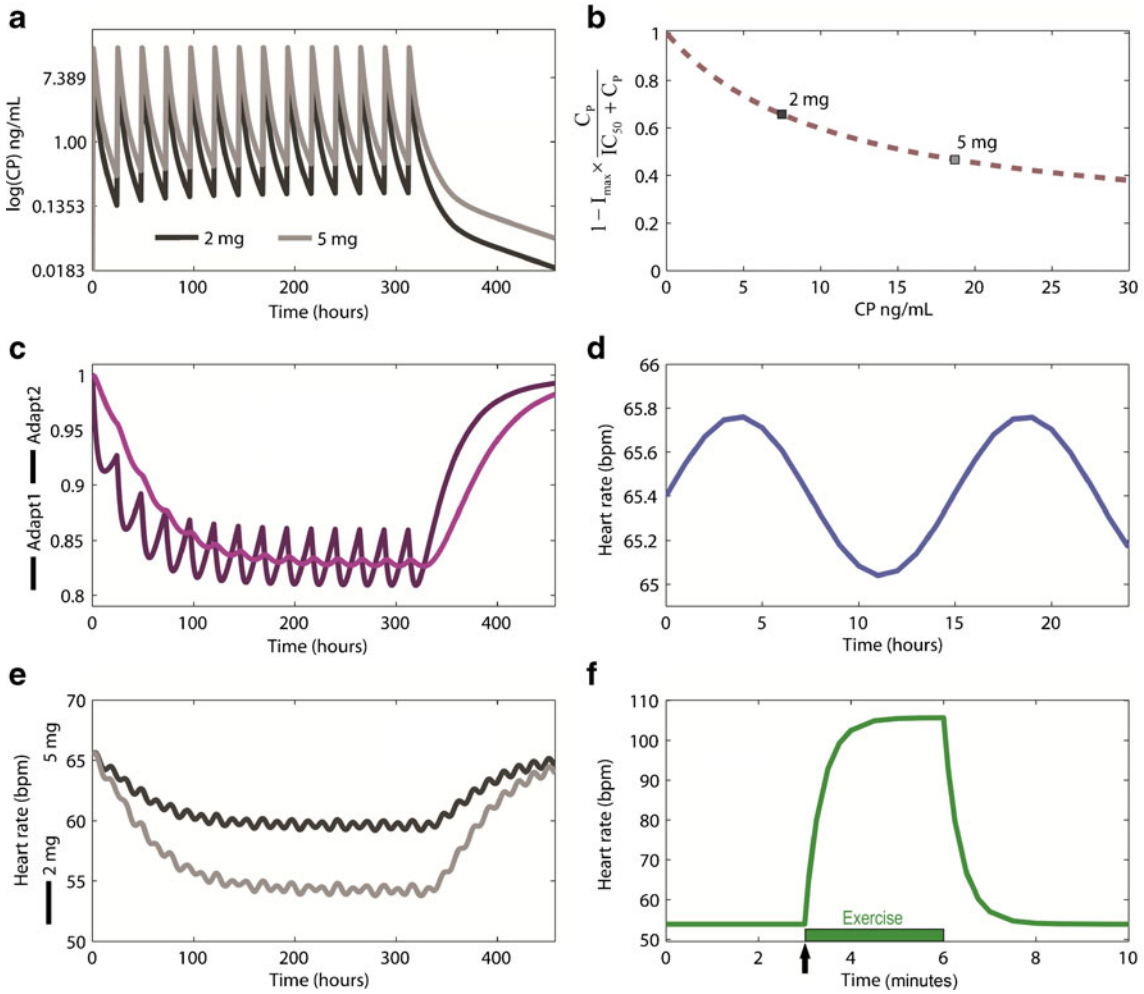

Fig. 6 Detailed model exploration. (**a**)Simulated typical PK profiles after once daily oral doses of 2 mg and 5 mg. (**b**) Pharmacodynamic relationship. *Squares* identify the maximum concentration reached after the 2 and 5 mg doses at the end of the treatment. (**c**) TR_1 and TR_2 dynamics during the entire study period corresponding to the dose of 5 mg. (**d**) Typical time profile for HR measured at rest during 24 h corresponding to the placebo group. (**e**) Typical time profiles for HR measured at rest during the entire study period corresponding to the 2 and 5 mg dose groups. (**f**) Predicted increase in HR during 3 min of exercise. The simulation was performed at 12 h after the last 5 mg administration.

analysed simultaneously HR at rest and at the end of exercise using the following expression $HR_{exercise} = HR_{rest} + \theta_{WKL} \times WKL$, which assumes that there is an instantaneous impact of WKL on HR. However, this assumption contradicts the fact that exercise has to be performed at the same workload for approximately three minutes to reach target HR. Here the derived value of $t_{1/2}k_{D\_HR}$ was 0.0041 h (0.2476 min) which means, that steady-state HR conditions after constant exercise are achieved in approximately 1.2 min.

Information available to develop the model included the individual workload and the duration of exercise, both were incorporated in the dataset for the development of the model. The model developed in the current study can be used to investigate the effect of different magnitudes of workload and duration of exercise. The model can also be applied when individual workload and duration are unknown but the time at which exercise began is known. In that case, an extra typical parameter representing the duration of the perturbation is required. Thus, the model described might help to identify model parameters in trials in which measurements were obtained more sparsely. In general the model developed can be applied to optimize further study designs (e.g. sampling timepoints, sample size).

In summary, a population PKPD model has been developed for the HR effects of cilobradine during multiple oral administrations. Results showed that cilobradine shows PK parameters that are dose and time independent. HR response was found to be concentration-dependent and appeared with a significant delay with respect to the time course of cilobradine in plasma. The predicted steady state for the HR is achieved latest 14 days after the start of the treatment.

HR measured at rest and at the end of exercise was modeled simultaneously using a semi-mechanistic PKPD model, in which a single set of drug-dependent (I_{MAX}, and

IC_{50}) and system-dependent parameters (HR_{basal}, k_{D_HR}, k_{TR}, and θ_{WKL}) were estimated. The selected model provides a useful modeling tool for cases where the PD response measured is the result of a temporal experimental induced perturbation. However, confirmation of the system-dependent nature of the parameters would require validation with data obtained for other drugs of the same class.

ACKNOWLEDGMENTS AND DISCLOSURES

Nieves Vélez de Mendizábal has received a postdoctoral fellowship from the University of Navarra, Pamplona, Spain.

Iñaki F. Trocóniz has received financial research support from Boehringer Ingelheim Pharma GmbH & Co KG.

Alexander Staab, Hans Günter Schäfer, Christiane Döge, Dirk Trommeshauser, Juliet Roberts, Matthias Klüglich are employes of Boehringer Ingelheim Pharma GmbH & Co KG, Germany or its affiliates.

REFERENCES

1. Laperche T, Logeart D, Cohen-Solal A, Gourgon R. Potential interests of heart rate lowering drugs. Heart. 1999;81(4):336–41.
2. Van Bogaert PP, Pittors F. Use-dependent blockade of cardiac pacemaker current (I_f) by cilobradine and zatebradine. Eur J Pharmacol. 2003;478(2–3):161–71.
3. Borer JS. Therapeutic effects of I(f) blockade: evidence and perspective. Pharmacol Res. 2006;53(5):440–5.
4. Liang M, Puri A, Devlin G. Heart rate and cardiovascular disease: an alternative to Beta blockers. Cardiol Res Pract. 2009;2009:179350.
5. De Caterina AR, Leone AM. Why beta-blockers should not be used as first choice in uncomplicated hypertension. Am J Cardiol. 2010;105(10):1433–8.
6. Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. Clin Pharmacol Ther. 1979;25(3):358–71.
7. Ragueneau I, Laveille C, Jochemsen R, Resplandy G, Funck-Brentano, Jaillon P. Pharmacokinetic-pharmacodynamic modeling of the effects of ivabradine, a direct sinus node inhibitor, on heart rate in healthy volunteers. Clin Pharmacol Ther. 1998;64(2):192–203.
8. Duval V, Laveille C. Ivabradine and S18982 activities on heart rate: a population PK/PD analysis. PAGE 14; 2005: Abstr 811 [www.page-meeting.org/?abstract=811]
9. Fliss G, Staab A, Tillmann C, Trommeshauser D, Schaefer HG, Kloft C. Population pharmacokinetic data analysis of cilobradine, an I_f channel blocker. Pharm Res. 2008;25(2):359–68.
10. Karlsson MO, Sheiner LB. The importance of modeling interoccasion variability in population pharmacokinetic analyses. J Pharmacokinet Biopharm. 1993;21(6):735–50.
11. Ludden TM, Beal SL, Sheiner LB. Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection. J Pharmacokinet Biopharm. 1994;22(5):431–45.
12. Savic RM, Jonker DM, Kerbusch T, Karlsson MO. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. J Pharmacokinet Pharmacodyn. 2007;34(5):711–26.
13. Dayneka NL, Garg V, Jusko WJ. Comparison of four basic models of indirect pharmacodynamic responses. J Pharmacokinet Biopharm. 1993;21(4):457–78.
14. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN) A Perl module for NONMEM related programming. Comput Methods Progr Biomed. 2004;75(2):85–94.