

Original article

A novel predictive pharmacokinetic/pharmacodynamic model of repolarization prolongation derived from the effects of terfenadine, cisapride and E-4031 in the conscious chronic av node—ablated, His bundle-paced dog

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Abstract

Introduction: Terfenadine, cisapride, and E-4031, three drugs that prolong ventricular repolarization, were selected to evaluate the sensitivity of the conscious chronic atrioventricular node—ablated, His bundle-paced Dog for defining drug induced cardiac repolarization prolongation. A novel predictive pharmacokinetic/pharmacodynamic model of repolarization prolongation was generated from these data. **Methods:** Three male beagle dogs underwent radiofrequency AV nodal ablation, and placement of a His bundle-pacing lead and programmable pacemaker under anesthesia. Each dog was restrained in a sling for a series of increasing dose infusions of each drug while maintained at a constant heart rate of 80 beats/min. RT interval, a surrogate for QT interval in His bundle-paced dogs, was recorded throughout the experiment. **Results:** E-4031 induced a statistically significant RT prolongation at the highest three doses. Cisapride resulted in a dose-dependent increase in RT interval, which was statistically significant at the two highest doses. Terfenadine induced a dose-dependent RT interval prolongation with a statistically significant change occurring only at the highest dose. The relationship between drug concentration and RT interval change was described by a sigmoid E_{\max} model with an effect site. Maximum RT change (E_{\max}), free drug concentration at half of the maximum effect (EC_{50}), and free drug concentration associated with a 10 ms RT prolongation ($EC_{10\text{ ms}}$) were estimated. A linear correlation between $EC_{10\text{ ms}}$ and HERG IC_{50} values was identified. **Discussion:** The conscious dog with His bundle-pacing detects delayed cardiac repolarization related to I_{Kr} inhibition, and detects repolarization change induced by drugs with activity at multiple ion channels. A clinically relevant sensitivity and a linear correlation with in vitro HERG data make the conscious His bundle-paced dog a valuable tool for detecting repolarization effect of new chemical entities.

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Keywords: Cardiovascular; Dog; His bundle-pacing; Methods; Pharmacokinetic/pharmacodynamic modeling; QT interval; Repolarization

1. Introduction

In recent years the effects of new chemical entities (NCEs) on cardiac repolarization has been the subject of ever-increasing regulatory review. Pre-clinical safety assessment of NCEs must include tests for their ability to prolong

ventricular repolarization, typically measured as QT interval. QT interval serves as an imperfect biomarker for the potential to induce proarrhythmic events including Torsade de Pointes, a potentially life-threatening polymorphic ventricular tachyarrhythmia characterized by beat-to-beat changes in the QRS complex conformation around the isoelectric axis (Roden, 2004).

Measurement of the QT interval is non-invasive and usually easily accomplished. The QT interval translates

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across species and provides an approximation of action potential duration, but is confounded by changes in heart rate (Aytemir et al., 1999; Malik, 2002). For any compound with a chronotropic effect, changes in QT interval are related to both the direct effects of the compound on cardiac repolarization, and indirect responses to a change in heart rate. Eliminating heart rate change as a confounding variable provides for a more precise characterization of ventricular repolarization and a potentially more sensitive model to define rate-independent drug effects (Olivier et al., 2003; Sanders, Bailie, & Olivier, 2004). The purpose of this study was to assess the sensitivity of the conscious chronic canine model of atrioventricular dissociation and His bundle-pacing for the detection of drug-induced repolarization prolongation. E-4031, an experimental class III antiarrhythmic, cisapride, a gastrointestinal promotility agent, and terfenadine, an H₁ antagonist, have all been associated with QT prolongation in patients, culminating in market withdrawal for the latter two drugs. These three agents, a “pure” I_{Kr} blocker (Fujiki, Tani, Mizumaki, Shimono, & Inhoue, 1994), a relatively selective I_{Kr} blocker (Michalets & Williams, 2000) and a multiple ion channel blocker (Ming & Nordin, 1995), respectively, were administered to the His bundle-paced dog to examine the model’s ability to detect changes in ventricular repolarization following I_{Kr} inhibition, and more importantly, the sensitivity to detect repolarization change associated with multiple ion channel blockers. Finally, pharmacokinetic/pharmacodynamic modeling was applied to explore the relationship between free drug concentration and repolarization prolongation in the His bundle-paced dog. The estimated free drug concentrations associated with repolarization prolongation of 10 ms were compared with HERG IC₅₀ values to assess in vitro and in vivo data correlations of the selected three drugs.

2. Materials and methods

2.1. Statement on use and care of animals

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Surgical methods

Three male beagle dogs weighing between 11 and 15 kg and of 18–30 months of age were selected for this investigation. For a comprehensive description of surgical methods, statistical power analysis to support a group size of 3, and the details of postoperative care please refer to Sanders et al., 2004. Briefly, dogs were anesthetized with intravenous propofol and an inhaled mixture of isoflurane

in oxygen. A radiofrequency ablation catheter was advanced to the AV node and radiofrequency energy was applied to induce complete and permanent AV dissociation. A temporary ventricular pacing lead and external stimulator was used to stabilize the dog for the surgery to follow. Through a right thoracotomy, a custom made active fixation pacing lead was inserted into the His bundle. This unipolar lead was tunneled to a mid-scapular subcutaneous pocket and connected to an implantable programmable pacing generator. Dogs were allowed a minimum of 2-week recovery period before inclusion in this study.

2.3. Experimental methods

His bundle-paced dogs determined to be clinically acceptable and demonstrating no biologically significant hematology or clinical biochemistry abnormalities were selected for this experiment. Dogs were restrained in slings for the administration of 4 doses of each of the following treatments: 30% SBE-CD vehicle control (sulfobutylether-cyclodextrin, Pfizer Inc., Ann Arbor, MI), terfenadine (Sigma-Aldrich, St. Louis, MO), cisapride (Pfizer Inc., Sandwich, Kent), and E-4031 (Pfizer Inc., Sandwich, Kent). Infusion protocols were taken from previously published work (Fossa, Depasquale, Raunig, Avery, & Leishman, 2002). Briefly, baseline ECG information was recorded prior to the initiation of test compound infusion. The four rising doses of compound were administered sequentially while an electrocardiogram was continuously recorded. Infusions of test compound were administered via a cephalic catheter at a rate of approximately 0.1 ml/min/kg (Table 1). Each infusion was

Table 1
Dose escalation protocol for the various drugs administered intravenously in the conscious His bundle-paced dogs

Treatment	Week	Dose	Infusion (5 min) µg/kg
Vehicle Control 30% SBE-CD	1	1	0.0
		2	0.0
		3	0.0
		4	0.0
Terfenadine in 30% SBE-CD	2	1	40
		2	120
		3	480
		4	1920
Cisapride in 30% SBE-CD	3	1	40
		2	120
		3	480
		4	1920
E-4031 in Saline	4	1	8
		2	24
		3	96
		4	384

The required volume of drug was administered intravenously at a rate of approximately 0.1 ml/min/kg.

given over 5 min. Blood samples were drawn at the end of each infusion to measure plasma drug concentration. A 7-day period was allowed for drug elimination between treatments based on the published half-lives of 17 h for terfenadine (McTavish, Goa, & Ferrill, 1990) and 7–10 h for cisapride (Michalets & Williams, 2000). No data were available for the half-life of E-4031 in the dog, therefore, this drug was administered last in the experimental protocol. Doses of experimental compounds were selected to provide measurable prolongation of ventricular repolarization. Previous studies with this model have assessed ventricular repolarization at multiple heart rates (Olivier et al., 2003; Sanders et al., 2004). During this investigation, heart rate was maintained at 80 beats/min to facilitate exploration of the relationship between drug concentration and repolarization prolongation.

2.4. Data collection

A Lead II electrocardiogram was recorded using surface electrodes, amplified and digitally stored at a sampling rate of 500 Hz. Raw ECG data were analyzed using the EMKA system (EMKA Technologies, Paris, France). All interpretable ECG complexes from each 5-min infusion were analyzed and 1-min means were generated. Mild septal pre-excitation can be evident for His bundle-paced dogs complicating precise identification of the beginning of the QRS complex. Therefore, RT intervals defined as the time between the peak of R-waves and the end of T-waves are routinely substituted for the traditional QT measurement in this model. The peak of the R-wave and the end of the T-wave were initially identified on a sample beat as the zero crossing of the first derivative of the ECG signal. This “library” beat was used as a template for EMKA’s shape-based algorithm to

identify R-peak and T-end for all remaining beats within the 1 min epochs of interest. Complexes with no distinguishable end of T or with disassociated P–T superimposition were excluded from analysis. Typically 5–10 complexes each min were excluded due to the above, and the 1-min means were generated from the remaining 70–75 beats (Fig. 1).

2.5. Plasma drug analysis

At the end of each 5-min infusion, 1 mL blood samples were drawn to determine plasma drug concentration. For terfenadine and cisapride analysis, the plasma proteins were denatured with acetonitrile and the resulting extract analyzed. E-4031 was extracted from plasma using a liquid–liquid procedure by acidifying the plasma with acetic acid followed by partitioning with MTBE/Ethanol, (95/5, v/v). A mass spectrometer was used to perform HPLC/MS/MS analysis. The same mobile phase, acetonitrile–methanol–40 mM ammonium acetate (50:25:25, v/v/v), pH7, was used for all the three drugs. Blood collected during the 30% SBE-CD vehicle treatment was not analyzed. Analyst LC/MS software, (Applied Biosystem/MDS SCIEX, Ontario, Canada) was used to measure peak areas. Watson LIMS software (InnaPhase, Philadelphia, PA) was used for data reduction. Peak area ratios (PARs) of drug to internal standard were calculated, and a calibration curve was constructed using PARs of the calibration standards by applying weighted regression analysis. All concentrations were calculated from their PARs against the regression line. The analytical runs were considered acceptable when no less than five quantitation standards were within 15% of nominal, and two thirds of the quality control samples, with at least one at each control level, were within 20% of nominal.

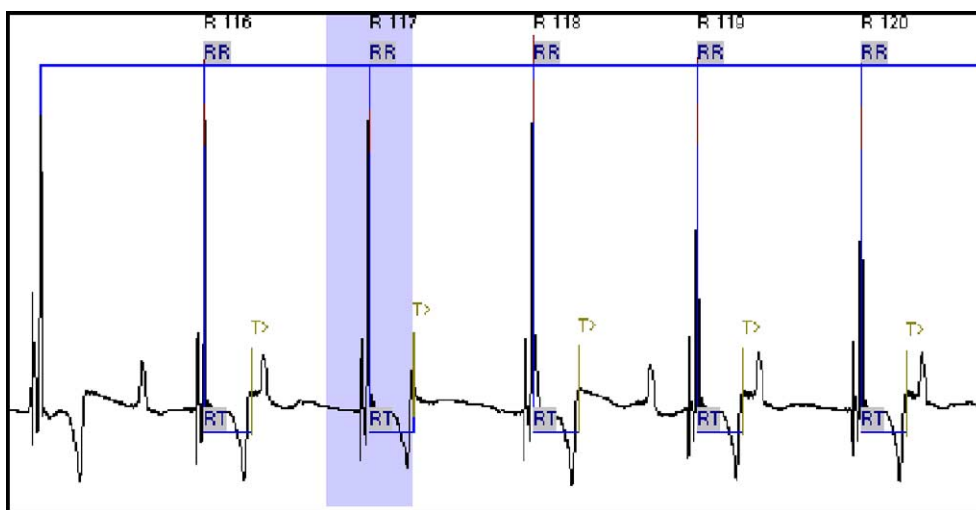


Fig. 1. An example of typical His bundle-paced ECG tracings and fiduciary points identified by the EMKA system. A “library” beat was used as a template for EMKA’s shape-based algorithm to identify R-peak and T-end for beats of interest. Complexes with no distinguishable end of T or with disassociated P–T superimposition (highlighted waveform) were excluded from analysis.

Free drug concentrations in plasma were calculated as total plasma concentration \times f_u (unbound fraction in dog plasma), assuming that plasma protein binding was constant across the entire concentration range studied. The f_u values were 2%, 5% and 31% for terfenadine, cisapride and E-4031, respectively as previously reported (Webster et al., 2001). Free plasma drug concentrations were used for all pharmacokinetic and pharmacodynamic analyses. Prior to model analysis, individual dog changes in RT interval during drug treatment were corrected for the corresponding changes observed during that dog's vehicle treatment.

2.6. Statistical methods

Statistical analyses were performed using the mean RT interval from the last minute of each 5-min infusion. Analysis of variance (ANOVA) procedures were used to evaluate the effect of drug and dose (SAS, Cary, North Carolina). Mean percent change from baseline RT interval for each dose of each compound was compared to the corresponding vehicle control. All tests were performed at the 0.05 level of significance.

2.7. Pharmacokinetic/pharmacodynamic (PK/PD) analysis

Nonlinear Mixed Effects Modeling software (NONMEM version V) (UCSF and GloboMax LLC, Hanover MD) was used for pharmacokinetic (PK) and PK/PD modeling. Observed free plasma drug concentrations at the end of each 5 min infusion were fitted to a one compartment PK model with a constant rate of intravenous (IV) drug infusion and a first order of elimination. Free plasma drug concentrations corresponding to measured mean RT intervals at each minute during infusion were estimated. Inspection of free plasma concentration and RT change (Δ RT) versus time profiles suggested a delayed RT change for one drug (E-4031). Thus a hypothetical effect compartment was postulated to link the observed Δ RT to free drug concentrations in the central (plasma) compartment (Fig. 2). As shown, K_{10} represents the first order rate constant for elimination of drug from the plasma compartment, and K_{e0} is the first order constant determining the rate of equilibration between plasma and the effect site,

respectively. The model was parameterized such that free drug concentrations in the effect compartment (C_e) would be equal to free drug concentrations in the central compartment (C_p) at a steady state during constant rate infusion. The relationship between free drug concentrations in the effect compartment and Δ RT data was modeled using a sigmoid E_{max} PK/PD model as described in Fig. 2 and by Eq. (1):

$$E = (E_{max} * C_e^\gamma) / (C_e^\gamma + EC_{50}^\gamma) + \varepsilon \quad (1)$$

where E represented the measured RT change (Δ RT), E_{max} was the maximum effect, and EC_{50}^γ was the effect site free drug concentration (nM) at half of the maximum effect. Gamma (γ) expressed the sigmoidicity factor or steepness of the concentration–effect relationship.

The inter-animal variability of the parameters was assessed using an exponential function:

$$P_i = \theta * \exp(\eta_i) \quad (2)$$

where θ was the population value for the parameters described in Eq. (1), P_i was the individual animal value and η_i was the random deviation of P_i from θ . The values of η_i were assumed to be independently normally distributed with a mean of zero and a variance of ω^2 . An additive error model estimated residual error:

$$Y = Y_p + \varepsilon \quad (3)$$

where Y was the observed value and Y_p was the model predicted value. ε represented the residual deviation of the model predicted response from the observed value.

3. Results

3.1. In vivo results

E-4031 induced a robust prolongation of RT interval that was statistically significant at the highest three doses (Fig. 3). Doses of 24, 96 and 384 μ g/kg produced corresponding free plasma concentrations of 22.3, 84.2, and 323 nM (Table 2), or 5–50 fold the free plasma concentration associated

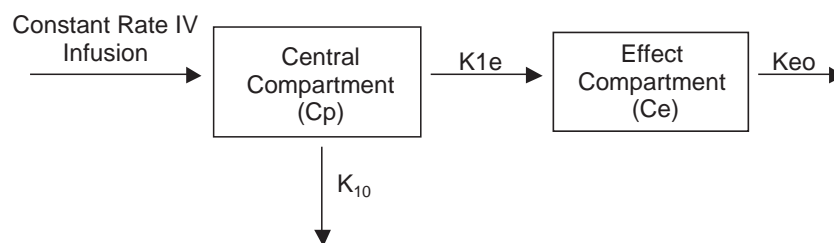


Fig. 2. A sigmoid E_{max} model with an effect site was used for PK/PD modeling. It was assumed that the free drug concentrations at the effect compartment (C_e) would be equal to the concentrations in the central (plasma) compartment (C_p) at steady state. K_{10} and K_{e0} were the apparent elimination rate constants from the central and the effective compartments, respectively.

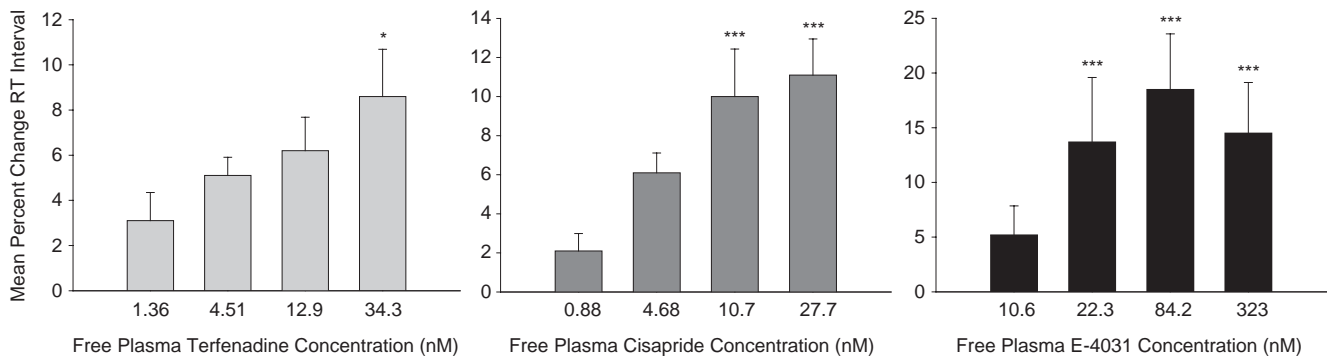


Fig. 3. Effects of an intravenous rising dose infusion of Terfenadine, Cisapride, and E-4031 on RT interval. RT interval changes are expressed as a percent change over baseline for each drug across four rising doses. Values represent means \pm S.E.M. of three His bundle-paced dogs with a heart rate of 80 beats/min. * $P < 0.05$ and *** $P < 0.001$ compared with vehicle. Mean plasma drug levels at the end of each 5-min infusion are expressed as free nM.

with delayed repolarization in patients (Fujiki et al., 1994). These exposures resulted in mean RT interval increases of 13.7–18.5% of baseline or 30.1–40.5 ms. The mean RT interval increase associated with a dose of 8 $\mu\text{g}/\text{kg}$ and an exposure of 10.6 nM represented approximately an 11.3 ms increase (* $p = 0.053$). No Torsade de Pointes was detected in any of the dogs. One dog exhibited two ventricular premature contractions at the highest dose. All exhibited a change in T wave morphology as a function of increasing dose, with the T wave overall becoming broader and shallower (Fig. 4).

Cisapride resulted in a dose-dependent RT interval prolongation, with significance occurring at the two highest

doses. Doses of 480 and 1920 $\mu\text{g}/\text{kg}$ resulted in corresponding free plasma exposures of 10.7 and 27.7 nM or about 2–5 fold the therapeutic free plasma level (Gladziwa et al., 1991; Van Haarst et al., 1998). These exposures prolonged the mean RT interval by 10.0% and 11.1% over baseline, and 22.8 and 25.4 ms (Table 2). A 14-ms mean prolongation of RT interval followed a dose of 120 $\mu\text{g}/\text{kg}$ and an exposure of 4.68 nM. Cisapride administration did not result in any ventricular arrhythmias, or in a change in T wave morphology.

Terfenadine induced a dose-dependent prolongation of RT interval with a significant change occurring only at the highest dose. The dose of 1920 $\mu\text{g}/\text{kg}$ produced a free

Table 2

Mean raw millisecond RT change, percent RT change, and total and free plasma concentration (nM) at the end of infusion of terfenadine, cisapride, and E-4031

Test compound	Dose ($\mu\text{g}/\text{kg}$)	N	Mean RT (ms)	S.E.M	Mean % change RT	S.E.M	Total drug concentration (nM)	S.D. (nM)	Free drug concentration (nM)	S.D. (nM)
Vehicle	Baseline	3	224.5	4.3						
	0	3	227.2	4.1	1.2	0.3				
	0	3	230.2	4.1	2.5	0.4				
	0	3	231.0	4.5	2.9	0.4				
	0	3	232.1	4.5	3.4	0.6				
Terfenadine	Baseline	3	216.7	6.7						
	40	3	223.4	6.7	3.1	1.2	67.8	47.8	1.36	0.96
	120	3	227.7	7.2	5.1	0.8	225	229	4.51	4.58
	480	3	230.1	5.7	6.2	1.5	642	442	12.9	8.8
	1920	3	235.4*	9.7	8.6*	2.1	1712	1220	34.3	24.4
Cisapride	Baseline	3	229.4	2.2						
	40	3	234.3	3.7	2.1	0.9	17.6	5.0	0.88	0.25
	120	3	243.4	4.7	6.1	1.0	93.7	74.6	4.68	3.73
	480	3	252.2***	3.6	10.0***	2.4	213	93	10.7	4.7
	1920	3	254.8***	4.6	11.1***	1.9	555	116	27.7	5.8
E-4031	Baseline	3	221.4	6.0						
	8	3	232.7	2.6	5.2	2.7	34.0	4.8	10.6	1.5
	24	3	251.5***	11.8	13.7***	5.9	71.8	35.3	22.3	11.0
	96	3	261.9***	8.8	18.5***	5.1	272	157	84.2	48.8
	384	3	253.2***	8.5	14.5***	4.6	1041	525	323	163

Dogs were maintained at a His bundle-paced ventricular rate of 80 beats/min. RT values represent means \pm S.E.M. of three dogs.

* $P < 0.05$.

*** $P < 0.001$ compared with vehicle.

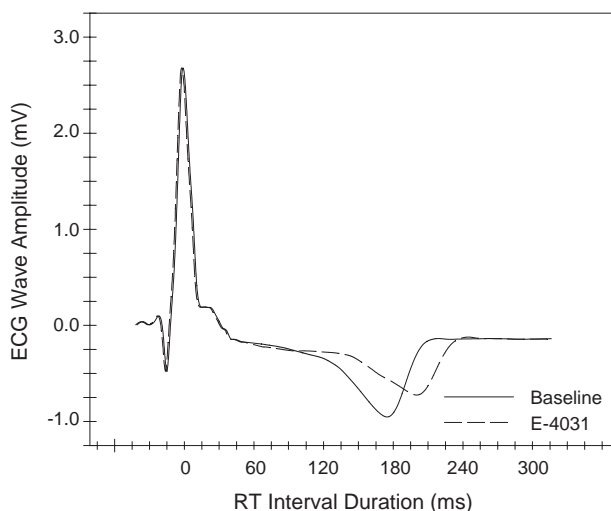


Fig. 4. Single animal prolongation of repolarization following administration of the highest dose of E-4031. All dogs exhibited similar changes in T wave morphology as a function of increasing dose, with the T wave becoming broader and shallower.

plasma concentration of 34.3 nM, which is about 8.7 times the free level associated with marked ECG changes in humans (Honig et al., 1993). This resulted in an 8.6% increase in mean RT interval from baseline and a raw ms

change of 18.7 ms (Table 2). No arrhythmias or changes in the T wave were observed.

Vehicle control resulted in an increase in RT interval duration as time in the sling increased (Table 2). Maximal RT change at the end of vehicle infusion represented a 3.4% mean change from baseline or an increase of 7.6 ms.

3.2. Pharmacokinetic/pharmacodynamic (PK/PD) modeling

A one-compartment PK model with a constant rate of IV drug infusion and first order of elimination described the concentration–time profiles of the three compounds. A sigmoid E_{\max} model with an effect site correctly described the relationship between drug concentration and RT interval change in the dogs. Observed and model-predicted RT changes versus free drug concentration in the effect compartment are presented in Fig. 5. Relevant mean PK/PD parameters (E_{\max} , EC_{50} , and K_{eo}) are summarized in Table 3. A 10-ms RT prolongation was considered biologically relevant in the His bundle-paced dog, based on earlier work characterizing the model (Sanders et al.) and unpublished data from our laboratories. The free drug concentration (nM) associated with a 10 ms RT prolongation in the His bundle-paced dog was estimated using the PK/PD parameters, and the results are summarized in Table 3. The model-computed mean E_{\max} , EC_{50} , and $EC_{10\text{ ms}}$

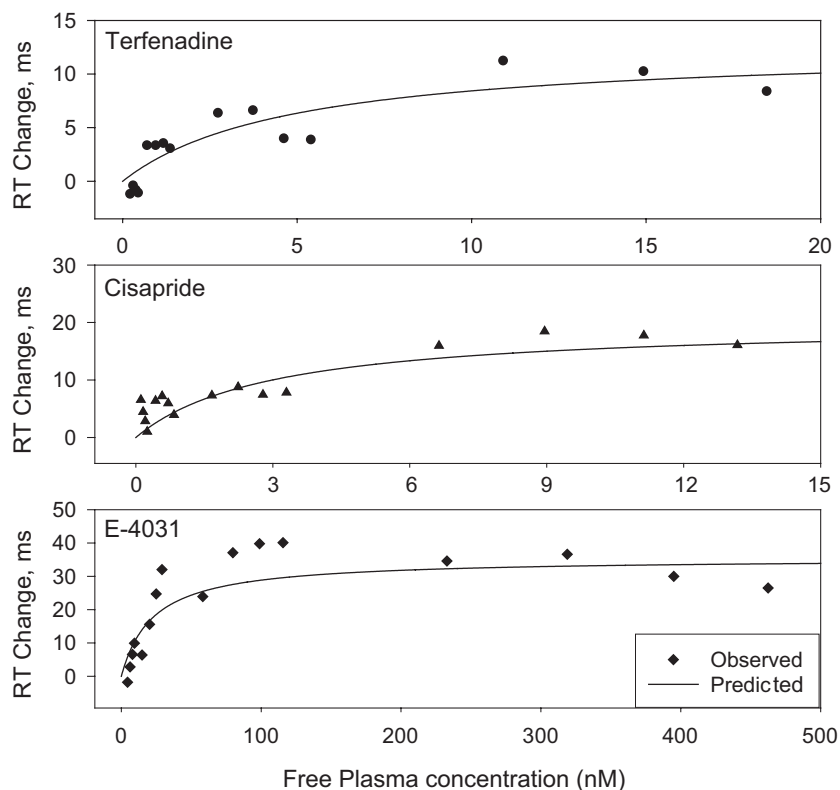


Fig. 5. Correlation between free drug concentrations (nM) at the effect site and changes in RT interval at 80 beats/min in a representative His bundle-paced dog. A sigmoid E_{\max} model with an effect site was used for analysis. Changes in RT interval in individual dogs were corrected for the corresponding RT changes observed during vehicle treatment.

values were 12.6, 13.4 and 23.5 ms; 1.5, 1.2, and 8.3 nM; and 17.3, 5.3, and 8.9 nM for terfenadine, cisapride and E-4031, respectively. The $EC_{10\text{ ms}}$ was approximately 1-, 2-, and 6- fold the free concentration associated with QT change in patients for cisapride (2–5 nM), E-4031 (3–4 nM), and terfenadine (3 nM) (Fujiki et al., 1994; Gladziwa et al., 1991; Honig et al., 1993).

Inter-animal variability of E_{max} was observed for all three drugs. The E_{max} ranged from 10.0–28.6, 11.1–18.7, and 14.3–39.6 ms for terfenadine, cisapride, and E-4031, suggesting a 2- to 3-fold variability in drug response across the three dogs. The inter-animal variability of EC_{50} was small for terfenadine and E-4031, but was larger for cisapride with values ranging from 0.45 to 12.2 nM. Unpublished d-sotalol data from our laboratories demonstrates a similar degree of inter-animal variability in E_{max} and EC_{50} in His bundle-paced dogs at a single paced rate.

The PK/PD model-estimated K_{eo} was 0.17 min^{-1} for E-4031. The relatively small value of K_{eo} suggests a delayed RT change relative to plasma drug concentration with a half-life of approximately 4 min. The estimated K_{eo} was $>10\text{ min}^{-1}$ for terfenadine and cisapride, which indicates an absence of delay in the drugs' distribution to the effect compartment and therefore an immediate effect on ventricular repolarization. Free drug concentrations in plasma (C_p) and the effect compartment (C_e) were essentially equivalent for the two drugs.

The PK/PD model estimated $EC_{10\text{ ms}}$ values were 17.3, 8.9, and 5.3 nM for terfenadine, E-4031, and cisapride, respectively (Table 3). This is in the same rank order as HERG IC_{50} values of 78.3, 11.0, and 7.2 nM for the 3 drugs (Fossa et al., 2004; Redfern et al., 2003). The correlation between model predicted $EC_{10\text{ ms}}$ and in vitro HERG current inhibition potential (HERG IC_{50}) is presented in Fig.

Table 3
Mean pharmacokinetic/pharmacodynamic parameters for terfenadine, cisapride, and E-4031

	Terfenadine	Cisapride	E-4031
E_{max} (SE)	12.6 (4.0)	13.4 (4.0)	23.5 (5.3)
EC_{50} (SE) (nM)	1.5 (0.76)	1.2 (0.72)	8.3 (1.2)
Gamma (γ)	1.0	1.0	2.3
$EC_{10\text{ ms}}$ (nM)	17.3	5.3	8.9
K_{eo} (min^{-1})	>10	>10	0.17
Free plasma concentration (nM) associated with QT prolongation in humans	3*	2–5**	3–4***

E_{max} : Maximum RT prolongation in milliseconds; EC_{50} : effective compartment free drug concentration (nM) at half the maximum effect; SE: model estimated standard error for E_{max} or EC_{50} ; Gamma (γ): sigmoidicity of the concentration-effect relationship; $EC_{10\text{ ms}}$: effective compartment free plasma concentration (nM) associated with RT interval prolongation of 10 ms; K_{eo} : the first order constant determining the rate of equilibration between plasma and the effect site.

* Honig et al., 1993.

** Gladziwa et al., 1991; Van Haarst et al., 1998.

*** Fujiki et al., 1994.

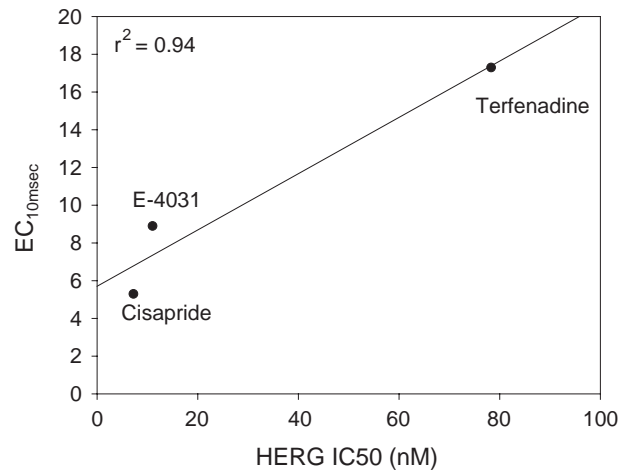


Fig. 6. Correlation between effect site free concentrations (nM) associated with RT prolongation of 10 ms ($EC_{10\text{ ms}}$) and in vitro HERG current inhibition potential (HERG IC_{50}) (nM). Values for in vitro HERG current inhibition potential were taken from Redfern et al. (2003) and Fossa et al. (2004). There was a linear relationship ($r^2=0.94$) between $EC_{10\text{ ms}}$ and HERG IC_{50} .

6. There was a linear correlation ($r^2=0.94$) between $EC_{10\text{ ms}}$ concentrations and HERG IC_{50} .

4. Discussion

The ability to define a NCE's propensity for causing delayed ventricular repolarization is of great importance in the pharmaceutical industry. Unfortunately current animal models may lack requisite sensitivity and specificity necessary to accurately predict repolarization-exposure relationships in humans (Redfern et al., 2002). The conscious dog with AV ablation and His bundle-pacing offers a more sensitive and more precise option for detecting drug-induced changes in repolarization independent of confounding intrinsic heart rate changes (Olivier et al., 2003; Sanders et al., 2004). This model detects repolarization change following I_{K_r} inhibition, and more importantly, detects changes in repolarization induced by multiple ion channel blockers, which have historically posed detection problems in preclinical investigations.

E-4031 is a class III antiarrhythmic with a pure I_{K_r} effect. In preclinical models, the plasma concentrations causing repolarization abnormalities are often close to therapeutic plasma concentration (Redfern et al., 2003). In the current study, the PK/PD model-estimated free drug concentration associated with a 10 ms RT prolongation ($EC_{10\text{ ms}}$) was about 2 times the range of 3–4 nM that is associated with QT prolongation in humans (Fujiki et al., 1994). A statistically significant RT prolongation of 13.7 ms was observed at about 5.5 times the therapeutic free plasma concentration. In future studies exposures closer to the clinically relevant level need to be explored.

QT prolongation following cisapride administration is concentration-related in humans, and 2 nM free drug is associated with a 6 ms mean increase in QTc (Van Haarst et al., 1998). Although primarily an I_{Kr} blocker, cisapride affects multiple types of ion channels, and in a previous conscious canine model significant QT prolongation was only seen at 31.6 nM, or about 15 times the free drug concentration associated with QTc prolongation clinically (Fossa et al., 2002). The His bundle-paced dog demonstrated RT prolongation when free drug concentrations were similar to those achieved during steady state drug administration in humans (4–5 nM) (Gladziwa et al., 1991). RT interval prolongation in the His bundle-paced dog was statistically significant and of similar magnitude at free plasma concentrations associated with a 28 ms mean QT prolongation in patients (12 nM) (Gladziwa et al., 1991; Van Haarst et al., 1998). These results therefore closely match the QT profile of cisapride in humans.

Terfenadine has been reported to have effects on multiple cardiac ion channels, including calcium, sodium and potassium, and therefore can both shorten and prolong the cardiac action potential resulting in variable QT effects (Ming & Nordin, 1995). In patients normal therapeutic plasma concentration ranges from 0.06 to 0.2 nM, but prolonged ventricular repolarization and TDP is not seen until concentrations approach 3 nM (Honig et al., 1993). Historically, detecting terfenadine-related QT prolongation in preclinical models has been extremely difficult, with significant changes in ventricular repolarization seen only at very high plasma concentrations (Usui et al., 1998) or with the concurrent administration of cytochrome *P*-450 inhibitors (Gras & Llenas, 1999; Laine, Perez, Dubreuil, & Gillet, 1998). To our knowledge the conscious His bundle-paced dog is the first canine model of its kind able to detect significant terfenadine related changes in cardiac repolarization at exposures within an order of magnitude of those causing repolarization changes in humans. A 10-ms RT change was predicted at free plasma concentration of 17.3 nM while a statistically significant effect was detected at 34 nM. This exposure is still 8–10 times the free plasma concentration associated with ECG changes in humans (Honig et al., 1993), but nonetheless represents an improvement in sensitivity over previous conscious dog models (Fossa et al., 2002; Laine et al., 1998). Further, a significant effect was identified with an *N* of 3; future inclusion of more animals would be expected to increase the power of detection of repolarization effect (Sanders et al., 2004).

A vehicle effect was observed in this study. During vehicle treatment there was an increase of 7.6 ms from baseline, a 3.4% change. There are two possible explanations for this effect. First, 30% SBE-CD may itself have some effect on cardiac repolarization dynamics and cause RT interval prolongation. Likely however is a change in autonomic tone as dogs begin to adjust to and relax in the sling. This effect appears to be duration dependent and

unpublished data from our laboratories indicate that His bundle-paced dogs in slings given no drug or vehicle treatment have shown a progressive lengthening of the RT interval directly related to time in the sling. Potentially a change in autonomic tone is responsible for this effect. In future studies a longer acclimation period in the sling prior to data collection may be of benefit.

The pharmacokinetic–pharmacodynamic correlation for all three drugs was analyzed based on a sigmoid E_{max} model with an effect site. Results from PK/PD modeling indicated a high correlation of free drug levels and RT interval changes as shown in Fig. 5. The model suggested an approximate 4-min half-life delay in RT changes relative to E-4031 plasma concentration, which may be related to a relatively slower distribution of E-4031 from the central compartment to the ventricular myocardium, the slow production of an active metabolite, or other mechanism. The absence of a delayed effect on ventricular repolarization following terfenadine and cisapride administration was indicated by the large K_{eo} for the two compounds. Delayed effects on ventricular repolarization have been reported previously for tacrolimus and quinidine. A counter-clockwise hysteresis in the relationship between tacrolimus blood level and QT change in the guinea pig was previously observed. Tacrolimus concentrations at the effect site, the ventricular myocardium, were closely correlated with the effect on ventricular repolarization, which suggested a delayed distribution to the effect site (Minematsu et al., 2001). A similar delay in ventricular repolarization was observed for Quinidine (Holford, Coates, Guentert, Riegelman, & Sheiner, 1981). The authors concluded that a slow distribution of quinidine from the blood to the ventricular myocytes and the formation of active metabolite(s) could both contribute to the delayed effects on repolarization prolongation.

The HERG assay is an *in vitro* method used to assess the effects of compounds on cardiac ion channel function. This study suggests that the HERG assay, in concert with the conscious His bundle-paced dog, provides a predictive model of repolarization effect for the three drugs selected. The linear relationship between the $EC_{10\text{ ms}}$ and HERG IC_{50} suggests two important characteristics of three compounds studied. First, the free concentrations of the three drugs are responsible for repolarization prolongation. Second, the *in vivo* potency in His bundle-paced dog correlates strongly with the *in vitro* potency of HERG channel inhibition regardless of whether a compound is a “pure” I_{Kr} blocker or has mixed ion channel effects.

Several aspects of this model must be addressed in future studies. First, a more robust pharmacological validation should be undertaken that assesses the effects of a broader range of drugs on ventricular repolarization both under normal physiological conditions and those in which Torsade de Pointes becomes more likely. Validation under conditions of bradycardia (Chiladakis, Karapanos, & Manolis, 1998; Khan & Nair, 2004), hypokalemia (Yelamanchi, Molnar,

Ranade, & Somberg, 2001), altered autonomic state (Bexton, Vallin, & Camm, 1986; Huang, Hull, Foreman, Lazzara, & Wolf, 1992; Magnano, Holleran, Ramakrishnan, Reiffel, & Bloomfield, 2002), and potentially other pathophysiologicals would be important in confirming the model's sensitivity in detecting repolarization effects.

Second, QT interval is an imperfect biomarker for proarrhythmia, and several studies demonstrate that prolongation of ventricular repolarization is not the sole determinant of the potential of a drug to cause arrhythmic events (Antzelevitch, 2004; Hondeghem, Carlsson, & Duker, 2001). Although the three investigated drugs induced prolongation of the RT interval, no Torsades de Pointes was detected in any of the dogs. Therefore, to date the model has been validated only to detect changes in cardiac repolarization, and not necessarily to differentiate between repolarization prolongation and proarrhythmia. The AV node—ablated, His bundle-paced dog's value as a proarrhythmic model is currently under investigation. Although the mechanistic association between QT prolongation and proarrhythmia is incompletely understood, the association between QT prolongation and the predisposition for arrhythmia is well established (Oto & Breithardt, 2001). As such, the QT interval remains the routinely evaluated parameter in both preclinical and clinical investigations of NCEs.

The conscious His bundle-paced dog has several advantages that may be employed in a QT screening strategy. Foremost, the model facilitates the assessment of compounds with chronotropic effects. Compounds with concurrent changes in rate and the QT/RR relationship are particularly challenging to evaluate in unpaced animal models, because the corrections applied to such drugs can inaccurately produce a false positive or false negative result (Malik, 2001). Unpublished data from our laboratories have identified chronotropes with effects on ventricular repolarization detected by the His bundle-paced dog that were missed by correcting QT/RR data from standard dog models. Further, many compounds have rate-dependent repolarization effects, and a drug effect can be missed entirely if the rate in question is never achieved during study. The programmable pacemaker facilitates evaluation of repolarization effects of drugs at one or over a wide range of selected heart rates, as desired.

Although initial instrumentation of these dogs is challenging, once completed, dogs can be maintained indefinitely in a stock colony to be used repeatedly in a variety of studies. The model appears to be free of ventricular remodeling (for complete discussion refer to the echocardiographic findings discussed in Sanders et al., 2004) which lends greater value to the model for chronic use. His-bundle pacing provides a morphologically normal QRS complex and utilizes the “normal” cardiac conduction pathway, important to the assessment of repolarization characteristics (Olivier et al., 2003; Sanders et al., 2004). The multifactor effects of general anesthesia are eliminated, allowing for the activation of normal behavioral and autonomic influences,

important modifiers of repolarization seen only in conscious animals. Finally, mixed effect PK/PD modeling showed a high level of sensitivity and some strong early correlations with in vitro HERG data, making the conscious His bundle-paced dog a promising model for detecting cardiac repolarization effect of new chemical entities.

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