An Integrative Approach for Improved Assessment of Cardiovascular Safety Data

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d) Non-standard abbreviations:
BP – blood pressure
BSL – baseline
CO – cardiac output
CR – circadian rhythm
dBP – diastolic blood pressure
EFF – drug effect
EM – expectation-maximization
FB – feedback
HD – handling effect
HR – heart rate
MAP – mean arterial blood pressure
NLME – non-linear mixed effects
PDE5 – phosphodiesterase-5
PDE3 – phosphodiesterase-3
SAN – sinoatrial node
sBP – systolic blood pressure
SV – stroke volume
TPR – total peripheral resistance
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 co-dependencies 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 modelled jointly including different compounds, exposure- and response-time courses using a non-linear mixed effects-approach. Estimated fractional turnover rates (h$^{-1}$, %RSE within brackets) were 9.45 (15), 30.7 (7.8), 3.8 (13) and 0.115 (1.7) of QT, HR, TPR and SV, respectively. Potencies (nM, %RSE within brackets) were $IC_{50}$=475 (11), $IC_{50}$=4.01 (5.4), $EC_{50}$=50.6 (93) and $IC_{50}$=47.8 (16), and efficacies (%RSE within brackets) 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 pre-clinical 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 pimobondan), where 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 e.g., EC\textsubscript{20} 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 co-dependencies between safety variables are typically shown, simultaneous modelling of all data results in more informative insights about the test substance (e.g., potency $EC_{50}$) and systems parameters (e.g., fractional turnover rates $k_{out}$). Co-dependencies act both directly, as in QT-adaptation to HR change, and indirectly, e.g. via baroreflex feedback (Cleophas, 1998; Holzgrefe et al., 2014). In addition, useful information about primary drug effects is neglected when co-dependencies 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 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/pharmacodynamic PK/PD modelling has proven to be a valuable tool, combining quantities such as HR, BP and QT, using a non-linear mixed effects (NLME) approach.

Semi-mechanistic models of cardiovascular physiology have been used for over 60 years, beginning with pivotal work by Noble and Guyton et al. (Noble, 1962; 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 et al., 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 analysis extends the pivotal Guyton et al 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 semi-mechanistic 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 Figure 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, sildenafil, dofetilide, and pimobendan) that have different mechanisms of action are analyzed using all biomarker data and different tool compounds simultaneously, thereby yielding separate estimates of drug- and system properties. In Step 3, equilibrium predictions of concentration-biomarker response relationships are calculated, visualized graphically, and quantified (e.g., in terms of model derived $EC_{20}$ for drug effects on HR, BP and QT-interval). This step serves as the basis for future safety assessment.

FIGURE 1
Materials and Methods

Animals

Male rats (Han-Wistar, Charles River) weighing 450 to 600 g, 3 to 19 months old, were used. Rats were chronically instrumented for the telemetric collection of arterial pressure, electrocardiogram (ECG) and body temperature as described previously (Schierok et al., 2000). Groups of up to 3 rats were housed together in cages (type IV-1815 cm$^2$) in a room with a 12-hour light/dark cycle and controlled temperature and humidity, 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 consists of elements of a Data Science International system (DSI, Minnesota, USA) for cardiovascular measurements (BP, HR, temperature and ECG). A calibration amplifier was added 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). Afterwards, the transmitters were sterilized with ethylene oxide and after 24 - 48 h of venting, kept under aseptic conditions until implantation.

Implant Surgery

Rats were anaesthetized 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, Narcoquip (Völcker, Germany) and anesthesia was maintained with 2% isoflurane. Under aseptic conditions a midline abdominal incision (2 - 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, (Novalgin, Hoechst, Germany), intraperitoneally (i.p.) 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 following intravenous (i.v.) and oral (p.o.) administration. A single dose of test compound was either administered orally (volume administered: 2 mL/100 g body weight suspended in Natrosol 0.5% + 0.015% Tween80, n=3 subjects per study) or intravenously (volume administered: 0.5 mL/100g body weight; dissolved in a 20% HP-β-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, 5, 0.5 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 min at 8,300 x g 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 (LC/MS/MS) with an analytical range of 1 to 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, and sildenafil, at 3 dose levels (volume administered: 1 mL/100 g body weight suspended in Natrosol 0.5% + 0.015% Tween80) orally (Table 1). Each study comprised 8 individual animals except for the sildenafil study, which comprised 31 individual animals.

TABLE 1

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 signal was confirmed with the help of a commercially available radio receiver. Systolic and diastolic blood pressure (sBP, dBP) and ECG were continuously measured. From these data, three cardiovascular variables were derived: HR, MAP (derived as 1/3 sBP + 2/3 dBP) and the QT-interval. All measurements began 1.5 hour 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) as well as ECG signals. From the arterial BP signal, systolic, diastolic, and mean arterial BP (MAP) 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 Modelling

Drug Exposure Model

A schematic plot of the absorption- and disposition models of ivabradine, sildenafil, dofetilide and pimobendan is shown in Figure 2.

FIGURE 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:

\[
\frac{dA_g}{dt} = -k_a \cdot A_g 
\]

(1a)

\[
V_p \cdot \frac{dC_p}{dt} = k_a \cdot A_g \cdot F - CL \cdot C_p + CL_d \cdot C_t - CL_d \cdot C_p 
\]

(1b)

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

(1c)

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

(1d)

\( A_g, C_p, \) and \( C_t \) denote amount in the gut compartment, concentration in plasma, and concentration in tissue, respectively. The model parameters \( k_a, CL, F, V_p, CL_d, \) and \( V_t \) correspond to absorption rate constant, plasma clearance, oral bioavailability, plasma volume of distribution, inter-compartmental distribution, and tissue volume, respectively.
Note that a hypothetical metabolite compartment (Equation 1d) was added to capture active metabolite(s) from the pimobendan parent compound. The rate constant $k_{\text{met}}$ 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, dofetilide, and pimobendan is shown in Figure 3. The model consists of four biomarker responses: $HR$, $TPR$, $SV$ 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 adaption of $QT$ to $HR$ (Figure 3).

**FIGURE 3**

The pharmacodynamic model is based on the approach presented in (Guyton et al., 1972; Snelder et al., 2014), consisting of three coupled turnover models, describing $HR$, stroke volume $SV$, and $TPR$, respectively. Each turnover model contains its individual turnover rate $k_{\text{in}}$ and fractional turnover rate $k_{\text{out}}$. The coupling of individual processes occurs via $MAP$. The time course of $MAP$ is modelled as a non-linear function according to Equation 2.

\[
CO = HR \cdot SV \cdot \left(1 - HR_{SV} \cdot \log\left(\frac{HR}{BSL_{HR}}\right)\right) \tag{2a}
\]

\[
MAP = CO \cdot TPR \tag{2b}
\]

$CO$ denotes cardiac output, $HR_{SV}$ is a scaling constant and $BSL_{HR}$ the baseline value of heart rate. The term “baseline value” refers to the equilibrium value of a biomarker response (here $HR$) when no influences from handling, circadian variations or drugs are present. This means that the baseline values are constant over time. $MAP$ is coupled, via feedback, to an inhibitory action on $HR$, $SV$, and $TPR$. The pharmacodynamic model also includes circadian rhythm ($CR$) modelled as a 24h sinus function by means of Equations 3 and 4:
\[ CR_{HR} = \text{amp}_{HR} \cdot \cos \frac{2\pi (t + \text{hor}_{HR})}{24} \]  
(3)

\[ CR_{TPR} = \text{amp}_{TPR} \cdot \cos \frac{2\pi (t + \text{hor}_{TPR})}{24} \]  
(4)

with \( \text{amp}_{HR} \), \( \text{hor}_{HR} \), \( \text{amp}_{TPR} \), and \( \text{hor}_{TPR} \) representing amplitude and phase of \( HR \) and \( TPR \). Handling effects (\( HD \)) from the dosing procedures are modelled as an exponential process for \( HR \)

\[ HD_{HR} = \begin{cases} P_{HR} \cdot e^{-k_{HD}(t-t_0)}, & t \geq t_0 \\ 0, & t < t_0 \end{cases} \]  
(5)

and \( TPR \)

\[ HD_{TPR} = \begin{cases} P_{TPR} \cdot e^{-k_{HD}(t-t_0)}, & t \geq t_0 \\ 0, & t < t_0 \end{cases} \]  
(6)

with \( P \) being the amplitude, \( k_{HD} \) the time constant for the exponential decay, and \( t_0 \) handling time. \( CR \) and \( HD \) are assumed to affect the turnover rate \( k_r \) of \( HR \) and \( TPR \) only, with no direct effect on \( SV \). Drug intervention on \( HR \), \( TPR \), or \( QT \) is either stimulatory action \( S(C) \) given drug exposure \( C \)

\[ S(C) = \frac{E_{max}C^\gamma}{EC_{50}^\gamma + C^\gamma} \]  
(7)

or inhibitory action \( I(C) \) given drug exposure \( C \) and metabolite concentration \( C_m \) (only present for pimobendan).

\[ I(C) = \frac{I_{max}(C+C_m)^\gamma}{IC_{50}^\gamma + (C+C_m)^\gamma} \]  
(8)
For stimulatory effects on $SV$ (from pimobendan), a linear model with a single slope parameter $SL$ was used:

$$\bar{S}(C) = (C + C_m) \cdot SL$$  \hspace{1cm} \text{(9)}$$

Table 2 and Equations 7 and 8 quantify each drug action by means of its own set of drug parameters $E_{max}/I_{max}$, $EC_{50}/IC_{50}$ and $\gamma$.

### TABLE 2

The turnover rate of $HR$ is expressed as

$$\frac{dHR}{dt} = k_{in,HR}(1 + CR_{HR})(1 - FB \cdot MAP)(1 + EFF_{HR} + HD_{HR}) - k_{out,HR} \cdot HR$$  \hspace{1cm} \text{(10)}$$

where turnover rate of $HR$ is modelled by baseline turnover $k_{in,HR}$, circadian rhythm $CR_{HR}$, $FB$ from $MAP$, handling $HD_{HR}$, and fractional turnover rate $k_{out,HR}$. The drug effects $EFF_{HR}$ can be either stimulatory ($EFF_{HR}=S(C)$ Equations 7) or inhibitory ($EFF_{HR}=I(C)$, Equation 8), with corresponding definitions for $EFF_{TPR}$ and $EFF_{QT}$. $EFF_{SV}$ follows Equation 9. The turnover of the $SV$ is expressed as

$$\frac{dSV}{dt} = k_{in,SV}(1 - FB \cdot MAP)(1 + EFF_{SV}) - k_{out,SV} \cdot SV$$  \hspace{1cm} \text{(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

$$\frac{dTPR}{dt} = k_{in,TPR}(1 + CR_{TPR})(1 - FB \cdot MAP)(1 + EFF_{TPR} + HD_{TPR}) - k_{out,TPR} \cdot TPR$$  \hspace{1cm} \text{(12)}$$
which is assumed to have the same structure to that of \( HR \). Initial conditions for Equations 10-12, as well as Equation 18 below, were determined numerically as described in the section Pharmacodynamic Model Parameters below.

The feedback \( FB \) from \( MAP \) is modelled as dependent on the individual baseline value \( BSL_{MAP} \) for \( MAP \), according to

\[
FB = FB_0 \left( \frac{BSL_{MAP}}{BSL_0,MAP} \right)^{FB_{0,MAP}} \tag{13}
\]

where \( FB_0 \) regulates the amount of feedback from \( MAP \), and \( BSL_0,MAP \) and \( FB_{0,MAP} \) are empirically derived constants regulating the shape of the feedback relationship.

Turnover rates \( k_{in} \) 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 \( k_{out} \) and the feedback parameter \( FB \):

\[
k_{in,HR} = \frac{k_{out,HR} BSL_{HR}}{1 - FB \cdot BSL_{MAP}} \tag{14}
\]

The turnover rate of the \( SV \) was described according to

\[
k_{in,SV} = \frac{k_{out,SV} BSL_{SV}}{1 - FB \cdot BSL_{MAP}} \tag{15}
\]

The turnover rate of the \( TPR \) was described according to

\[
k_{in,TPR} = \frac{k_{out,TPR} BSL_{TPR}}{1 - FB \cdot BSL_{MAP}} \tag{16}
\]
where $BSL_{HR}$, $BSL_{SV}$, $BSL_{TPR}$, 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 Section 6 of the Supplemental material.

**Modelling 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

$$\frac{dQT}{dt} = k_{in,QT} (1 - QT \cdot HR) \cdot (1 + EFF_{QT} + HD_{QT}) - k_{out,QT} \cdot QT$$ (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 Equations 5 and 6:

$$HD_{QT} = \begin{cases} P_{QT} \cdot e^{-k_{HD,QT}(t-t_0)}, & t \geq t_0 \\ 0, & t < t_0 \end{cases}$$ (19)

The turnover rate of the QT-interval was expressed as

$$k_{in,QT} = \frac{k_{out,QT} \cdot BSL_{QT}}{1 - QT_{HR} \cdot BSL_{HR}}$$ (20)

where $BSL_{QT}$ and $BSL_{HR}$ denote baseline values for QT and HR, respectively.
Modelling 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 material 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 sets of residuals for HR and one sets 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 Equations 1a-1c.

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 where 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 inter-individual variability directly from data. Specifically, the analysis was done using a custom implementation of the EM-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 \( BSL_{HR}, BSL_{MAP} \), and \( BSL_{QT} \) were assumed to vary across individuals. No inter-individual variability was added to drug parameters \( E_{\text{max}}, EC_{50} \) and \( \gamma \) of the biomarker responses (\( HR, SV, TPR \) and \( QT \)). A Gaussian prior with a mean of 0.126 and a standard deviation of 0.013 was used to constrain \( k_{\text{out}, SV} \) 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 modelled biomarker to its baseline value (\( BSL \)) and simulating the model over a long time span (336 h), 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 where the unbound plasma concentration is constant and where 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 (\( t = 96h \)) with a logarithmically spaced exposure range. Parameters for handling (\( amp_{HR}, amp_{TPR} \)), diurnal variations (\( P_{HR}, P_{TPR}, P_{QT} \)) 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 Equation 22a or 22b below, was then regressed to the simulated concentration-biomarker response data, i.e.,

\[
E_T = E_0 + \frac{E_{\text{max}} \cdot C_T^\gamma}{EC_{50}^\gamma + C_T^\gamma} \tag{22a}
\]
\[ E_T = E_0 - \frac{I_{\text{max}} \cdot C_T}{IC_{50} + C_T} \]  

(22b)

depending on if 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 where \( C_T = EC_{50} \cdot ((E_T - E_0)/(E_0 + E_{\text{max}} - E_T))^{1/\gamma} \), 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 modelled as linear according to Equation 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 24h vehicle control study, are shown in Figure 4.

HR and MAP responses randomly deviated towards higher values and were superimposed on a diurnal baseline wave (example results for one of the animals are shown in Figure 5). This is particularly evident for \( HR \) (upper panel, Figure 4), and \( MAP \) (lower panel, Figure 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 datasets. 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 Figure 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. 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 (depicted in Figure 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 \( MAP \) (data not shown) was found (see Supplemental Figure S1). 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 (Figure 2 and Equations 1a – 1c) was simultaneously fitted to intravenous and oral data shown in Figure 6 for each compound. Final parameter estimates of disposition (clearance Cl, inter-compartmental distribution Clb, volumes of the central and peripheral compartments) and absorption parameters (absorption rate constant ka and bioavailability F) are given in Table 3.

FIGURE 6

TABLE 3

The Goodness-of-Fit was assessed by means of residual analysis. Note that several of the parameters are poorly estimated (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 in order to have a pharmacokinetic model that predicts exposure data well and can serve as a 'driver' of biomarker response data. Concentration-time courses of experimental and model-predicted data are shown in Figure 6. The oral model was then used to predict the time courses of unbound plasma concentrations for the actual dosing regimens used in Step 2 of pharmacodynamic assessment of data. A common concentration-time course was used for all animals in each drug study.

Pharmacodynamic Model of Biomarker Responses

The agreement between observed and model-predicted data was assessed using predictive plots presented in Figures 7-10. 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 over-predict 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 relative standard error RSE% are presented in Table 4. Additional fixed model parameters are listed in Supplemental Table S1.

TABLE 4

For the systems parameters, estimated baseline values for heart rate $BSL_{HR}$, mean arterial pressure $BSL_{MAP}$, QT-interval $BSL_{QT}$, the parameter regulating baroreflex feedback $FB_0$, and handling, $P_{HR}$ and $P_{TPR}$, were estimated with good precision. The available data exhibited a very weak relationship between $HR$ and $QT$ (see Supplemental Figure S1) which led us to exclude the $HR_{QT}$ from the estimation by fixing it to 0. The inter-individual variability was low (5%). Drug- specific parameters, such as $E_{max}$, $EC_{50}$ and $\gamma$, had generally high precision, except for the pimobendan action on stroke volume (RSE% > 100%) and dofetilide $IC_{50}$ (RSE% = 93%). The potencies of ivabradine ($HR$) and pimobendan ($SV$) were estimated to be 475 and 604 nM, respectively, which is beyond the observed exposure range, but still within published data (Kitzen and Winbury, 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 gamma 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 was assessed for each test compound (Figures 11-13). Supplemental Section 5 shows how data were generated.

![FIGURE 11](image1)

![FIGURE 12](image2)

![FIGURE 13](image3)

$E_{\text{max}} / I_{\text{max}}, E_{C_{50}} / I_{C_{50}}$ and $\gamma$ of the equilibrium concentration-response relationships we estimated from model-generated data in Table 4 (Figures 11-13, Table 5) by regressing Equations 22a or 22b to the simulated data. In the regression, 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.

**TABLE 5**
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, 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 (200-300g) and showed slightly different pharmacokinetic properties compared to the larger Han-Wistar (450-600g) used here (Figure 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 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), generally reported to be ≥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 inter-relationships 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 compared to Sprague-Dawley rats (0.0053 versus 0.0029).

Predicted half-lives based on estimated fractional turnover rates $k_{out}$ were consistent with published data: 3.6 min of HR, 5.5h of SV, and 12 min of TPR, which 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 non-rodent species.

Table 4 shows estimated parameters related to their specific action on HR, SV, TPR, or QT (Equations 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 (Equations 2 and 10-12), while values in Table 4 do not.
Ivabradine is a selective blocker of the $I_f$-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 (Figure 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, while the corresponding value for $IC_{20} (MAP)$ demonstrates a 300-fold margin. Although estimated steady state $IC_{50}$ 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 (Figure 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 $C_u$, close to the upper limit of the therapeutic interval (Table 5).

Dofetilide is a highly potent blocker of the rapid component of the $I_{Kr}$-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 $EC_{20}$ and $EC_{50}$ well below the free peak concentration $C_{max}$ of the lowest dose. The rat is controversial as model for assessing QT-prolongation due to 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 I_{Kr}-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 lag after the plasma exposure by a half-life of about 4 minutes (Table 5). The time to establish equilibrium between plasma and target site exposure will be 3-4 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 and Winbury, 1988). Estimated values for SLSV (inotropic effects) and IC_{50,TPR} (vasodilator effects) show a higher potency for vasodilation compared to 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, Figure 10 and supplemental Figure S9 show that the model has difficulties adapting to high-dose effects on blood pressure. The EC_{20}/IC_{20} estimates correspond to a nine-fold increase from the lowest C_u. Pimobendan has been reported to lower MAP and increase HR in several animal species (Kitzen and Winbury, 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 modelled percentiles can be observed in Figures S3-S9 (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 (Figure 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 $EC_{20}$ and $EC_{50}$. 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, $EC_{20}$ 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 pre-clinical assessment of safety margins.
Acknowledgments

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Authorship Contributions

*Participated in research design:* SS, NP, EM

*Conducted experiments:* NP, SS

*Contributed new reagents or analytic tools:* -

*Performed data analysis:* MW, JG, MJ

*Wrote or contributed to the writing of the manuscript:* MW, JG, EM, SS, NP, MJ
References


Deedwania P (2013) Selective and Specific Inhibition of If with Ivabradine for the Treatment of Coronary Artery Disease or Heart Failure. Drugs 73:1569–1586.


Footnotes

Conflict of Interest Statement: No author has an actual or perceived conflict of interest with the contents of this article.

Financial Disclosure: This work received no external funding.
Legends for Figures

**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., potencies) parameters. **Step 3:** Steady-state concentration-biomarker response relationships are established for heart-rate, blood pressure and QT-response.

**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 (Equation 1d) was added to capture active metabolite(s) from parent compound. The rate constant $k_{\text{met}}$ was estimated from biomarker response-time data and is not part of the plasma exposure analysis (Kitzen and Winbury, 1988).

**Fig. 3.** Schematic diagram of the extended model. Heart rate $HR$, stroke volume $SV$, total peripheral resistance $TPR$, and QT-interval $QT$ are described with their respective zero-order turnover rates (e.g., $k_{0,HR}$) and first-order fractional turnover rates (e.g., $k_{\text{out},HR}$). The experimentally measured biomarker responses, such as heart rate, mean arterial pressure and QT-interval are shown as dark grey boxes. There are no experimental data on total peripheral resistance and stroke volume (light grey boxes). Mean arterial pressure is also a derived function of $HR$, $SV$, and $TPR$. $MAP$ acts on $HR$, $SV$, and $TPR$ via negative feedback (dashed lines). The QT-interval is inhibited by $HR$ (dashed line). Drug action on $HR$, $SV$, $TPR$ and/or QT-interval is either via stimulatory action (e.g., $S(P)$, where pimobendan stimulates the turnover rate of $SV$) or via inhibitory action (e.g., $I(I)$, where ivabradine inhibits turnover rate of $HR$). Pimobendan and sildenafil both exhibit inhibitory action turnover rate of $TPR$ ($I(P, S)$). Dofetilide stimulates the turnover rate of the QT-interval ($S(D)$). Solid arrows are production and loss processes, and dashed arrows are control actions.
Fig. 4. **Upper panel:** High-resolution response-time data of heart rate during a 24-hour baseline cycle. **Lower panel:** High-resolution response-time data of blood pressure (MAP) during a 24-hour baseline cycle. Dashed vertical black lines indicate start and stop of the 12-hour light and dark cycle. Red arrow shows time of oral (saline) dosing.

Fig. 5. **Upper row:** Observed heart rate and blood pressure (MAP) versus 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) versus 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.

Fig. 7. Experimental data (gray dots), model-predicted average (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 (grey line) is superimposed on experimental data.
**Fig. 8.** Experimental data (gray dots), model-predicted average (solid line) and 95% prediction interval (dashed lines) response of heart rate and 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$^{-1}$. The predicted vehicle-control response (grey line) is superimposed on experimental data.

**Fig. 9.** Experimental data (gray dots), model-predicted average (solid line) and 95% prediction interval (dashed lines) response of QT-interval 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$^{-1}$. The predicted vehicle-control response (grey line) is superimposed on experimental data.

**Fig. 10.** Experimental data (gray dots), model-predicted average (solid line) and 95% prediction interval (dashed lines) response of heart rate and blood pressure (MAP) 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$^{-1}$. The predicted vehicle-control response (grey line) is superimposed on experimental data.

**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.

**Fig. 12.** Model-predicted equilibrium concentration-blood pressure (MAP) response (solid red line), population variability (black dotted lines) and 20% change in blood pressure (black dashed line) for ivabradine, sildenafil, dofetilide, and pimobendan, respectively.

**Fig. 13.** Model-predicted equilibrium concentration-QT response (solid red line), population variability (black dotted lines) and 20% change in QT-response (black dashed line) for ivabradine, sildenafil, dofetilide, and pimobendan, respectively.
# Tables

## TABLE 1
Compound, mode of action, doses, study period, molecular weight and unbound fraction in rats.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mode of action</th>
<th>Doses (mg/kg)</th>
<th>Study period (h)</th>
<th>Molecular weight (g·mol⁻¹)</th>
<th>$f_u$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivabradine</td>
<td>blocks $I_f$ and HCN channels in SAN</td>
<td>0, 3, 10, 30</td>
<td>24</td>
<td>468.6</td>
<td>0.25</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>PDE5-inhibitor</td>
<td>0, 3, 10, 30</td>
<td>7</td>
<td>474.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>$I_{Kr}$-channel blocker</td>
<td>0, 1, 3, 10</td>
<td>7</td>
<td>441.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Pimobendan</td>
<td>Ca²⁺ sensitizer, PDE3-inhibitor</td>
<td>0, 1, 3, 10</td>
<td>24</td>
<td>334.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Unbound fraction of test compounds in rats ($f_u$); Funny current ($I_f$); Sino-atrial node (SAN); Hyperpolarization-activated cyclic nucleotide–gated channels (HCN); Sinoatrial (SA) node; Phosphodiesterase-5 (PDE5); Phosphodiesterase-3 (PDE3); rapid delayed rectifier current ($I_{Kr}$). Unbound fractions from in-house data and FDA approval documentation (www.pharmapendium.com).
<table>
<thead>
<tr>
<th>Compound name</th>
<th>Drug action</th>
<th>$I_{HR}$</th>
<th>$S_{SV}$</th>
<th>$I_{TPR}$</th>
<th>$S_{QT}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ivabradine</td>
<td>↓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>sildenafil</td>
<td></td>
<td>-</td>
<td>-</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>dofetilide</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>pimobendan</td>
<td></td>
<td>-</td>
<td>↑</td>
<td>↓</td>
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</table>
### TABLE 3
Final pharmacokinetic parameter estimates and their relative standard errors (RSE%)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cl</th>
<th>Vp</th>
<th>Cl_d</th>
<th>V_t</th>
<th>k_a</th>
<th>F</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(L·h⁻¹·kg⁻¹)</td>
<td>(L·kg⁻¹)</td>
<td>(L·h⁻¹·kg⁻¹)</td>
<td>(L·kg⁻¹)</td>
<td>(h⁻¹)</td>
<td>(%)</td>
</tr>
<tr>
<td>Ivabradine</td>
<td>2.88 (24)</td>
<td>1.45 (25)</td>
<td>0.408 (&gt;100)</td>
<td>0.427 (&gt;100)</td>
<td>0.61 (31)</td>
<td>19 (26)</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>3.55 (33)</td>
<td>1.29 (19)</td>
<td>0.137 (&gt;100)</td>
<td>0.135 (&gt;100)</td>
<td>0.80 (31)</td>
<td>1.4 (33)</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>3.50 (24)</td>
<td>1.52 (18)</td>
<td>0.328 (&gt;100)</td>
<td>0.397 (&gt;100)</td>
<td>0.60 (26)</td>
<td>37 (22)</td>
</tr>
<tr>
<td>Pimobendan</td>
<td>4.08 (18)</td>
<td>2.71 (22)</td>
<td>2.03 (&gt;100)</td>
<td>0.861 (&gt;100)</td>
<td>1.5 (20)</td>
<td>21 (19)</td>
</tr>
</tbody>
</table>
TABLE 4
Estimated systems and drug parameters in rats, and their precision (RSE%)

<table>
<thead>
<tr>
<th>Drug vs. system</th>
<th>Parameters</th>
<th>Estimates (RSE%)</th>
<th>IIV (RSE%)</th>
<th>$t_{1/2,k_{out}}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>System parameters</td>
<td>$BSL_{HR}$ (beats min$^{-1}$)</td>
<td>317. (1.4)</td>
<td>24. (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$BSL_{MAP}$ (mmHg)</td>
<td>102. (1.7)</td>
<td>7.2 (13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$BSL_{QT}$ (ms)</td>
<td>64.3 (2.1)</td>
<td>3.9 (11)</td>
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<tr>
<td></td>
<td>$FB_0$</td>
<td>0.00532 (0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P_{HR}$</td>
<td>3.25 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P_{TPR}$</td>
<td>1.21 (2.0)</td>
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<tr>
<td></td>
<td>$k_{out,OT}$ (h$^{-1}$)</td>
<td>9.45 (15)</td>
<td>4.4</td>
<td></td>
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<tr>
<td></td>
<td>$k_{out,HR}$ (h$^{-1}$)</td>
<td>30.7 (7.8)</td>
<td>1.4</td>
<td></td>
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<tr>
<td></td>
<td>$k_{out,TPR}$ (h$^{-1}$)</td>
<td>3.8 (13)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_{out,SV}$ (h$^{-1}$)</td>
<td>0.115 (1.7)</td>
<td>360</td>
<td></td>
</tr>
<tr>
<td>Ivabradine</td>
<td>$I_{max,HR}$</td>
<td>0.944 (1.7)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>$IC_{50,HR}$ (nM)</td>
<td>475. (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\gamma_{HR}$</td>
<td>0.511 (3.6)</td>
<td></td>
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<tr>
<td>Sildenafil</td>
<td>$I_{max,TPR}$</td>
<td>1.00 (1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$IC_{50,TPR}$ (nM)</td>
<td>4.01 (5.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\gamma_{TPR}$</td>
<td>0.63 (2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dofetilide</td>
<td>$E_{max,QT}$</td>
<td>0.195 (9.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$EC_{50,QT}$ (nM)</td>
<td>50.6 (93)</td>
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<td></td>
<td>$\gamma_{QT}$</td>
<td>0.156 (21)</td>
<td></td>
<td></td>
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<tr>
<td>Pimobendan</td>
<td>$SL_{SV}$ (nM$^{-1}$)</td>
<td>$5.01*10^{-3}$ (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$I_{max,TPR}$</td>
<td>0.745 (4.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$IC_{50,TPR}$ (nM)</td>
<td>47.8 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\gamma_{TPR}$</td>
<td>0.546 (4.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_{met}$ (h$^{-1}$)</td>
<td>0.116 (14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RSE% – relative standard error. IIV – Inter-Individual Variability, standard deviation of the parameter within the population. $t_{1/2,k_{out}}$ – equivalent half-lives for $k_{out}$. All $IC_{50}/EC_{50}$ refer to unbound concentrations.
### TABLE 5
Estimated equilibrium parameters and predicted $EC_{20}$ values in rats, and free concentrations $C_u$ in human

<table>
<thead>
<tr>
<th>Compound</th>
<th>Response</th>
<th>$E_{\max}/I_{\max}$</th>
<th>$EC_{50}/IC_{50}$</th>
<th>$\gamma$</th>
<th>$EC_{20}/IC_{20}$</th>
<th>$C_u$</th>
<th>$t_{1/2,kout}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(nM)</td>
<td>(nM)</td>
<td></td>
<td>(nM)</td>
<td></td>
<td>(min)</td>
</tr>
<tr>
<td>Ivabradine</td>
<td>HR (I)</td>
<td>306</td>
<td>1100</td>
<td>0.403</td>
<td>35.7</td>
<td>5.3 – 23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>MAP (I)</td>
<td>53</td>
<td>400000</td>
<td>0.336</td>
<td>6460</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>HR (E)</td>
<td>1160</td>
<td>837</td>
<td>0.478</td>
<td>46.1</td>
<td>10 – 47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>MAP (I)</td>
<td>103</td>
<td>837</td>
<td>0.478</td>
<td>46.1</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>QT (E)</td>
<td>12.5</td>
<td>50.6</td>
<td>0.156</td>
<td>0.0073</td>
<td>0.45 – 4.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.4</td>
</tr>
<tr>
<td>Pimobendan</td>
<td>HR (E)</td>
<td>138</td>
<td>144</td>
<td>0.489</td>
<td>8.50</td>
<td>0.95 – 1.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>MAP (I)</td>
<td>12.4</td>
<td>144</td>
<td>0.489</td>
<td>8.50</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

E denotes an excitatory response, I denotes an inhibitory response. $t_{1/2,kout}$ from Table 4 are included for convenience. $C_u$ denotes clinical plasma concentration in humans. All concentrations are unbound.

<sup>a</sup>(Deedwania, 2013), <sup>b</sup>VIAGRA, Pfizer summary, <sup>c</sup>(Allen et al., 2000), <sup>d</sup>(Chu et al., 1995)
Ivabradine
Sildenafil
Dofetilide

\[ K_a \]
\[ F \]
\[ CL_d \]
\[ V_c \]
\[ V_t \]
\[ CL \]

Input (IV, PO)

Pimobendan

\[ K_a \]
\[ F \]
\[ CL_d \]
\[ V_c \]
\[ V_t \]
\[ CL \]
\[ k_{met} \]

metabolite

\[ k_{met} \]

Figure 2
Figure 4
Figure 5
Figure 6

Ivabradine

IC₅₀ (HR)

Sildenafil

i.v. data

IC₅₀ (TPR)

Dofetilide

EC₅₀ (QT)

Pimobendan

IC₅₀ (TPR)

Simulated max dose

Model fit

Plasma conc. (nM)

Time (h)

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Figure 8
Figure 9

Dofetilide, 0mg/kg

1mg/kg

3mg/kg

10mg/kg

QT-interval (ms) vs Clock time
Figure 10
Figure 11