An Integrative Approach for Improved Assessment of Cardiovascular Safety Data

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ABSTRACT

Cardiovascular adverse effects in drug development are a major source of compound attrition. Characterization of blood pressure (BP), heart rate (HR), stroke volume (SV), and QT-interval prolongation are therefore necessary in early discovery. It is, however, common practice to analyze these effects independently of each other. High-resolution time courses are collected via telemetric techniques, but only low-resolution data are analyzed and reported. This ignores codependencies among responses (HR, BP, SV, and QT-interval) and separation of system (turnover properties) and drug-specific properties (potencies, efficacies). An analysis of drug exposure-time and high-resolution response-time data of HR and mean arterial blood pressure was performed after acute oral dosing of ivabradine, sildenafil, dofetilide, and pimobendan in Han-Wistar rats. All data were modeled jointly, including different compounds and exposure and response time courses, using a nonlinear mixed-effects approach. Estimated fractional turnover rates (\( k_{out} \)) within parentheses were \( I_{max} = 0.944 (1.7), I_{max} = 1.00 (1.3), E_{max} = 0.195 (9.9), \) and \( I_{max} = 0.745 (4.6) \) for ivabradine, sildenafil, dofetilide, and pimobendan. Hill parameters were estimated with good precision and below unity, indicating a shallow concentration-response relationship. An equilibrium concentration–biomarker response relationship was predicted and displayed graphically. This analysis demonstrates the utility of a model-based approach integrating data from different studies and compounds for refined preclinical safety margin assessment.

SIGNIFICANCE STATEMENT

A model-based approach was proposed utilizing biomarker data on heart rate, blood pressure, and QT-interval. A pharmacodynamic model was developed to improve assessment of high-resolution telemetric cardiovascular safety data driven by different drugs (ivabradine, sildenafil, dofetilide, and pimobendan), wherein system- (turnover rates) and drug-specific parameters (e.g., potencies and efficacies) were sought. The model-predicted equilibrium concentration–biomarker response relationships and was used for safety assessment (predictions of 20% effective concentration, for example) of heart rate, blood pressure, and QT-interval.

Introduction

In drug development, cardiovascular effects are one of the major sources of compound attrition. Identifying these effects at an early stage is therefore a focus of significant effort (Piccini et al., 2009; Gintant et al., 2016). Preclinical data on cardiovascular variables, such as blood pressure (BP), heart rate (HR), and QT-interval prolongation, have long been routinely collected in animals and humans (Piccini et al., 2009). Although these variables are often obtained simultaneously, they are commonly analyzed separately. However, since significant codependencies between safety variables are typically shown, simultaneous modeling of all data results in more informative insights about the test substance (e.g., potency \( IC_{50} \)) and systems parameters (e.g., fractional turnover rates \( k_{out} \)). Codependencies act both directly, as in QT adaptation to HR change, and indirectly (e.g., via baroreflex feedback) (Cleophas, 1998; Holzgreve et al., 2014). In addition, useful information about primary drug effects is neglected when codependencies are ignored. A consequence of this is inaccurate results. It is also common practice to analyze the outcomes of experiments independently from each other in spite of repeatedly using the same

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ABBREVIATIONS:

BP, blood pressure; BSL, baseline; CL, clearance; CLint, intercompartmental distribution; Cu, clinical free concentration; DSI, Data Science International; \( EC_{20} \), 20% effective concentration; ERG, ether-a-go-go-related gene; F, bioavailability; FB, feedback; hERG, human ERG; HR, heart rate; \( IC_{20} \), 20% inhibitory concentration; \( k_{d} \), rapid delayed rectifier current; \( K_{e} \), absorption rate constant; \( k_{e} \), turnover rate; \( k_{out} \), fractional turnover rate; MAP, mean arterial BP; NLME, nonlinear mixed-effects; PDE, phosphodiesterase; PK, pharmacokinetic; RSE, relative standard error; SV, stroke volume; TPR, total peripheral resistance.

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test population, which partly ignores valuable baseline behavior. Furthermore, high-resolution time courses of biomarker data are collected using telemetry, but only low-resolution data are analyzed and reported. To remedy some of these shortcomings, pharmacokinetic (PK)/pharmacodynamic modeling has proven to be a valuable tool combining quantities, such as HR, BP, and QT, using a nonlinear mixed-effects (NLME) approach.

Semimechanistic models of cardiovascular physiology have been used for over 60 years, beginning with pivotal work by Noble (1962) and Guyton et al. (1972). Their work has been developed in a number of ways in response to improved understanding, new measurement techniques, and computational power (Kappel and Peer, 1993; Winslow et al., 1999; Ursino and Magosso, 2003; ten Tusscher, 2004; ). Models that accommodate drug effects have been introduced more recently (Zemzemi et al., 2013; Snelder et al., 2014; Colatsky et al., 2016). The present extension analyzes the pivotal Guyton et al. (1972) framework used within many discovery projects and emphasizes the applicability of presently used models with high-resolution data. The ambition is not to use the collected data for model discrimination purposes but rather to demonstrate how a semimechanistic platform well known among physiologists and (safety) pharmacologists can be used with their data.

The model-based approach for improved assessment of cardiovascular safety that we propose here is shown schematically in Fig. 1. In step 1, high-resolution telemetric data are collected from Han-Wistar rats, and exploratory and regression analyses are done to visualize and quantify baseline properties of biomarker data, such as HR, BP, and QT-interval (Kramer and Kinter, 2003). In step 2, low-resolution biomarker data obtained with different drugs (ivabradine, silde-

Fig. 1. Schematic overview of study analyses. Step 1: High-resolution time courses of biomarker response are obtained telemetrically from animals. These data are used to assess baseline biomarker response. Step 2: Low-resolution data combined with baseline model serve as input to a meta-analysis of biomarker-response time courses, compounds, and experiments, generating final estimates of systems (e.g., turnover rates) and drug (e.g., potentcies) parameters. Step 3: Steady-state concentration–biomarker response relationships are established for heart rate, blood pressure, and QT response.

Materials and Methods

Animals

Male rats (Han-Wistar, Charles River) weighing 450–600 g, 3–19 months old, were used. Rats were chronically instrumented for the telemetric collection of arterial pressure, ECG, and body temperature as described previously (Schierok et al., 2000). Groups of up to three rats were housed together in cages (type IV—1815 cm²) in a room with a 12-hour light/dark cycle and controlled temperature and humidity, which was identical for all experiments. The rats had access to normal rodent chow and water ad libitum and were not fasted prior to experiments.

Telemetric System

The telemetric system consisted of elements of a Data Science International (DSI) system (DSI, Minnesota, USA) for cardiovascular measurements (BP, HR, temperature, and ECG). A calibration amplifier was added that was capable of converting the signals from the DSI system for transfer to a computer-based acquisition and analysis system (HEM v.3.3 or v.4.3, Notocord, Paris, France). Two of these systems were run in parallel to allow simultaneous measurement of eight animals.

Miniature transmitters (DSI TA PA-C40) for the measurement of BP and HR, temperature, and ECG were used. Before implantation, the transmitter was calibrated using an assigned receiver (RLA 1020 DSI). Afterward, the transmitters were sterilized with ethylene oxide, and after 24–48 hours of venting, they were kept under aseptic conditions until implantation.

Implant Surgery

Rats were anesthetized in a box ventilated with 4% isoflurane. After the onset of the anesthesia, they were transferred to the head chamber of the inhalation apparatus for anesthesia in small animals, Narco-

Völcker, Germany), and anesthesia was maintained with 2% isoflurane. Under aseptic conditions, a midline abdominal incision (2 and 3 cm) along the linea alba was made, and the intestines were retracted. The lower abdominal aorta was isolated and temporarily occluded with a ligature. A small hole was punctured into the aorta near the iliac bifurcation using a 20-gauge hypodermic needle, and the catheter end of the transmitter was inserted. After the puncture site was dried thoroughly, the catheter was sealed in place using a tissue adhesive (Histoacryl, Braun, Germany). The intestines were gently put back in place, and the transmitter body was attached to the abdominal muscle during closing of the abdominal cavity and
the skin incision. To reduce any pain or infection risk, the animals were treated with dypyrone 50 mg/kg i.p. (Novalgin, Hoechst, Germany) prior to the surgery, and a prophylactic dose of penicillin 0.05 ml/100 g (Tardomyocel, Bayer, Germany) was administered. The surgical preparation was followed by a 4-week recovery period before experiments.

**Study Description**

Data from four separate drug provocation studies in rats were used: ivabradine (Sigma-Aldrich, US), sildenafil (Neuraxpharm, Germany), dofetilide (Sigma-Aldrich), and pimobendan (Sigma-Aldrich). Each compound was used in a pharmacokinetic and a pharmacodynamic study. We are consistently reporting and utilizing unbound plasma concentrations throughout this work, with unbound fractions listed in Table 1.

**Pharmacokinetic Assessment**

The plasma PK of all four compounds was investigated in rats after intravenous and oral administration. A single dose of test compound was either administered orally (volume administered: 2 ml/100 g b.wt. suspended in Natrosol 0.5% + 0.015% Tween80, n = 3 subjects per study) or intravenously (volume administered: 0.5 ml/100 g b.wt.; dissolved in a 20% HP-ß-cyclodextrin solution with pH adjustment to pH 6, n = 2 subjects per study). For ivabradine, sildenafil, dofetilide, and pimobendan, doses were 1, 10, 1, and 3 mg/kg (oral) and 0.5, 0.1, 0.3, and 0.33 mg/kg (intravenous), respectively. Plasma drug concentrations were analyzed in samples drawn at 0.08, 0.25, 0.5, 1, 2, 4, 8, and 24 hours. Lower limits of quantification were 1 nM for ivabradine, dofetilide, and pimobendan and 2.5 nM for sildenafil.

Blood samples were collected in Microvette tubes 0.50 ml K3EDTA (Sarstedt). The tubes were ice-chilled prior to sampling. After collection, the blood was gently mixed by inverting the tube several times and stored upright on ice until centrifugation. Blood samples were centrifuged for 5 minutes at 8300g at 4°C. After centrifugation, plasma was aliquoted and stored below -20°C pending chemical analyses. Drug plasma concentrations of all four compounds were analyzed using liquid chromatography/tandem mass spectrometry with an analytical range of 1–2000 nM.

**Pharmacodynamic Assessment**

Four separate groups of rats (n = 8 per group) received vehicle control (Natrosol 0.5% + 0.015% Tween80) and one of the test compounds—ivabradine, dofetilide, pimobendan, or sildenafil—at three dose levels (volume administered: 1 ml/100 g b.wt. suspended in Natrosol 0.5% + 0.015% Tween80 orally (Table 1). Each study comprised eight individual animals except for the sildenafil study, which comprised 31 individual animals.

Eight implanted animals were transferred to eight separate cages placed on the previously assigned DSI receivers on the day of each experiment. The transmitters were activated magnetically, and the

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**TABLE 1**

| Compound   | Mode of Action                  | Doses (mg kg⁻¹) | Study Period (h) | Molecular Weight (g mol⁻¹) | Unbound Fraction (fu) |
|------------|---------------------------------|-----------------|------------------|----------------------------|-----------------------|
| Ivabradine | Blocks I_f and HCN channels in SAN | 0, 3, 10, 30    | 24               | 468.6                      | 0.25                  |
| Sildenafil | PDE5-inhibitor                  | 0, 3, 10, 30    | 7                | 474.6                      | 0.05                  |
| Dofetilide | IKr-channel blocker             | 0, 1, 3, 10     | 7                | 441.6                      | 0.2                   |
| Pimobendan | Ca²⁺ sensitizer, PDE3-inhibitor | 0, 1, 3, 10     | 24               | 334.4                      | 0.05                  |

fu, unbound fraction of test compounds in rats; HCN, hyperpolarization-activated cyclic nucleotide–gated channels; I_f, funny current; SAN, sinoatrial node.

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![Fig. 2. Schematic diagram of the extended model. Left plot: absorption and disposition model used for plasma exposure-time data of ivabradine, sildenafil, and dofetilide. Right plot: absorption and disposition model used for plasma exposure-time data of pimobendan. For pimobendan, a hypothetical metabolite compartment (eq. 1d) was added to capture active metabolite(s) from parent compound. The rate constant k_met was estimated from biomarker response–time data and is not part of the plasma exposure analysis (Kitzen et al., 1988).](image-url)
signal was confirmed with the help of a commercially available radio receiver. Systolic blood pressure (sBP), diastolic blood pressure (dBP), and ECG were continuously measured. From these data, three cardiovascular variables were derived: HR, mean arterial BP (MAP) (derived as one-third sBP + two-thirds dBP), and the QT-interval. All measurements began 1.5 hours before dosing and continued for variable lengths of recording (Table 1).

Data Acquisition
The Acquisition Software HEM version 3.3 or 4.3 (Notocord, Paris, France) was used to record the experimental data continuously (sampled at 1000 Hz) in real time and store it on the local hard disk. HEM is used to acquire hemodynamic arterial BP signals as well as ECG signals. From the arterial BP signal, systolic, diastolic, and mean arterial BP was calculated as well as HR. These signals are denoted high-resolution biomarkers. Body temperature was recorded directly. At the end of each experiment, the raw data were saved on a system server. The data were summarized by calculating the median value of sequential events over 10 minutes for each biomarker, giving low-resolution biomarkers.

Pharmacodynamic Modeling

Drug Exposure Model. A schematic plot of the absorption and disposition models of ivabradine, sildenafil, doftelidine, and pimobendan is shown in Fig. 2.

Drugs (also called test compounds) were administered intravenously or orally, and a two-compartment disposition model with first-order input/output was fitted to plasma concentration data:

\[
dCm = -k_{in} \cdot Cm\text{ }dt
\]

\[
V_p \cdot \frac{dCp}{dt} = K_a \cdot A_p - CL \cdot C_p + CL_d \cdot C_t - CL_d \cdot C_p
\]

\[
V_t \cdot \frac{dCt}{dt} = CL_d \cdot C_p - CL_d \cdot C_t
\]

\[
\frac{dC_m}{dt} = k_{met} \cdot C_p - k_{met} \cdot C_m
\]

where \(K_a\), \(C_p\), and \(C_t\) denote amount in the gut compartment, concentration in plasma, and concentration in tissue, respectively. The model parameters correspond to absorption rate constant \(K_a\), plasma clearance \(CL\), oral bioavailability \(F\), plasma volume of distribution \(V_p\), intercompartmental distribution \(CL_d\), and tissue volume \(V_t\).

A hypothetical metabolite compartment (eq. 1d) was added to capture active metabolite(s) from the pimobendan parent compound. The rate constant \(k_{met}\) was estimated from response-time data and is not a part of the plasma exposure analysis (Fasanmade and Jusko, 1995).

Pharmacodynamic Model

A schematic plot of the pharmacodynamic actions of ivabradine, sildenafil, doftelidine, and pimobendan is shown in Fig. 3. The model consists of four biomarker responses: HR, TPR, stroke volume (SV), and QT. HR, TPR, and SV are interconnected and drive MAP, which attenuates the turnover rates of these states, creating a feedback loop. HR also affects the turnover rate of the QT-interval, describing the length adaptation of QT to HR (Fig. 3).

The pharmacodynamic model is based on the approach presented in Guyton et al. (1972) and Snelder et al. (2014) consisting of three coupled turnover models describing HR, SV, and TPR, respectively. Each turnover model contains its individual turnover rate \(k_{in}\) and fractional turnover rate \(k_{out}\). The coupling of individual processes occurs via MAP. The time course of MAP is modeled as a nonlinear function according to eq. 2.
no direct effect on SV. Drug intervention on HR, TPR, or QT is either stimulatory action \((S(C))\) given drug exposure \((C)\)
\[
S(C) = \frac{E_{\text{max}}C}{EC_{50}+C^\gamma}
\]
(7)
or inhibitory action \((I(C))\) given drug exposure \((C)\) and metabolite concentration \((C_m)\) (only present for pimobendan).
\[
I(C) = - \frac{I_{\text{max}}(C + C_m)^\gamma}{IC_{50}+(C+C_m)^\gamma}
\]
(8)
For stimulatory effects on SV (from pimobendan), a linear model with a single slope parameter (SL) was used:
\[
S(C) = (C + C_m) \cdot SL
\]
(9)
Table 2 and eqs. 7 and 8 quantify each drug action by means of its own set of drug parameters: \(E_{\text{max}} \cdot I_{\text{max}}, EC_{50} \cdot IC_{50}\), and \(\gamma\).

The turnover rate of HR is expressed as
\[
\frac{dHR}{dt} = k_{\text{in,HR}}(1 + CR_{HR})(1 - FB \cdot MAP)(1 + EFF_{HR} + HD_{HR}) - k_{\text{out,HR}} \cdot HR
\]
(10)
in which turnover rate of HR is modeled by baseline turnover \((k_{\text{in,HR}})\), circadian rhythm \((CR_{HR})\), feedback \((FB)\) from MAP, handling \((HD_{HR})\), and fractional turnover rate \((k_{\text{out,HR}})\). The drug effects \((EFF_{HR})\) can be either stimulatory \((EFF_{HR} = S(C)\) eqs. 7) or inhibitory \((EFF_{HR} = I(C)\), eq. 8), with corresponding definitions for \(EFF_{TPR}\) and \(EFF_{SV}\). EFF follows eq. 9. The turnover of the SV is expressed as
\[
\frac{dSV}{dt} = k_{\text{in,SV}}(1 - FB \cdot MAP)(1 + EFF_{SV}) - k_{\text{out,SV}} \cdot SV
\]
(11)
The turnover rate of SV has a similar structure to that of HR but is assumed not to be affected directly by circadian rhythm or handling. The turnover of the TPR is expressed as
\[
\frac{dTPR}{dt} = k_{\text{in,TPR}}(1 + CR_{TPR})(1 - FB \cdot MAP)(1 + EFF_{TPR} + HD_{TPR}) - k_{\text{out,TPR}} \cdot TPR
\]
(12)
which is assumed to have the same structure as that of HR. Initial conditions for eqs. 10–12 as well as eq. 18 below were determined numerically as described in the section Pharmacodynamic Model Parameters.

The FB from MAP is modeled as dependent on the individual baseline value \((BSL_{MAP})\) for MAP, according to
\[
FB = FB_0 \left(\frac{BSL_{MAP}}{BSL_{MAP}}\right)^{FB_0_{MAP}}
\]
(13)
in which \(FB_0\) regulates the amount of feedback from MAP, and \(BSL_{MAP}\) and \(FB_0_{MAP}\) are empirically derived constants regulating the shape of the feedback relationship.

Turnover rates \((k_{\text{in}})\) for the biomarker responses of HR, SV, and TPR were expressed as a function of their baseline values and the baseline value of MAP together with their fractional turnover rates \((k_{\text{out}})\) and the feedback parameter \((FB)\):
\[
k_{\text{in,HR}} = k_{\text{out,HR}} \cdot BSL_{HR} \frac{FB_{0,MAP}}{1 - FB \cdot BSL_{MAP}}
\]
(14)
The turnover rate of the SV was described according to
\[
k_{\text{in,SV}} = k_{\text{out,SV}} \cdot BSL_{SV} \frac{FB_{0,MAP}}{1 - FB \cdot BSL_{MAP}}
\]
(15)
The turnover rate of the TPR was described according to
\[
k_{\text{in,TPR}} = k_{\text{out,TPR}} \cdot BSL_{TPR} \frac{FB_{0,MAP}}{1 - FB \cdot BSL_{MAP}}
\]
(16)
in which \(BSL_{HR}, BSL_{SV}, BSL_{TPR},\) and \(BSL_{MAP}\) denote baseline values for HR, SV, TPR, and MAP, respectively. Here, we also require that
\[
BSL_{MAP} = BSL_{TPR} \cdot BSL_{CO}
\]
(17a)
\[
BSL_{CO} = BSL_{SV} \cdot BSL_{HR}
\]
(17b)
The consistency of model equations with the definitions of the baseline values is further analyzed in Supplemental Section 6.

**Modeling of QT Interval**

The model was extended by adding the QT dynamics represented by a fourth turnover model. A feedback term was introduced to reflect the regulation of QT by means of HR, which is expressed according to
\[
\frac{dQT}{dt} = k_{\text{in,QT}}(1 - QT_{HR} \cdot HR)(1 + EFF_{QT} + HD_{QT}) - k_{\text{out,QT}} \cdot QT
\]
(18)
Here, \(QT_{HR}\) is a constant regulating the degree of QT adaptation to HR. The formulation of the handling effects, \(HD_{QT}\), follows those presented in eqs. 5 and 6:
\[
HD_{QT} = \begin{cases} 
P_{Q,T} \cdot e^{-\frac{k_{\text{out,QT}}(t-t_0)}{t_0}}, & t \geq t_0 \\
0, & t < t_0
\end{cases}
\]
(19)
The turnover rate of the QT-interval was expressed as
\[
k_{\text{in,QT}} = k_{\text{out,QT}} \cdot \frac{BSL_{QT}}{1 - QT_{HR} \cdot BSL_{HR}}
\]
(20)
in which \(BSL_{QT}\) and \(BSL_{HR}\) denote baseline values for QT and HR, respectively.

**Modeling of Concentration- and Biomarker Response–Time Data**

**Exploratory Analysis of High-Resolution Data.** An initial exploratory analysis of high-resolution and low-resolution biomarker–response data of HR, MAP, and QT-interval from the vehicle-control group included visual inspection. Biomarker responses displayed a slowly oscillating baseline response, which was repeatedly upwardly stimulated. A sinusoidal model (see Supplemental Section 1) was manually fitted to the slowly oscillating baseline response (for initial parameter estimates) until acceptable consistency was obtained between experimental and model-simulated data. Residuals between low-resolution data and sinus model predictions were computed, resulting in one set of residuals for HR and one set for MAP. These residuals were used to compute an initial distribution for the residual model in the NLME parameter estimation, as described below.

**Drug Exposure Model Parameters.** Deviations between experimental and model predictions were assumed to follow a Gaussian distribution for all exposure data. A maximum likelihood objective function was used to produce a single estimate per compound of the pharmacokinetic parameters in eq. 1, a–c.
Pharmacodynamic Model Parameters. The parameters of the pharmacodynamic model were estimated using an NLME approach, allowing simultaneous regression of all individual biomarker-response time courses (i.e., HR, MAP, and QT). Note that the stroke volume was not among the biomarkers measured in this work. In the cases in which the model was regressed to effects on stroke volume (pimobendan), it was done based on the secondary effects on the measured biomarkers. This technique allows the estimation of interindividual variability directly from data. Specifically, the analysis was done using a custom implementation of the expectation-maximization algorithm (Kuhn and Lavielle, 2005) in MATLAB (version R2019b, The Mathworks, Inc., Natick, Mass.) to maximize the likelihood, $L$, of the system and drug-specific parameters ($\theta$) given the measured data, $d$:

$$L(\theta) = \prod_{i=1}^{N} \int P(d_i|\theta, \eta_i)P(\eta_i|\theta)$$ (21)

Here, $N$ denotes the total number of individuals, and $\eta_i$ denotes the vector of individual random-effects model parameters for individual ($i$). The distribution of the individual random effects was assumed to be Gaussian. For increased stability of the estimation algorithm, 1000 sampled parameter vectors for each individual were used in each iteration. This also obviated the need for smoothing the estimated parameter values at the cost of a higher computational demand. Convergence was considered to be attained when none of the parameters diverged more than 1% from their average across 10 iterations of the algorithm.

The baseline parameters $BSL\text{HR}$, $BSL\text{MAP}$, and $BSL\text{QT}$ were assumed to vary across individuals. No interindividual variability was added to drug parameters $E_{\text{max}}$, $EC_{50}$, and $\gamma$ of the modelled biomarker responses ($HR$, $SV$, $TPR$, and $QT$). A Gaussian prior with a mean of 0.126 and a S.D. of 0.013 was used to constrain $\hat{k}_{\text{out,SV}}$ to values around previously reported values for rat (Snelder et al., 2014) since this was found to improve estimation stability. After a change in any parameter value, new initial conditions were computed by initializing each modeled biomarker to its $BSL$ and simulating the model over a long time span (336 hours), allowing it to reach equilibrium.

Equilibrium Concentration-Response Relationships. The estimated pharmacokinetic/pharmacodynamic model was used to predict the equilibrium concentration–biomarker response relationship of each compound. Here, equilibrium refers to a state in which the unbound plasma concentration is constant and wherein no circadian rhythm is present. Since the model is nonlinear, the biomarker response cannot be expressed explicitly in terms of drug exposure. Instead, steady-state data were generated by means of model simulations ($t=96$ hours) with a logarithmically spaced exposure range. Parameters for handling ($P_{\text{HR}}$, $P_{\text{TPR}}$, $P_{\text{QT}}$), diurnal variations ($amp_{\text{HR}}$, $amp_{\text{TPR}}$), and residual errors were set to zero. Standard target-effect models for sigmoidal $E_{\text{max}}$ and $I_{\text{max}}$ models with baseline (Table 3.1, p.221 in Gabrielsson and Weiner (2016), according to eqs. 22a or 22b below, were then regressed to the simulated concentration–biomarker response data. In other words,

$$E_T = E_0 + \frac{E_{\text{max}} \cdot C_T}{EC_{50} + C_T}$$ (22a)

$$E_T = E_0 - \frac{I_{\text{max}} \cdot C_T}{IC_{50} + C_T}$$ (22b)

depending on whether the observed effect was of stimulatory or inhibitory action, respectively.

From the estimated drug parameters $EC_{50}$, $E_{\text{max}}$, and $\gamma$ at the equilibrium state, an effective target concentration ($C_T$) was calculated using the standard target concentration model for a sigmoidal $E_{\text{max}}$ model with baseline wherein $C_T = EC_{50}$ ($E_T - E_0$)/($E_0 + E_{\text{max}} - E_T$)$^{1/\gamma}$, which for a 20% change in response from baseline (i.e., $E_T = E_0 + 0.2 \cdot E_{\text{max}}$) results in

$$C_T = EC_{20} = EC_{50} \cdot 4^{-1/\gamma}$$ (23a)

and likewise, in the case of inhibitory action with $E_T = E_0 - 0.2 \cdot I_{\text{max}}$

$$C_T = IC_{20} = IC_{50} \cdot 4^{-1/\gamma}$$ (23b)

This analysis was not applicable to the effects on SV from pimobendan since these were modeled as linear according to eq. 9.

Results

Exploratory Analysis of High-Resolution Data. High-resolution biomarker-response data (obtained from ECG and blood pressure measured at 1000 Hz, downsampled to 500 Hz)
from four rats were analyzed in step 1 to understand baseline behavior and residual analysis. An example of high-resolution response of HR and MAP for a single animal obtained during a 24-hour vehicle-control study is shown in Fig. 4.

HR and MAP responses randomly deviated toward higher values and were superimposed on a diurnal baseline wave (example results for one of the animals are shown in Fig. 5). This is particularly evident for HR (upper panel, Fig. 4) and MAP (lower panel, Fig. 4). Light and dark periods differed clearly in duration, frequency, and amplitude of deviations. A sinusoidal model was fitted to the diurnal baseline wave obtained from high-resolution data sets. The residuals between the model-predicted and experimental data were obtained for low-resolution data. The high- and low-resolution responses and model regressions are compared in the upper panel of Fig. 5.

To capture both the asymmetry of HR and MAP around their respective baselines and the correlation between the two signals the bivariate log-normal distribution was chosen to describe the residual errors. Because of the systematic differences between light- and dark-hour response-time data, two separate residual error models were used to describe light and dark periods.

**Fig. 5.** Upper row: observed heart rate and blood pressure (MAP) vs. clock time of high- and low-resolution data (gray and orange lines, respectively) with a sinusoidal model fit to the resting state (black solid line). The light and dark periods are separated by vertical dashed lines. Bottom row: observed blood pressure (MAP) vs. heart rate residuals (solid symbols) between model and the low-resolution data. The solid (blue) lines represent fitted log-normal distributions of light (left) and dark (right) periods.

**Fig. 6.** Observed total concentration-time data after intravenous (solid triangles) and oral (solid squares) dosing of ivabradine, sildenafil, dofetilide, and pimobendan. Solid lines are model-fitted concentration time courses driving the pharmacodynamic model. Doses of ivabradine, sildenafil, dofetilide, and pimobendan were 2.13, 21.1, 2.26, and 8.97 μmol kg⁻¹ (oral) and 1, 1, 10, and 1 μmol kg⁻¹ (intravenous), respectively. The horizontal dashed red lines represent unbound potencies in Table 4 and are shown as a comparison between exposure data and the predicted drug parameter. The red, simulated plasma concentration-time curves are model simulations of the highest oral dose of each compound in the pharmacodynamic assessment of biomarker data.
dark conditions (depicted in Fig. 5, bottom panel). Parameters for the residual error model are given in Supplemental Section 2. For the QT-interval response, a normal distribution was used since no correlation to either HR or MAP (unpublished data) was found (see Supplemental Fig. 1). These residual error models were the main outcome of the exploratory analysis and were used in the pharmacodynamics analysis.

**Exposure Time Courses of Test Compounds.** The two-compartment disposition model (Fig. 2; eqs. 1, a–c) was simultaneously fitted to intravenous and oral data shown in Fig. 6 for each compound. Final parameter estimates of disposition (CL, CLd, and volumes of the central and peripheral compartments) and absorption parameters (Ka and bioavailability (F)) are given in Table 3.

The goodness of fit was assessed by means of residual analysis. Note that several of the parameters are poorly estimated (relative standard error (RSE) >100%), which is not commonly accepted for correct assessment of the pharmacokinetic properties. However, the goal was to correctly capture both high- and low-exposure data to have a pharmacokinetic model that predicts exposure data well and can serve capture both high- and low-exposure data to have a pharmacokinetic properties. However, the goal was to correctly not commonly accepted for correct assessment of the pharmacokinetic properties. However, the goal was to correctly estimate (relative standard error (RSE) >100%) from the estimated population distribution. Then, 1000 predictions of the residuals each of HR, MAP, and QT were sampled and added to the model output. Finally, for each time point, the 1000 simulated responses were ordered, and the 25 highest and lowest values were removed to construct a 95% prediction interval. Regressed exposure data showed high consistency between measured data and model output, with the majority of data points well within their prediction intervals. However, the model appears to systematically overpredict the drug blood pressure effect of the highest pimobendan dose.

Parameter estimates were obtained from low-resolution data, utilizing residual distributions for light and dark periods from the exploratory analysis of the high-resolution data as initial estimates. Estimated pharmacodynamic parameters and their RSE% are presented in Table 4. Additional fixed-model parameters are listed in Supplemental Table 1.

For the system’s parameters, estimated baseline values for heart rate (BSLHR), mean arterial pressure (BSLMAP), QT-interval (BSLQT), the parameter regulating baroreflex feedback (Fb), and handling (PbHR and PTPR) were estimated with good precision. The available data exhibited a very weak relationship between HR and QT (see Supplemental Fig. 1), which led us to exclude the HRQT from the estimation by fixing it to zero. The interindividual variability was low (5%). Drug-specific parameters, such as Emax, EC50, and γ, had generally high precision except for the pimobendan action on stroke volume (RSE% >100%) and dofetilide IC50 (RSE% = 93%). The potencies of ivabradine (HR) and pimobendan (SV) were

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**TABLE 3**

| Compound   | CL (l h\(^{-1}\) kg\(^{-1}\)) | Vp (l kg\(^{-1}\)) | CLd (l h\(^{-1}\) kg\(^{-1}\)) | Vf (l kg\(^{-1}\)) | Ks (h\(^{-1}\)) | F (%) |
|------------|-------------------------------|-------------------|-------------------------------|-------------------|----------------|-------|
| Iva,radine | 2.88 (24)                     | 1.45 (25)         | 0.408 (>100)                  | 0.427 (>100)      | 0.61 (31)      | 19 (26) |
| Sildenafil | 3.55 (33)                     | 1.29 (19)         | 0.137 (>100)                  | 0.125 (>100)      | 0.80 (31)      | 1.4 (33) |
| Dofetilide | 3.50 (24)                     | 1.52 (18)         | 0.328 (>100)                  | 0.397 (>100)      | 0.60 (26)      | 37 (22) |
| Pimobendan | 4.08 (18)                     | 2.71 (22)         | 2.03 (>100)                   | 0.861 (>100)      | 1.5 (20)       | 21 (19) |

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The data shown in the predictive plots were computed from model simulations based on 1000 parameter sets sampled from the estimated population distribution. Then, 1000 predictions of the residuals each of HR, MAP, and QT were sampled and added to the model output. Finally, for each time point, the 1000 simulated responses were ordered, and the 25 highest and lowest values were removed to construct a 95% prediction interval. Regressed exposure data showed high consistency between measured data and model output, with the majority of data points well within their prediction intervals. However, the model appears to systematically overpredict the drug blood pressure effect of the highest pimobendan dose.

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Parameter estimates were obtained from low-resolution data, utilizing residual distributions for light and dark periods from the exploratory analysis of the high-resolution data as initial estimates. Estimated pharmacodynamic parameters and their RSE% are presented in Table 4. Additional fixed-model parameters are listed in Supplemental Table 1.

For the system’s parameters, estimated baseline values for heart rate (BSLHR), mean arterial pressure (BSLMAP), QT-interval (BSLQT), the parameter regulating baroreflex feedback (Fb), and handling (PbHR and PTPR) were estimated with good precision. The available data exhibited a very weak relationship between HR and QT (see Supplemental Fig. 1), which led us to exclude the HRQT from the estimation by fixing it to zero. The interindividual variability was low (5%). Drug-specific parameters, such as Emax, EC50, and γ, had generally high precision except for the pimobendan action on stroke volume (RSE% >100%) and dofetilide IC50 (RSE% = 93%). The potencies of ivabradine (HR) and pimobendan (SV) were

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**Fig. 7.** Experimental data (gray dots), model-predicted avg. (solid line), and 95% prediction interval (dashed lines) response of heart rate and blood pressure (MAP) after a single oral dose of ivabradine at 0, 3, 10, and 30 mg kg\(^{-1}\) corresponding to 0, 6.40, 21.3, and 64.0 μmol kg\(^{-1}\). The predicted vehicle-control response (gray line) is superimposed on experimental data.
estimated to be 475 and 604 nM, respectively, which is beyond the observed exposure range but still within published data (Kitzen et al., 1988; Du et al., 2004). The potencies of dofetilide (QT) and sildenafil (TPR) were estimated to be 50.6 and 4.01 nM, respectively, which is within the observed exposure range and consistent with published data (Mounsey and DiMarco, 2000; Gresser and Gleiter, 2002). The \( g \) parameter was relatively low for all compounds and biomarker responses and particularly for dofetilide and QT effect, suggesting a shallow equilibrium concentration–biomarker response relationship around its potency value (Table 5).

**Equilibrium Concentration–Biomarker Response Analyses.** The equilibrium concentration–biomarker response (heart rate, blood pressure, and QT-interval) relationships were assessed for each test compound (Figs. 11–13). Supplemental Section 5 shows how data were generated.

\[ E_{max} = \frac{EC_{50}/IC_{50}}{\gamma} \]

The values of \( E_0 \) in these equations correspond to the baseline values \( BSL_{HR}, BSL_{QT}, \) and \( BSL_{MAP} \) listed in Table 4. The clinical free concentration (\( C_u \)) ranges are included for comparisons.

**Discussion**

This analysis covers exposure- and response-time data from HR and MAP data after acute oral dosing of ivabradine, sildenafil, and pimobendan in Han-Wistar rats. Data on QT-interval duration were also obtained from dofetilide. The analysis allowed system parameters (turnover rates, etc.) to be shared across test compounds and studies, whereas drug parameters (such as \( EC_{50} \)) were compound-specific and shared across dosing regimens. Finally, the equilibrium concentration–response relationships were established and visualized.

**Exploratory Analysis of High-Resolution Biomarker-Response Data.** The exploratory data analysis covered both high- and low-resolution biomarker-response data, revealing two structural features. One was a slowly oscillating baseline, which was assumed to originate from intrinsic circadian variations in biomarker response. The second feature was that fluctuations from baseline increased both in amplitude and duration during dark periods, which are believed to originate from acute baroreflex resetting due to increased
animal activity (Potts and Mitchell, 1998; Dampney, 2017). Data suggested that a model of residual errors would be asymmetric and correlated, which led to selection of a translated log-normal distribution model with dark/light-specific parameters.

**Exposure Time Courses of Test Compounds.** The exposure time courses of ivabradine (Zhang et al., 2016), sildenafil (Sawatdee et al., 2018), dofetilide (Smith et al., 1992), and pimobendan (Asakura et al., 1993) were previously studied in Sprague-Dawley rats (200–300 g) and showed slightly different pharmacokinetic properties compared with the larger Han-Wistar rats (450–600 g) used here (Fig. 6). This highlights the necessity of exposure time courses obtained in the actual animal strain for model development. The primary goal was to apply an exposure model that accurately captures both high- and low-exposure time data for each test compound.

**Pharmacodynamic Model.** A series of pharmacodynamic models of cardiovascular effects in rodents have been developed for BP- (Hao et al., 2007; Bertera et al., 2012; Kiriyama et al., 2016), HR- (van Steeg et al., 2007; Bertera et al., 2012; Kiriyama et al., 2016), and ECG-based biomarkers (Bol et al., 1997; Ohtani et al., 2000; Kiriyama et al., 2016). The majority of these neglect the intertwined dependencies

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**Table 4**

| Drug vs. System | Parameters | Estimates (RSE%) | IIV (RSE%) | t1/2, Konf |
|----------------|------------|------------------|------------|-----------|
| System parameters | BSLHR (beats min⁻¹) | 317.1 (1.4) | 24. (12) |  |
| | BSLMAP (mm Hg) | 102.1 (1.7) | 7.2 (13) |  |
| | BSLQT (ms) | 64.3 (2.1) | 3.9 (11) |  |
| | Fβ0 | 0.00532 (0.7) |  |  |
| | FHR | 3.25 (1.1) |  |  |
| | PR | 1.21 (2.0) |  |  |
| | kout,QT (h⁻¹) | 9.45 (15) | 4.4 |  |
| | kout,HR (h⁻¹) | 30.7 (7.8) | 1.4 |  |
| | kout,TPR (h⁻¹) | 3.8 (13) | 11 |  |
| | kout,SV (h⁻¹) | 0.115 (1.7) | 360 |  |
| Ivabradine | Imax,HR | 0.944 (1.7) |  |  |
| | IC50,HR (nM) | 475. (11) |  |  |
| | γHR | 0.511 (3.6) |  |  |
| Sildenafil | Imax,TPR | 1.00 (1.3) |  |  |
| | IC50,TPR (nM) | 4.01 (5.4) |  |  |
| | γTPR | 0.63 (2.5) |  |  |
| Dofetilide | Emax,QT | 0.195 (9.9) |  |  |
| | EC50,QT (nM) | 50.6 (93) |  |  |
| | γQT | 0.156 (21) |  |  |
| Pimobendan | SLAV (nM⁻¹) | 5.01*10⁻⁵ (25) |  |  |
| | Imax,TPR | 0.745 (4.6) |  |  |
| | IC50,TPR (nM) | 47.8 (16) |  |  |
| | γTPR | 0.546 (4.7) |  |  |
| | kout (h⁻¹) | 0.116 (14) |  |  |

IIV, interindividual variability, S.D. of the parameter within the population; t1/2, Konf, equivalent half-lives for k_out.
across cardiovascular measures, making comparison of results difficult. For instance, \( \gamma \) parameters in Tables 4 and 5 were generally lower than previously reported values in human (Chu et al., 1999; Duffull et al., 2000; Mehrotra et al., 2007; Gotta et al., 2016), which were generally reported to be \( \geq 1 \), but it is unclear whether this stems from differences in species or model. A few studies (Francheteau et al., 1993; Upton and Ludbrook, 2005; Snelder et al., 2014; Kamendi et al., 2016) have applied an integrative approach, including interrelationships between TPR, SV, and MAP.

Estimated baseline values of \( MAP, HR, \) and \( QT \) and their variabilities are consistent with previously published data (Howgate, 2013; Snelder et al., 2014). The estimated feedback coefficient \( FB_0 \) shows a more effective regulation in Han-Wistar rats compared with Sprague-Dawley rats (0.0053 vs. 0.0029). Predicted half-lives based on estimated fractional turnover rates \( (k_{out}) \) were consistent with published data: 3.6 minutes of \( HR \), 5.5 hours of \( SV \), and 12 minutes of \( TPR \). This suggests more rapid dynamics in \( HR \), \( QT \), and \( SV \) and slower dynamics in \( TPR \). All system parameters were estimated with good precision (1.1%–21% RSE). Although \( HR_{QT} \) was excluded from estimation because of the weak \( HR-QT \) relationship in rodents, it was included in the model structure for completeness and in anticipation of studies in nonrodent species.

Table 4 shows estimated parameters related to their specific action on \( HR, SV, TPR, \) or \( QT \) (eqs. 10–12, and 18). In contrast, Table 5 shows estimates obtained from the predicted equilibrium concentration–response relationship based on simulated steady-state data. Thus, values in Table 5 take into account the systemic interaction between the biomarkers via \( MAP \) (eqs. 2 and 10–12), whereas values in Table 4 do not.

Ivabradine is a selective blocker of the funny current that specifically reduces heart rate (Tardif et al., 2009). However, with high doses, the drastic negative chronotropic effects also affect the cardiac output, lowering the arterial blood pressure (Fig. 7). Because of the indirect effect on blood pressure, only drug-specific parameters for heart rate are presented in Table 4. However, the steady-state analysis presented in Table 5 provides potencies for both heart rate and blood pressure. The unbound plasma concentration in Table 5 shows an \( IC_{20}(HR) \) close to clinical peak (unbound) concentration, whereas the corresponding value for \( IC_{50}(MAP) \) demonstrates a 300-fold margin. Although estimated steady-state \( IC_{50} \) values for heart rate are higher than values reported for human (Duffull et al., 2000), they are 100-fold lower than corresponding values for \( MAP \), being consistent with the observation that ivabradine does not give a significant decrease in blood pressure at therapeutic plasma concentrations (Deedwania, 2013; Koruth et al., 2017). Despite a pronounced reduction in heart rate at high doses, no relevant change in QT-interval duration was observed. A weak relationship between QT and RR intervals has been previously reported in mice (Roussel et al., 2016) and more recently in unanesthetized rats (Mulla et al., 2018), suggesting that the influence of HR on QT is marginal in rodents.

Sildenafil promotes peripheral vasodilation. Simulations (Fig. 8) showed a marked increase in \( HR \) and a minor reduction in \( MAP \), consistent with reported results at therapeutic concentrations (Vardi et al., 2002). For sildenafil, \( EC_{20}(HR) \) and \( IC_{50}(MAP) \) were reached by a 5-fold increase from the lowest \( C_{ss} \), which was close to the upper limit of the therapeutic interval (Table 5).

**Fig. 11.** Model-predicted equilibrium concentration–heart rate response (solid red line), population variability (black dotted lines), and 20% response in heart rate (black dashed line) for ivabradine, sildenafil, dofetilide, and pimobendan, respectively.

### TABLE 5

| Compound      | Response | \( E_{max}/I_{max} \) | \( EC_{50}/IC_{50} \) | \( \gamma \) | \( EC_{20}/IC_{20} \) | \( C_{ss} \) | \( t_{1/2,\text{hout}} \) |
|---------------|----------|-----------------------|-----------------------|------------|-----------------------|------------|--------------------------|
| Ivabradine    | HR (I)   | 306                   | 1100                  | 0.403      | 35.7                  | 5.3–23\(^a\) | 1.4                       |
|               | MAP (I)  | 53                    | 400,000               | 0.336      | 6460                  | —          | —                        |
| Sildenafil    | HR (E)   | 1160                  | 817                   | 0.478      | 46.1                  | 10–47\(^b\) | 1.4                       |
|               | MAP (I)  | 105                   | 837                   | 0.478      | 46.1                  | —          | —                        |
| Dofetilide    | QT (E)   | 12.5                  | 50.6                  | 0.156      | 0.0073                | 0.45–4.5\(^b\) | 4.4                       |
| Pimobendan    | HR (E)   | 138                   | 144                   | 0.489      | 8.50                  | 0.95–1.9\(^d\) | 1.4                       |
|               | MAP (I)  | 12.4                  | 144                   | 0.489      | 8.50                  | —          | —                        |

\(^a^\)Excitatory response; I, inhibitory response; \( t_{1/2,\text{hout}} \), equivalent half-lives for \( k_{\text{hout}} \).

\(^b^\)VIAGRA, Pfizer summary.

\(^d^\)Allen et al. (2000).

\(^e^\)Chu et al. (1995).
Dofetilide is a highly potent blocker of the rapid component of the IKr current carried by hERG channel in humans, thereby increasing the action-potential duration and QT-interval (Trudeau et al., 1995). In this study, dofetilide induced a pronounced prolongation of the QT-interval duration, with steady-state values for both EC20 and EC50 well below the free peak concentration Cmax of the lowest dose. The rat is controversial as model for assessing QT prolongation because of its low expression or weak functionality of hERG-like channels in the ventricles (Rees and Curtis, 1996; McDermott et al., 2002). However, expression of ERG in rat heart tissue has been indicated using RNase protection assays and confirmed by blocking the tail current by E-4031 and dofetilide in rat myocytes (Wymore et al., 1997). Likewise, compounds with different modes of action (e.g., erythromycin) have been observed to affect QT prolongation in rodents (Ohtani et al., 2000; McDermott et al., 2002). These findings suggest that the contribution of the IKr component to the ventricular repolarization in rodents is present but weak, making the detection of QT prolongation in vivo challenging. The Hill coefficient of the dofetilide exposure–QT response relationship was low, suggesting a shallow equilibrium concentration–response relationship but, more importantly, an extended duration of QT response whenever the effect has been established in spite of rapidly declining plasma exposure. On the other hand, below critical exposure at the target site, rapid plasma concentration fluctuations may not be deleterious because target exposure will always be above a half-life of about 4 minutes (Table 5). The time to establish equilibrium between plasma and target-site exposure will be 3 to 4 half-lives (10–15 minutes).

Pimobendan is a calcium sensitizer and a selective PDE3 inhibitor with positive inotropic and vasodilator effects primarily used in veterinary medicine (Kitzen et al., 1988). Estimated values for the slope parameter SLSV (inotropic effects) and IC50,TPR (vasodilator effects) show a higher potency for vasodilation compared with stroke volume, which are negligible within the investigated exposure range (Table 4). This suggests that the model impacts the vasodilator effect as a cause of the observed changes in blood pressure and heart rate. However, Fig. 10 and Supplemental Fig. 9 show that the model has difficulties adapting to high-dose effects on blood pressure. The EC20/IC20 estimates correspond to a 9-fold increase from the lowest Cw. Pimobendan has been reported to lower MAP and increase HR in several animal species (Kitzen et al., 1988) but to a lesser extent in humans (Kubo et al., 1992; Chu et al., 1999).

Although the model is able to mimic both vehicle-control and drug responses, some systematic deviations between measured and modeled percentiles can be observed in Supplemental Figs. 3–9 (Supplemental Material). This might indicate a need for further model refinement and that extrapolating model predictions should be done with caution.

**New Features of the Proposed Model.** The current approach in safety pharmacology studies is to test compounds as a single dose at two to three dose levels defined to ensure that plasma exposure is at least 3- to 30-fold greater than the estimated therapeutic peak exposure. We extended previously published models to also incorporate turnover of QT response (Fig. 2). This allows quantification of pivotal safety measures, such as the margin between therapeutic and extended exposure with a risk of adverse effects, exemplified by the equilibrium EC20 and EC50. The rationale for including the QT-interval achieved in rats is primarily of translational safety reasons. This study constitutes a first evaluation of the model on rat data, but further applications of the proposed model on data from other species are ongoing.
The exploratory analysis revealed a more rational description of shifting biomarker responses during light and dark conditions, allowing the model to naturally replicate previously observed asymmetric variations in HR and MAP.

The integrated model was used to predict the concentration-response relationships of the four reference compounds and to quantify readouts pivotal for safety assessment, such as onset, intensity, and duration of response as well as the equilibrium exposure–response relationships. Here, \( EC_{50} \) was used as an example safety measure, but any appropriate measure can be used within the presented methodology.

The proposed model may be applied to highlight the plasma exposure associated with potential adverse effects within and beyond therapeutic exposure and predictions of safety margins without necessarily additional preclinical studies. This analysis demonstrates the utility of a model-based approach that integrates data from different studies and compounds for refined preclinical assessment of safety margins.

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Authorship Contributions

Participated in research design: Scheuerer, Martel, Pairet.
Conducted experiments: Scheuerer, Pairet.
Performed data analysis: Wallman, Jirstrand, Gabrielsson.
Wrote or contributed to the writing of the manuscript: Wallman, Scheuerer, Martel, Pairet, Jirstrand, Gabrielsson.

References

Allen MJ, Nichols DJ, and Oliver SD (2000) The pharmacokinetics and pharmacodynamics of oral doxifluridine after twice daily and three times daily dosing. Br J Clin Pharmacol 50:247–252.
Asakura M, Nakagura A, Tarui S, and Matsumura R (1993) Simultaneous determination of the entanionomers of pimobendan and its main metabolite in rat plasma by high-performance liquid chromatography. J Chromatogr A 614:135–141.
Bertera F, Di Vernacio MA, Mayer MA, Chiappetta D, Busentino F, Polizio AH, Taira CA, and Hich C (2012) Pharmacokinetic and pharmacodynamic properties of carvedilol in fructose hypertensive rats. Xenobiotica 42:206–219.
Bol CIJG, Danhof M, Staniski DR, and Mandema JW (1997) Pharmacokinetic-pharmacodynamic characterization of the cardiovascular, hypnic, EEG and ventilatory responses to dexmedetomidine in the rat. J Pharmacol Exp Ther 283:107–117.
Chu KM, Hu OY-P, and Shieh SM-M (1999) Cardiovascular effect and simultaneous pharmacokinetic and pharmacodynamic modeling of pimobendan in healthy normal subjects. Drug Metab Dispos 27:791–709.
Chu KM, Shieh SM-M, and Hu OY-P (1995) Pharmacokinetics and pharmacodynamics of enantiomers of pimobendan in patients with dilated cardiomyopathy and congestive heart failure after single and repeated oral dosing. Clin Pharmacol Ther 57:610–623.
Cleophas TJ (1998) Mechanisms offsetting the beneficial effects of antihypertensive drugs: a problem increasingly considered but incompletely understood. Am J Ther 5:413–419.
Colatsky T, Fermini B, Gintant G, Pierson JB, Sager P, Sekino Y, Strauss DG, and Stockbridge N (2016) The comprehensive in vitro proarrhythmia assay (CiPA) – a good manufacturing practice approach to improving cardiac safety testing in drug discovery. J Pharmacol Toxicol Methods 73:158–167.
Cross-species Scaling of Cardiovascular Safety Pharmacology – PKPD Modelling and Simulation, The University of Manchester, Manchester, UK.
Kamendi H, Barthlow H, Lengel M, Beaudoin M-E, Brody M, Kolin P, Shanes J, et al.; The Pimobendan Multicenter Research Group (1992) Beneficial effects of pimobendan on exercise tolerance and quality of life in patients with heart failure. Results of a multicenter trial. Circulation 85:942–949.
Kuhn E and Lavielle M (2005) Maximum likelihood estimation in nonlinear mixed effects models. Comput Stat Data Anal 49:150–163.
McDermott JS, Salmen HJ, Cox BF, and Gintant GA (2002) Importance of species selection in arrhythmogenic models of Q-T interval prolongation. Antimicrob Agents Chemother 46:593–599.
Mehrotra N, Gupta M, Kavara A, and Meibom B (2007) The role of pharmacokinetics and pharmacodynamics in phosphodiesterase-5 inhibitor therapy. Int J Impot Res 19:253–261.
Mounsey JP and DuMarco JP (2000) Cardiovascular drugs. Doxifluridine. Circulation 102:2665–2670.
Nella B, Gillis R, Murninkas W, Beaudoin M-E, Brody M, Kolin P, Shanes J, et al.; The Pimobendan Multicenter Research Group (1992) Beneficial effects of pimobendan on exercise tolerance and quality of life in patients with heart failure. Results of a multicenter trial. Circulation 85:942–949.
Kuhn E and Lavielle M (2005) Maximum likelihood estimation in nonlinear mixed effects models. Comput Stat Data Anal 49:150–163.
angina receiving beta-blocker therapy: a 4-month, randomized, placebo-controlled trial. Eur Heart J 30:540–548.

van Steeg TJ, Freijer J, Danhof M, and de Lange ECM (2007) Pharmacokinetic-pharmacodynamic modelling of S(-)-atenolol in rats: reduction of isoprenaline-induced tachycardia as a continuous pharmacodynamic endpoint. Br J Pharmacol 151:356–366.

Upton RN and Ludbrook GL (2005) Pharmacokinetic-pharmacodynamic modelling of the cardiovascular effects of drugs - method development and application to magnesium in sheep. BMC Pharmacol 5:5.

Zhang Y, Sun W, Pan Y, Li T, Yang X, Xu R, and Qiu X (2016) Pharmacokinetics changes of ivabradine and N-desmethylivabradine after oral administration with puerarin in rats. Int J Clin Exp Med 9:8369–8374.

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An Integrative Approach for Improved Assessment of Cardiovascular Safety Data
Mikael Wallman, Stefan Scheuerer, Eric Martel, Nicolas Pairet, Mats Jirstrand, and Johan Gabrielsson

Supplemental Material

1. Sinusoidal Model

The sinusoidal model employed for initial fitting to high- and low-resolution data during the exploratory data analysis was defined according to

\[ M_S(t) = B_S + A_S \cdot \cos \left( \frac{2\pi(t+P_S)}{24} \right) \]

where \( S \) can be either HR or MAP, \( B \) denotes the average biomarker value, \( A \) is the amplitude of circadian variations and \( P \) is the phase of the circadian variations.

2. Residual Error Model

Due to the systematic differences between light- and dark-hour response-time data, two separate residual error models were used to describe light- and dark conditions, each following a log-normal distribution. The maximum likelihood estimates of parameters from the exploratory data analysis corresponding to light and dark hours were

\[ m_{light} = (3.12 \ 2.13), \quad C_{light} = \begin{pmatrix} 0.432 \\ 0.195 \\ 0.195 \\ 0.381 \end{pmatrix} \]

\[ m_{dark} = (3.89 \ 2.59), \quad C_{dark} = \begin{pmatrix} 0.983 \\ 0.495 \\ 0.495 \\ 0.374 \end{pmatrix} \]

These were used as initial values in the parameter estimation. During parameter estimation, the mode of the lognormal distribution was kept at zero.

3. Relationship Between Heart Rate and QT-Interval

Relationship between heart rate and QT-interval for placebo and the highest dose (30 mg/kg) of ivabradine.
Fig. S1. Experimental data (circles) for heart rate and QT-interval over 24 h for placebo (left panel) and 30 mg/kg of ivabradine (right panel).

4. Fixed Model Parameters

| Parameters         | Values |
|--------------------|--------|
| $BSL_{CO}$ (mL/min) | 129    |
| $FB_{0,MAP}$       | -1.98  |
| $BSL_{0,MAP}$ (mmHg) | 155    |
| $HR_{SV}$ (min$^{-1}$) | 0.312  |
| $k_{HD}$ (h$^{-1}$) | 4.70   |
| $amp_{HR}$         | 0.0918 |
| $hor_{HR}$ (h)     | 10     |
| $amp_{TPR}$        | 0.0918 |
| $hor_{TPR}$ (h)    | 9      |

5. Equilibrium Concentration-Biomarker Response Relationships

Drug concentration-biomarker response ($HR$, $TPR$, $SV$, and $MAP$) relationships in steady state (SS) were predicted for each compound. This is equivalent to solving following system of equations:

\[
HR_{SS} = (1 + EFF_{HR})(1 - FB \cdot MAP_{SS})k_{in,HR}/k_{out,HR} \tag{S1}
\]

\[
SVT_{SS} = (1 + EFF_{SVT})(1 - FB \cdot MAP_{SS})k_{in,SVT}/k_{out,SVT} \tag{S2}
\]

\[
TPR_{SS} = (1 + EFF_{TPR})(1 - FB \cdot MAP_{SS})k_{in,TPR}/k_{out,TPR} \tag{S3}
\]

\[
QT_{SS} = k_{in,QT}(1 - QT_{HR} \cdot HR_{SS})(1 + EFF_{QT})/k_{out,QT} \tag{S4}
\]
\[ MAP_{SS} = HR_{SS} \cdot TPR_{SS} \cdot SV_{SS} \cdot (1 - HR_{SV} \cdot \log \frac{HR_{SS}}{BSL_{HR}}) \] (S5)

The pharmacodynamic model does not admit an explicit analytical solution for a concentration-biomarker response to be derived. Here, the system was solved using the fminsearch function in MATLAB (version R2019b, The Mathworks, Inc., Natick, Mass.), but any standard numerical solver can be used.

6. Baseline values

The baseline values (BSL) are defined as the equilibrium values of biomarker responses (HR, SV, TPR, MAP or QT) when no influences from handling, circadian variations, or drugs are present (i.e., CR = 0, EFF = 0, HD = 0) (Snelder et al. 2014). In this section we show that this definition is consistent with the pharmacodynamic model equations.

When HR, TPR, SV, and QT are constant in time and equal to their corresponding baseline parameter values (and CR = 0, EFF = 0, HD = 0), Equation 2 gives

\[ BSL_{MAP} = BSL_{HR} \cdot BSL_{SV} \cdot BSL_{TPR} \cdot (1 - HR_{SV} \log \frac{BSL_{HR}}{BSL}) = BSL_{HR} \cdot BSL_{SV} \cdot BSL_{TPR} \] (S1)

Utilizing this in Equation 10 one gets

\[ \frac{dHR}{dt} = k_{in,HR}(1 + 0)(1 - FB \cdot BSL_{MAP})(1 + 0 + 0) - k_{out,HR} \cdot BSL_{HR} \]
\[ = \frac{k_{out,HR}BSL_{HR}}{1 - FB \cdot BSL_{MAP}}(1 - FB \cdot BSL_{MAP}) - k_{out,HR} \cdot BSL_{HR} = 0 \] (S2)

where we have used Equation 14 in the second equality. Similarly, for Equations 11, 15 and S1 (SV, S3), for Equations 12, 16 and S1 (TPR, S4), and Equations 18, 20 and S1 (QT, S5), one gets

\[ \frac{dSV}{dt} = k_{in,SV}(1 - FB \cdot BSL_{MAP})(1 + 0) - k_{out,SV} \cdot BSL_{SV} \]
\[ = \frac{k_{out,SV}BSL_{SV}}{1 - FB \cdot BSL_{MAP}}(1 - FB \cdot BSL_{MAP}) - k_{out,SV} \cdot BSL_{SV} = 0 \] (S3)

\[ \frac{dTPR}{dt} = k_{in,TPR}(1 + 0)(1 - FB \cdot BSL_{MAP})(1 + 0 + 0) - k_{out,TPR} \cdot BSL_{TPR} \]
\[ = \frac{k_{out,TPR}BSL_{TPR}}{1 - FB \cdot BSL_{MAP}}(1 - FB \cdot BSL_{MAP}) - k_{out,TPR} \cdot BSL_{TPR} = 0 \] (S4)

\[ \frac{dQT}{dt} = k_{in,QT}(1 - QT_{HR} \cdot BSL_{HR})(1 + 0 + 0) - k_{out,QT} \cdot BSL_{QT} \]
\[ = \frac{k_{out,QT}BSL_{QT}}{1 - QT_{HR} \cdot BSL_{HR}}(1 - QT_{HR} \cdot BSL_{HR}) - k_{out,QT} \cdot BSL_{QT} = 0 \] (S5)

Since Equations S2-S5 all evaluate to zero, this demonstrates that the baseline parameters correspond to a steady state solution (a.k.a. equilibrium values) of the differential equations when circadian rhythm, handling, and drug effects are set to zero.
7. Visual predictive checks

In this section we provide visual predictive checks (VPCs) of the estimation results for ivabradine, sildenafil, dofetilide and pimobendan, based on 4000 model simulations each, including individual and residual variability. All figures include 87.5% percentiles (7/8 animals) and median values for measured biomarkers (solid lines) and simulated data (dashed lines). Colored fields correspond to 95% confidence interval of percentiles (blue) and of median (red). Black dots correspond to data points. An overlap between all three fields is present in all figures, visible as a central purple field.

**Fig. S3.** VPC for response in heart rate (HR) after a single oral dose of ivabradine at 0, 3, 10 and 30 mg·kg\(^{-1}\) corresponding to 0, 6.40, 21.3 and 64.0 µmol·kg\(^{-1}\).

**Fig. S4.** VPC for response in blood pressure (MAP) after a single oral dose of ivabradine at 0, 3, 10 and 30 mg·kg\(^{-1}\) corresponding to 0, 6.40, 21.3 and 64.0 µmol·kg\(^{-1}\).

**Fig. S5.** VPC for response in heart rate (HR) after a single oral dose of sildenafil at 0, 3, 10 and 30 mg·kg\(^{-1}\) corresponding to 0, 6.32, 21.1 and 63.2 µmol·kg\(^{-1}\).
Fig. S6. VPC for response in blood pressure (MAP) after a single oral dose of sildenafil at 0, 3, 10 and 30 mg·kg⁻¹ corresponding to 0, 6.32, 21.1 and 63.2 µmol·kg⁻¹.

Fig. S7. VPC for response in QT-interval (QT) after a single oral dose of dofetilide at 0, 3, 10 and 30 mg·kg⁻¹ corresponding to 0, 6.79, 22.6 and 67.9 µmol·kg⁻¹.

Fig. S8. VPC for response in heart rate (HR) after a single oral dose of pimobendan at 0, 3, 10 and 30 mg·kg⁻¹ corresponding to 0, 8.96, 29.9 and 89.7 µmol·kg⁻¹.

Fig. S9. VPC for response in blood pressure (HR) after a single oral dose of pimobendan at 0, 3, 10 and 30 mg·kg⁻¹ corresponding to 0, 8.96, 29.9 and 89.7 µmol·kg⁻¹.
8 Regression to equilibrium

In this section we illustrate the model regression described in the "Equilibrium Concentration-Biomarker Response Analyses" section of the Result, using heart rate effects from pimobendan as an example.

**Fig. S10.** Modelled equilibrium heart rate (circles) and regression to Equation 22a (solid line) for pimobendan effects on heart rate.