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Reverse engineering of drug induced QT(c) interval prolongation : towards a systems pharmacology approach

Dubois, V.F.S.

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Chapter 6:

Translating QT(c) interval prolongation from conscious dogs to humans.

VFS Dubois (1,2), G Smania (3), H Yu (1), R Graf (3), A Chain (1) , M Danhof (1) ,O Della Pasqua (3,4) on behalf of the Cardiovascular Safety Project Team, TI Pharma PKPD Platform.

(1) Leiden Academic Centre for Drug Research, Division of Pharmacology, Leiden University, Leiden, The Netherlands
(2) Pharmacometrics, Grunenthal GmbH, Aachen, Germany
(3) Clinical Pharmacology Modelling & Simulation, GlaxoSmithKline, Stockley Park, Uxbridge, United Kingdom
(4) Clinical Pharmacology & Therapeutics, UCL, London, United Kingdom

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Members of the TI Pharma Cardiovascular Safety Project involved in the preparation of this manuscript:
Eli Lilly & Co: Dinesh De Alwis
GlaxoSmithKline: Jackie Bloomer, Nick McMahon, Phil Milliken
Johnson & Johnson: David Gallagher, An Vermeulen
Pfizer: Piet van der Graaf, Mark Holbrook
AstraZeneca: Sandra Visser

Abstract

Aim: Despite screening procedures in early drug development, uncertainty remains about the propensity of new chemical entities (NCEs) to prolong the QT/QTc interval. The evaluation of pro-arrhythmic activity using a comprehensive *in vitro* pro-arrhythmia assay does not fully account for pharmacokinetic-pharmacodynamic (PKPD) differences *in vivo.* Here we evaluate the correlation between drug-specific parameters describing QT interval prolongation in dogs and in humans.

Methods: Using estimates of the drug-specific parameter, data on the slopes of the PKPD relationships of 9 compounds with varying QT prolonging effects (cisapride, sotalol, moxifloxacin, carabersat, GSK945237, SB237376 and GSK618334, and two anonymised NCEs) were analysed. Mean slope estimates varied between -0.98 and 6.1 ms/µM in dogs and -10 and 90 ms/µM in humans, indicating a wide range of effects on QT interval. Linear regression techniques were then applied to characterise the correlation between the parameters across species.

Results: For compounds without a mixed ion channel block a correlation was observed between the drug-specific parameter in dogs and humans (y=*-1.709+11.6x,* R^2*=0.989*).These results show that per unit concentration the drug effect on QT interval in humans is 11.6 fold larger than in dogs.

Conclusions: Together with information about the expected therapeutic exposure, the evidence of a correlation between the compounds-specific parameter in dogs and humans represents an opportunity for translating preclinical safety data before progression into the clinic. Whereas further investigation is required to establish the generalisability of our findings, this approach can be used with clinical trial simulations to predict the probability of QT prolongation in humans.

1. Introduction

An important matter of concern in drug development is the pro-arrhythmic potential of pharmaceutical compounds [1]. A number of drugs have had to undergo labelling revision or market withdrawal due to post-marketing reports of sudden cardiac death linked to the development of torsades de pointes (TdP) [2–8]. It has been demonstrated that an excessive lengthening of cardiac repolarisation (measured by the QT interval prolongation) may induce these life-threatening ventricular tachyarrhythmias [9].

A wide range of drugs from various therapeutic classes has been associated with QT prolongation and TdP including both antiarrhythmic drugs, non-antiarrhythmic (20) cardiovascular drugs and non-cardiovascular (50) drugs (e.g. antihistamine, antipsychotic, antidepressant, antifungal, anti-infective, and gastrointestinal prokinetic drugs) [4,10–12]. However, predicting the risk of these types of serious side effects has proven a major challenge in cardiac safety pharmacology, as not all drugs showing QT prolonging effects are associated with TdP. In fact, a new proposal is being considered which shifts the focus from evaluating QT prolongation to evaluating pro-arrhythmic activity using a comprehensive *in vitro* pro-arrhythmia assay (CiPA), but this new approach does not take into account the underlying pharmacokinetic-pharmacodynamic (PKPD) relationships and overlooks the implications of differences between *in vitro* and *in vivo* experimental protocols [13,14].

Whereas the characterisation of the relationship between drug concentration and QT interval prolongation *in vivo* in preclinical species may not be considered a surrogate for the risk of TdP, such protocols can be informative and provide the basis for predicting drug effects in humans in a strictly quantitative manner. In a previous investigation in which a general PKPD model was used to assess QT prolonging effects of reference compounds, we showed that there are differences in the concentration of moxifloxacin associated with the probability of ≥ 10ms increase in QTc prolongation between dogs, monkeys and humans [15]. In contrast to data-driven approaches, our method relies on a PKPD model with a generic parameterisation, which disentangles drug-specific properties from biological or physiological system properties, enabling extrapolation of the estimates across species. In addition, the approach readily allows for the incorporation of historical data on system-related parameters describing e.g., circadian variability as well as the effect of heart rate on QT interval. Given the Bayesian nature of the analysis, it also offers the possibility to estimate posterior parameter distributions, which reflect all acknowledged sources of uncertainty [16]. Here we attempt to demonstrate the feasibility of establishing an interspecies correlation between drug-

specific parameter estimates, which can be used subsequently as a scaling factor or predictor of the clinical effects before candidate molecules enter clinical development.

As shown in Figure 6.1, decisions about the progression of a candidate molecule during the drug development path demands a good understanding of arrhythmogenic signals. In this paper an integrated approach is proposed, along the same principles as suggested by Lowe *et al.* and Pollard *et al.* [17,18]According to these authors, to scale between *in vivo* species, multiple compounds need to be tested where quantifiable differences can be detected. This implies an iterative process between models and experiments [17,18]. Similarly, such an iterative process can be applied when considering findings in preclinical species and humans. For the scaling of drug effects on QT interval from preclinical species to humans, a correlation can be derived and continuously updated as new compounds progress to clinical development. Assuming that differences in QT prolonging effects across species reflect varying degree of target engagement (activation or inhibition) and/or homeostatic mechanisms, one could use such a correlation to predict the magnitude of the drug effects in humans as well as to better design clinical study protocols aimed at the characterisation of QT prolonging effects.

We envisage therefore that model-predicted estimates can provide the basis for go/no go decisions before taking the compound into clinical development, whilst reducing attrition due to false positive and false negative results, as often observed in the analysis of individual experimental protocols.

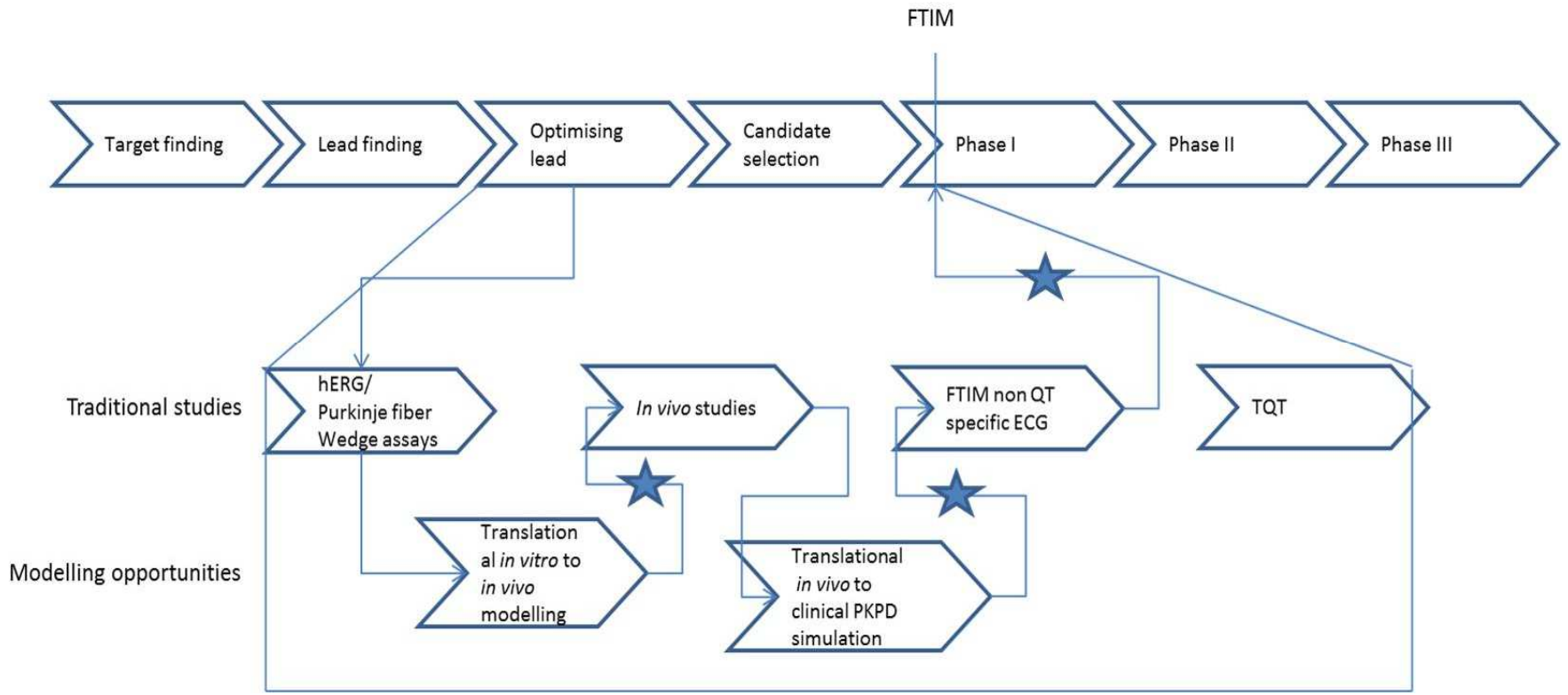

Figure 6.1. Opportunities (indicated with stars) where PKPD modelling can help in decision making in cardiovascular safety. *The possibility of discriminating between system- and drug-specific properties allows preclinical data to be used as basis for translating drug effects from animals to humans. Adapted from Pollard et al. [19].*

2. Methods

2.1. Data

Data on QTc interval, heart rate and plasma concentrations of nine different compounds were collected from multiple partners within safety pharmacology workgroup of the TI-Pharma PKPD platform. All available data were used for the purpose of PKPD modelling. An overview of the available data and the study designs is presented in Table 6.1. Whereas no predefined selection criteria were applied to the compounds included in this analysis, the data set consisted of compounds with different mechanisms of action, namely, moxifloxacin (DNA gyrase inhibitor), *d,l*-sotalol (non-selective β-adrenoreceptor antagonist), cisapride (5-HT_4-receptor agonist), carabersat (SB204269, a benzopyran derivative with undefined CNS binding site), SB237376 (potassium and calcium channel blocker), GSK945237 (topo-isomerase II inhibitor), GSK618334 (dopamine D_3 receptor antagonist), NCE03 and NCE04 (unknown mechanism of action) [19].

Table 6.1. *Summary of protocol and study designs used for PKPD modelling of the QT prolonging effects in dogs and humans. M= male, F= female.*

Drug	**Mechanism of action**	**Species**	**Sample size**	**Dosing Regimen**	**Route**	**Dose**	**PK Sampling (h)**	**PD Measurement**	**PD Sampling (h)**
Moxifloxacin	DNA gyrase inhibitor	Beagle dogs	N=8	Single dose min. 1 week btw. changes of dose	Oral gavage	10,30,100 mg/kg	0, 0.5, 1, 2, 4, 8, 24, 36, 48	Implanted telemetry measurements: blood pressure ECG	Every 1 min, averages over 48 h
Moxifloxacin	DNA gyrase inhibitor	Humans (healthy subjects)	F=88 M=49	Single dose	Oral	Placebo, 400mg	-1, -0.5, -0.83, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24		-1, -0.5, -0.83, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24
Sotalol	Non-selective β-adrenoreceptor antagonist	Beagle dogs	N=6	Single dose min. 1 week btw. changes of dose	Oral gavage	Vehicle, 4, 8 mg/kg	0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 24	Implanted telemetry measurements: blood pressure ECG	Every 5 min, averages over 24 h
Sotalol	Non-selective β-adrenoreceptor antagonist	Humans (healthy subjects)	F=12 M=18	Single dose	Oral	Placebo, 160 mg	-0.5, -0.25, 0.83, 0.25, 0.5, 0.75, 1, 2, 4, 8, 10, 18, 24		-2, -1.75, -1.5, -1, -0.75, -0.5, -0.25, 0.5, 1, 1.25, 2, 4, 6, 8, 10, 12, 18, 24
Cisapride	5-HT_4-receptor agonist	Beagle	N=8	Single dose min. 1 week btw. changes of dose	Oral gavage	Vehicle, 0.6, 2, 6 mg/kg	0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 24	Implanted telemetry measurements: blood pressure, ECG	Every 30 s, averages over 24 h
Cisapride	5-HT_4-receptor agonist	Humans (healthy subjects)	F=10 M=14	Dose escalation	Oral	Placebo, 10, 20, 40, 80, 120 mg	0, 1, 1.5, 2, 3, 4, 6, 12, 24		-24, -23, -22.5, -21, -20, -18, -12, 0, 1, 1.5, 2, 3, 4, 6, 12, 24
Carabersat (SB204269)	Benzopyran derivative	Beagle dogs	N=4	Single dose min. 1 week btw. changes of dose	Oral	Vehicle, 10, 30, 100, 1000 mg/kg	0, 0.5, 1, 2, 4, 8, 24, 36, 48	Implanted telemetry measurements: blood pressure , ECG	Every 30 min, for 20 h

Drug	**Mechanism of action**	**Species**	**Sample size**	**Dosing Regimen**	**Route**	**Dose**	**PK Sampling (h)**	**PD Measurement**	**PD Sampling (h)**
Carabersat (SB204269)	Benzopyran derivative	Humans (healthy subjects)	M=35		Oral	placebo, 100, 200, 400, 800, 1600, 2800, 4000, 5000 mg	0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 30, 48		0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24
NCE03	NA	Beagle dogs	N=4	Single dose min. 1 week btw. changes of dose	?	Vehicle, 2.15, 4.3 mg/kg	predose, 0.5, 1, 1.5, 2, 6, 24	Implanted telemetry measurements: blood pressure, ECG	-1,-0.75,-0.5,-0.25, 0,0.5,1,1.5,2,1.5, 2,2.5,3,4,5,6,10,12, 16,20
NCE03	NA	Humans	M=29		Oral	Placebo, 10, 30, 70, 90, 180, 360, 430, 500 mg	0, 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36, 48		-1, 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.25,1.5, 1.75, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, 24, 36
NCE04	NA	Beagle	N=6	Single dose min. 1 week btw. changes of dose	Oral/ subcut.	Vehicle, 4, 20, 100 mg/kg	0, 1, 3, 6,17, 24	Implanted telemetry measurements: blood pressure, ECG	0.5,1,2,3,4,6,8,12,16, 20,24
NCE04	NA	Humans	M=64		Oral/ Subcut.	Placebo, 3, 6, 12, 24, 48, 95, 190 mg	0, 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36, 48		Frequent sampling up to 2.5 hours, 3, 3.5, 4, 6, 8, 12, 24
SB237376	Potassium-calcium channel blocker	Beagle dogs	N=4	Single dose min. 1 week btw dose levels	Oral	0, 10 ,80 mg/kg		Implanted telemetry measurements: blood pressure, ECG	
SB237376	Potassium-calcium channel blocker	Humans (healthy subjects)	F=9 M=30	Single and repeat dose	Oral	Placebo, 25 , 50 mg			
GSK945237	Topo-isomerase II inhibitor	Beagle dogs	F=6 M=6	twice daily, ½ of dose, approx 6 hours btw doses	Oral	Placebo, 30, 100, 300 mg/kg/day	0, 0.25, 0.5, 1, 3, 6 6.25, 6.5, 7, 9, 24 (Days 1 and 14)	-	-

Drug	**Mechanism of action**	**Species**	**Sample size**	**Dosing Regimen**	**Route**	**Dose**	**PK Sampling (h)**	**PD Measurement**	**PD Sampling (h)**
GSK945237	Topo-isomerase II inhibitor	Beagle dogs	M=4	twice daily, ½ of dose, approx 6 hours btw doses, min. 1 week btw. dose levels	Oral	30, 100, 300 mg/kg/day	-	Implanted telemetry Measurements: HR, blood pressure, ECG , body temp.	0, 1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 15, 18, 21, 24
GSK945237	Topo-isomerase II inhibitor	Humans (healthy subjects)	45	Single dose	Oral	(6/dose) 50, 250, 500, 1000, 1750 mg/day	0,1,2,3,4,8, 24, 48	12-lead ECG, dual-lead cardiac monitoring	0,1,2,3,4,8, 24,48
GSK618334	Dopamine D_3 receptor antagonist	Beagle dogs	M=9 F=9	-	Oral	2, 5, 15 mg/kg	0.5, 1, 2, 4, 6, 8, 24	-	-
GSK618334	Dopamine D_3 receptor antagonist	Beagle dogs	M=4	Mind.6 days btw doses	Oral	2 ,5, 15 mg/kg		Implanted telemetry measurements: blood pressure ECG	0-24 Continuous all 30 sec.
GSK 618334	Dopamine D3 receptor antagonist	Humans (healthy subjects)	M=20 Divided into 2 cohorts	single ascending doses, min. 2 weeks btw doses	Oral	Cohort 1: 2.5, 25, 100, 400 mg Cohort 2: 10, 50, 200, 600 mg	0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, 72, 96	Lead II ECG; continuous 12-lead ECGs Measurements: blood pressure, HR, ECGs	Continuous from 0 to 6 0,1, 2, 3, 4, 6, 12, 24, 48,

2.2. Drug concentrations

Time-matched concentration and QT interval values were required for the characterisation of the PKPD relationships. When direct measurements were not available, individually predicted concentrations relative to each ECG recording time were simulated or interpolated using either non-linear mixed effects modelling techniques in NONMEM VII or VI (ICON, Maryland, USA) or deconvolution in WinNONLIN 4.2 (Pharsight Co., North Carolina, USA). Pharmacokinetic models were validated based on graphical and statistical criteria, including goodness of fit (GOF) and normalised prediction and distribution errors (NPDE) where applicable. Details on the analysis of pharmacokinetic data for relevant compounds can be found elsewhere [15,20,21].

2.3. Pharmacokinetic-pharmacodynamic modelling

A previously published model based on an adaptation of the work of Piotrovski *et al.* [22] was used to independently analyse QT interval and concentration data in dogs and humans. In contrast to numerous approaches where PKPD modelling has been used, this model relies on a common set of parameters to describe drug effects both in dogs and in humans. Model parameters, which discriminate between system- and drug-specific properties, were estimated using WinBUGS version 1.4.3 [23,24] (see appendix for model code). Details of the model can be found in Chain & Dubois *et al.* and Dubois *et al.* [20,21]. In brief, the model consists of three main components, namely (i) an individual correction factor to account for variability in the RR-interval, (ii) an oscillatory function describing diurnal variation in QT and (iii) a slope describing the linear relationship between concentration and drug effect on QT interval [25]. The model equation, including all these elements is shown in Figure 6.2 along with a diagram illustrating the interspecies differences in the linear relationship between drug levels and QT interval prolongation.

It should be noted that such a linear relationship seems to contrast with the theoretical views that PKPD relationships are best described by a sigmoidal function (e.g. Hill equation). However, linear relationships ensure that focus is given to the lower part of the concentration-effect relationship, i.e. the region of the curve which triggers clinical concern. Another practical limitation for the implementation of a sigmoidal function is the fact that safety considerations may prevent the estimation of maximum QT prolongation in humans. Usually, protocol stop criteria require subject withdrawal when the QTc interval exceeds 500 ms. Likewise in preclinical studies adverse events often prevents accurate estimation of maximum prolongation [15,20].

2.3.1. Model diagnostics

Details on the WinBUGS model code and evaluation procedures can be found elsewhere [20,21]. In brief, to assess the adequacy of the Bayesian PKPD model, two Markov Chain Monte Carlo (MCMC) chains [22] were run independently until at least 12 500 samples were obtained. Estimates from these runs were subsequently pooled and summarised, not only in terms of their point estimates (population mean), but also as posterior distributions and credible intervals. In Bayesian statistics, the availability of posterior distributions allows direct comparison between model predictions and observed values. In addition, goodness of fit and model performance criteria included the deviance information criterion (DIC) [26] and chain convergence. This latter criterion was assessed visually by monitoring the dynamic traces of Gibbs iterations and numerically by computing the Gelman-Rubin, Geweke, Raftery-Lewis and Heidelberger-Welch test statistics for all population parameters [26–28].

2.4. Interspecies correlation: linear and nonlinear regression

Linear and nonlinear regression methods were used to characterise the correlation between the estimates of the slope parameter (*S*) in dogs and humans (Figure 6.2). The ultimate goal of the analysis was to establish whether such a correlation can serve as a scaling factor for the differences between the two species and consequently enable the extrapolation of the drug effects on the QT interval from dogs to humans. During model selection, linear regression was prioritised to allow the evaluation of negative slopes, which reflects compounds with QT shortening activity. R2.12.3 was used for the purposes of the analysis, including statistical and graphical summaries. In addition to the regression coefficient (R^2), the slope of the correlation was selected as the parameter of interest for subsequent evaluation of the predictive performance of the interspecies correlation.

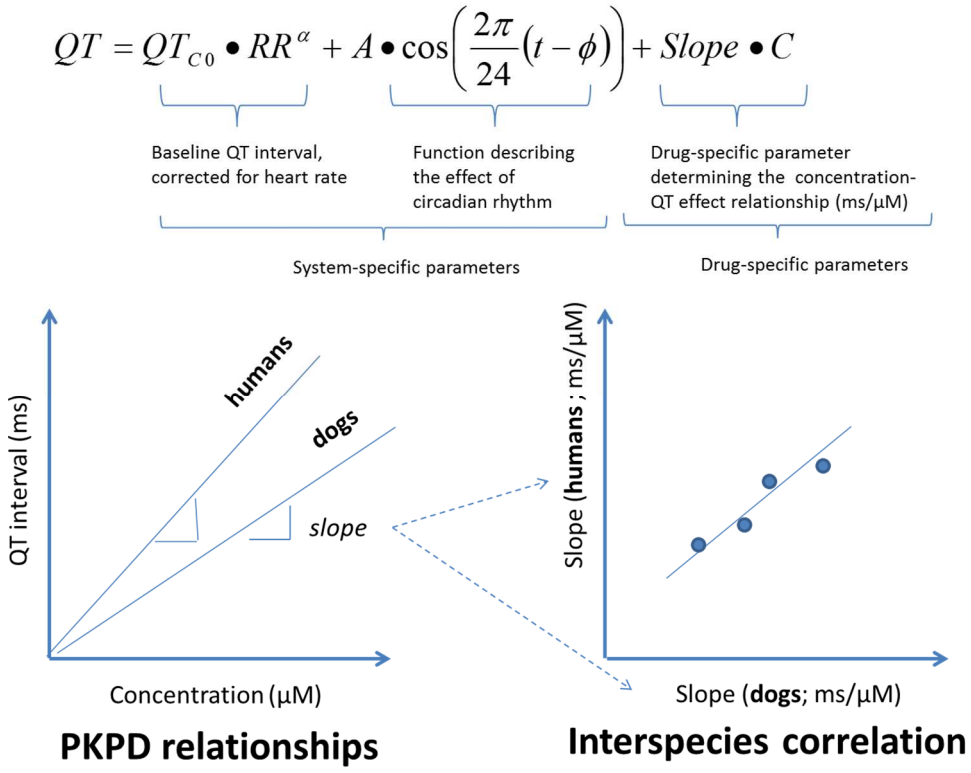

Figure 6.2.Schematic representation of the methods to determine the interspecies differences in the linear relationship between drug levels and QT interval prolongation. *The equation shows the three model components used to characterise drug-induced QT interval prolongation in dogs and humans. QT_{c0} [ms] is the individually corrected baseline QTc (sex will be included as a covariate in humans), RR [sec] is the interval between successive R peaks, α is the individual RR correction factor, A [ms] is the amplitude of the circadian rhythm, t is the clock time [decimal h],Φ [hr] is the phase and Slope is the drug-specific parameter describing the relationship between drug concentration and QT interval. The lower panels illustrate how linear regression techniques were applied to the drug-specific model parameters to define the interspecies correlation.*

3. Results

3.1. PKPD modelling of QT interval

All model parameters used as input in this analysis were derived from a PKPD model based on a generic parameterisation of drug- and system-specific properties. A summary of the parameter estimates per compound is presented in Tables 6.2 and 6.3. In addition to the typical Bayesian criteria for parameter convergence and model acceptance, graphical summaries using goodness-of-fit plots (i.e. observed *vs.* predicted QT values) are shown in Figure 6.3 for each compound and species. These results are complemented by a graphical summary of the model performance, i.e. how well the PKPD model predicts the experimental data (Figure 6.4). Model predictions accurately describe the time course of the observed QT interval for the different compounds both in dogs and humans

Our results clearly indicated that the so-called system-specific parameters, i.e. baseline QT (QT_{c0}), the QT-RR correction factor (α), the amplitude (A) and phase (Φ) are almost all within the same range of values for the different compounds within each species (Table 6.2). However, we should emphasise the fact that mean differences [95% credible intervals] for parameter values describing system-specific properties in dogs show larger variability than the estimated obtained in humans. Of interest is the baseline QTc (QT_{c0}) in dogs, which ranged between 238 [237-240] ms (cisapride) and 261 [252-270] ms (NCE04) and in humans from 379 [371-386] ms (NCE03) to 405 [377-435] ms (GSK618334). Similarly, the values of correction factor for RR (α) in dogs ranged from 0.18 [0.11-0.3] (sotalol) to 0.3 [0.22-0.4] (GSK945327) and in humans from 0.18 [0.14-0.23] (cisapride) to 0.4 [0.38-0.42] (moxifloxacin). Estimates obtained for the other two model parameters describing the circadian rhythm, namely the amplitude and phase, appear to be affected by experimental protocol design, with values for amplitude ranging from 2.13 [0.56-7.67] ms (GSK618334) to 9.2 [4.3-18.1] ms (NCE03) and in humans from 2.4 [1.7-2.9] ms (moxifloxacin) to 7.9 [6.8-9.3] ms (NCE04). For the phase parameter, values in dogs ranged from 4.4 [0.6-9.1] h (NCE04) to 31.4 [16-55] h (GSK618334) and in humans from 4.4 [3.3-5.7] h (NCE04) to 10 [7-13] h (moxifloxacin).

Table 6.2. *System- specific parameter estimates for dogs ( ) and humans ( ), where α is the RR correction factor; A is the amplitude and ϕ is the circadian oscillator and QT_{c0} is the corrected QT intercept following equation 1. N is the number of dogs/healthy subjects in each experimental protocol or clinical trial. Population mean parameter estimates are shown along with 95% credible intervals.*

		Moxi floxacin	Cisapride	Sotalol	NCE03	NCE04	GSK945237	SB237376	Carabersat	GSK618334
	N	8	8	6	4	6	4	4	4	4
	α	0.28 (0.22-0.35)	0.26 (0.2-0.33)	0.18 (0.11-0.3)	0.23 (0.14-0.38)	0.2 (0.31-0.48)	0.3 (0.22-0.4)	0.25 (0.17-0.34)	0.26 (0.17-0.42)	0.28 (0.17-0.46)
	A (ms)	4.6 (3.1-7.0)	5.6 (3.9-8.1)	6.6 (2.1-19.8)	9.2 (4.3-18.1)	4.3 (1.8-8.6)	8.6 (4.5-15.2)	7.4 (3.1-14.6)	4.2 (2.5-7.1)	2.13 (0.56-7.67)
	ϕ (h)	23.1 (15-35)	19.9 (16-26)	12.2 (7-36)	16.4 (10-26)	4.4 (0.6-9.1)	16.2 (9-28)	14 (10-19)	9 (5-16)	31.4 (16-55)
	QT_{c0} (ms)	240 (238-242)	238 (237-240)	255 (253-257)	244 (239-249)	261 (252-270)	258 (188-345)	246 (180-328)	250 (248-252)	246 (155-390)
	N	137	24	30	29	64	45	39	35	20
	α	0.4 (0.38-0.42)	0.18 (0.14-0.23)	0.27 (0.24-0.3)	0.3 (0.27-0.33)	0.22 (0.2-0.24)	0.22 (0.17-0.27)	0.33 (0.29-0.38)	0.24 (0.22-0.26)	0.28 (0.25-0.31)
	A (ms)	2.4 (1.7-2.9)	3.3 (1.1-6.0)	3.3 (2.4-4.3)	5.75 (2.6-10.6)	7.9 (6.8-9.3)	3.1 (2.1-4.2)	3.5 (1.9-5.3)	4.9 (3.8-6.3)	2.7 (1-4.6)
	ϕ (h)	10 (7-13)	4.3 (2.4-8.7)	6.22 (5.1-7.6)	28.2 (22.2-39.3)	4.4 (3.3-5.7)	7.2 (4.9-9.5)	9.2 (6.6-11.8)	9.7 (8.1-10.9)	8.3 (3.9-25.2)
	QT_{c0} (ms)	399 (394-403)	386 (382-390)	387 (383-392)	379 (371-386)	380 (378-382)	394 (360-431)	386 (371-402)	385 (379-392)	405 (377-435)

Table 6.3. *Drug-specific parameter estimates for dogs (🐕) and humans (🧍) where CP50= Concentration associated with a 50% probability of QT increase ≥ 10 ms. N is the number of dogs/healthy subjects in each experimental protocol or clinical trial. Population mean parameter estimates are shown along with 95% credible intervals.*

	Moxi floxacin	Cisapride	Sotalol	NCE03	NCE04	GSK945237	SB237376	Carabersat	GSK618334
N 🐕	8	8	6	4	6	4	4	4	4
N 🧍	137	24	30	29	64	45	39	35	20
Slope 🐕 [ms/µM]	0.56 (0.02- 1.4)	4.5 (0.96-9.8)	1.9 (0.6-8)	6.1 (2.2-16)	-0.98 (-2.1-0.6)	0.0098 (-0.01-0.03)	0.092 (0.07-0.11)	0.64 (-0.91-4.3)	0.814 (-0.3-3.4)
Slope 🧍 [ms/µM]	3.9 (3.3-4.4)	90 (87-120)	21 (17-26)	70 (50-80)	-10 (-13- -7)	0.0114 (0.008-0.02)	0.301 (0.297-0.304)	-0.2 (-1-0.7)	7.2 (5.3-9.2)
CP50 🐕	6.4	2.2	46.2	1.6	NA	5000	108.9	16.9	12.4
CP50 🧍	2.64	0.14	0.47	0.17	NA	4005	33.2	>9000	1.3

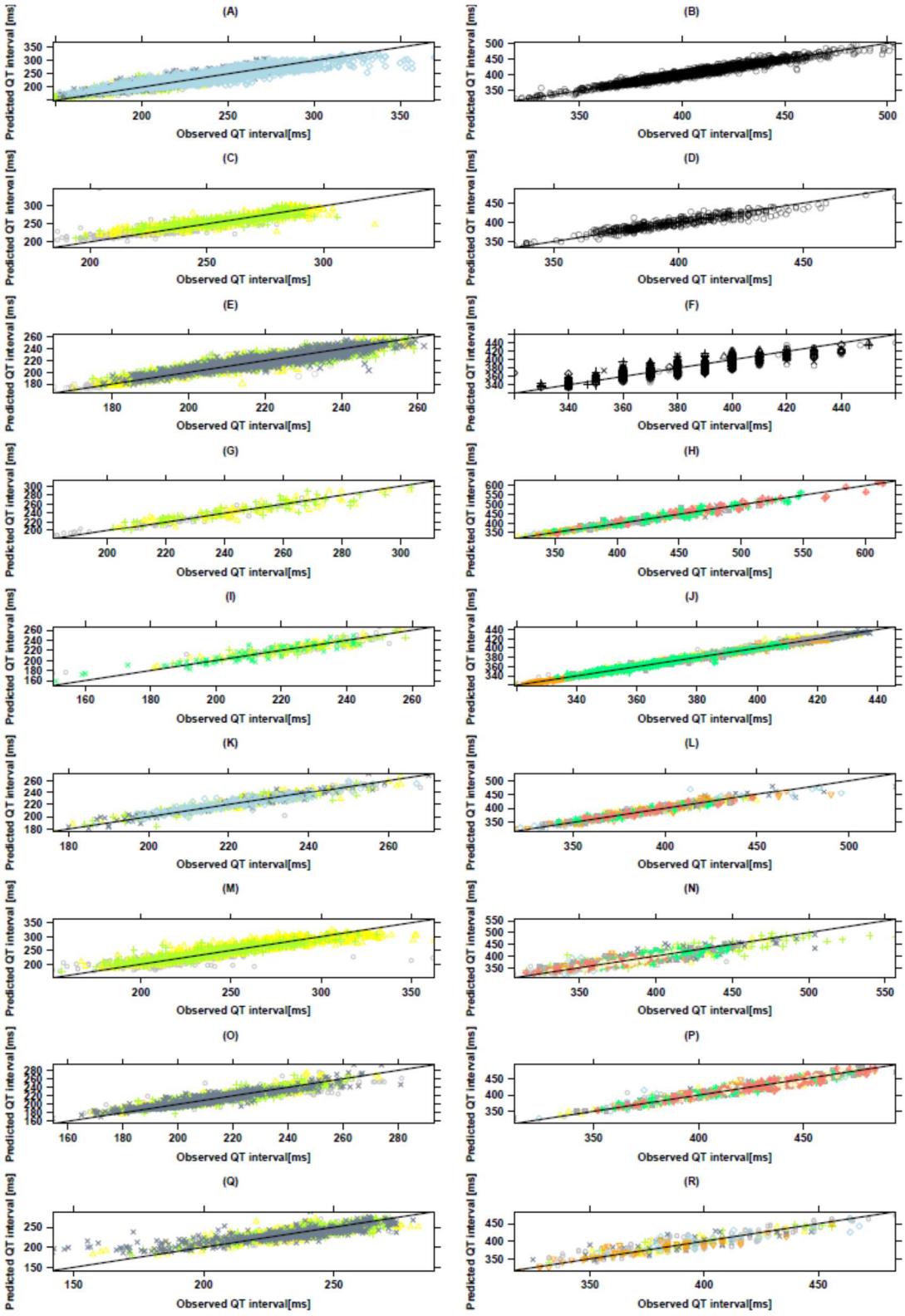

Figure 6.3. Plots describing the goodness-of fit for the PKPD relationships. *Individual predicted and observed QT interval for all 9 compounds after administration to dogs (left panels) and humans (right panels). From top to bottom, panels show data for moxifloxacin, sotalol, cisapride, NCE03, NCE04, carabersat, SB237376, GSK618334, GSK945237 different colours indicate different dose levels (for details see Table 6.1).*

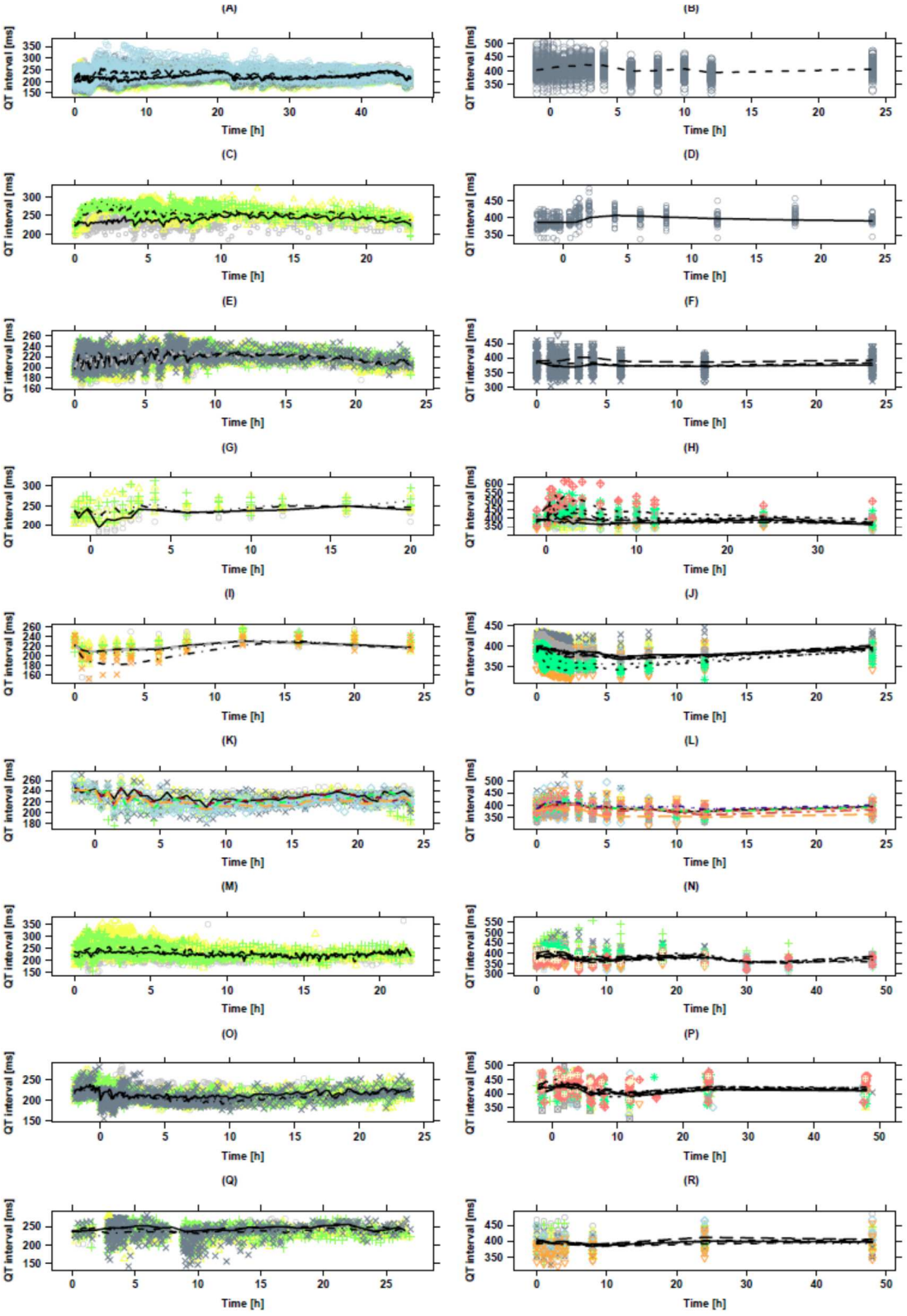

Figure 6.4. Plots describing the model predictions and observed QT interval vs. time for all 9 compounds after administration to dogs (left panels) and humans (right panels). *From top to bottom, panels show data for moxifloxacin, sotalol,cisapride, NCE03, NCE04, carabersat, SB237376, GSK618334, GSK945237 different colours and lines indicate different dose levels (for details see Table 6.1).*

3.2. Interspecies correlation

As indicated previously, the PKPD analysis showed that the main difference between compounds as well as between species was the slope of the linear concentration-effect relationship and consequently CP_{50} (the concentration associated with a 50% probability of QT increase ≥ 10 ms; Table 6.3).

The correlation between the slope parameter in dogs and humas was assessed by linear regression (R^2= 0.989)(Figure 6.5). However, data from cisapride were not included in the estimation steps due to the known differences in QT prolonging effects, i.e., it does not only block a single ion channel type, it also interacts with other ion channels (see Figure S6.1 for the relation including cisapride, appendix). For the compounds without a mixed ion channel block, the slope and intercept parameters describing the linear regression were 11.58 and -1.71ms/μM, respectively. From a translational perspective, these estimates can be considered as a scaling factor and as such used to extrapolate drug effects from dogs to humans in the drug specific effect. Of particular interest is the slope of the regression, which suggests that at comparable drug levels, dogs are on average, approximately 12 fold less sensitive to the drug-induced QT effects than humans.

Given the uncertainty in the parameter estimates obtained during the initial PKPD modelling, 95% credible intervals were also calculated to ensure a worst-case scenario is considered for subsequent scaling purpose (Figure 6.5).

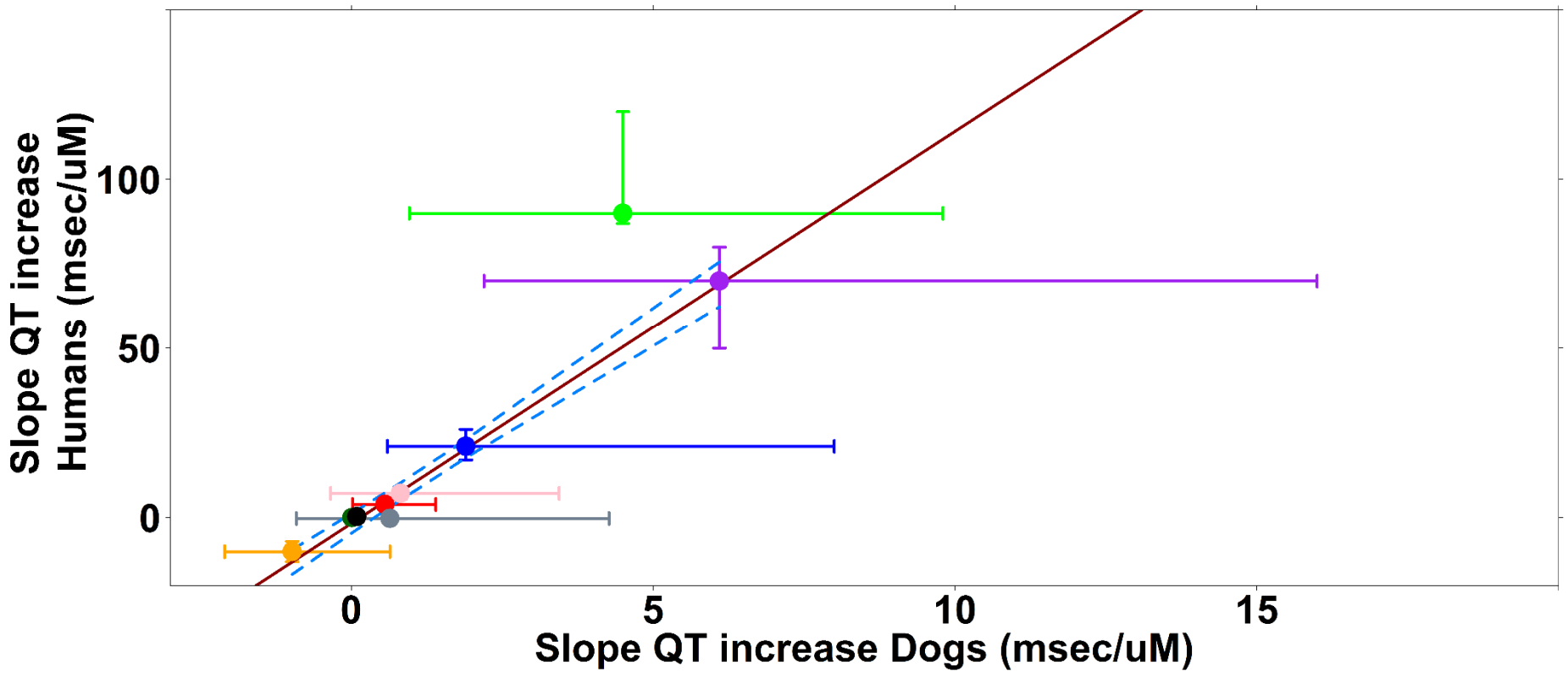

Figure 6.5. Unweighted linear correlation of the slope (ms/µM) in dogs and humans. *Compounds are shown with different colours: cisapride (green), moxifloxacin (red), sotalol (blue), NCE04 (orange), NCE03 (purple), carabersat (grey), GSK618334 (pink), GSK945237 (dark green), SB237376 (black). Dashed lines represents the 95% confidence interval around the mean, linear correlation (red line)= y=-1.709+11.586x, R^2=0.989*

4. Discussion

Evidence of limited or no pro-arrhythmic properties is critical for the progression of compounds into clinical development. A myriad of tests are performed both *in vitro* and *in vivo* before progressing into humans, during which hERG channel blockade, electrophysiological measures of drug activity of heart conductivity and telemetered QT interval are assessed in preclinical species. However, no quantitative measure is available that allows direct extrapolation and prediction of drug effects at therapeutic relevant concentrations in humans. The lack of a scaling factor to translate drug effects in humans represents therefore a major challenge for the assessment of the safety profile of molecules entering Phase I trials, during which doses are escalated to supra-therapeutic levels (29-31).

The difficulties in the prediction of QT prolongation in humans has been assigned to numerous factors, including the lack of suitable measures to characterise prodromic effects *in vitro* and the poor specificity of QT interval as a marker of pro-arrhythmic activity [32,33]. Despite such difficulties, for more than a decade QT prolongation has been recognised by regulators as the most appropriate marker of the risk of TdP and of the potential implications of hERG or ion channel inhibition on heart conductivity. In fact, the current regulatory requirements have contributed to efforts aimed at the assessment of the relationship between drug concentrations and QT interval prolongation. Various examples exist in the published literature, where PKPD modelling has been used as a tool to overcome the shortcomings of the traditional approaches based on statistical hypothesis testing. However, most PKPD models are data-driven and descriptive, i.e., the primary objective of such analyses is to obtain accurate parameter estimates and establish the magnitude of drug-induced effect [34]. Less common is the use of PKPD modelling as the basis for translational purposes.

Here we have attempted to assess the correlation between drug-specific parameter estimates obtained by PKPD modelling of QT interval data in dogs and humans. As shown in Figure 6.5, the identification of a linear correlation for the slope of the PKPD relationships provides an opportunity to revisit the approach for the assessing the liability for QT prolonging effects properties of novel molecules. We explore therefore its utility as a scaling factor or predictor of the clinical effects before candidate molecules enter clinical development.

4.1. Implications of interspecies differences in system-specific parameters

Whereas intrinsic differences are known to exist between species, such as baseline QT (QT_{C0}), changes in this parameter are also caused by physiological factors such as age and basal heart rate [35]. In fact, QT_{C0} in dogs varied from 238 ms for cisapride to 261 ms NCE04. Under comparable experimental conditions, these differences are most likely explained by differences in the age of the dogs. It is well known that heart rate varies with age and in dogs and such changes occur over a shorter time span relative to humans. By contrast, as only adult subjects were used in the clinical studies such effects are not visible in the clinical data (Table 6.2). Similarly, the individual QT-RR correction factor (α) also showed larger values and different ranges in humans, as compared to dogs. Despite such differences between species, the observed variability in the estimates of α should have no impact on the slope parameter, which captures the drug effect on the QT interval.

The other system-specific parameters in dogs and in humans, i.e., the amplitude (A) and the phase of the 24-h circadian rhythm (ϕ) were estimated within a similar range for all compounds (Table 6.2). The variation that was observed in these estimates is likely to be an artefact of the ECG sampling procedures. In fact, for some experiments, the phase parameters showed values greater than 24 h, which exceed the physiological boundaries of the circadian rhythm. These findings were probably due to the limited sampling scheme used in preclinical protocols, which are sparse between 6 and 24 h after dosing.

4.2. Implications of interspecies differences in the drug-specific parameter

In our analysis, the main differences between compounds and between species were found in the estimates of the slope of the PKPD relationships (Table 6.3). Such a systematic difference (i.e., > 10 fold ratio between dogs and humans) prompted us to further explore the correlation between species. We have also noticed that some compounds produce an exposure-dependent change in RR, yielding a different QT-RR correlation during treatment, as compared to the normal physiological changes in QT interval due to variability in the RR interval [36]. As most compounds included in the analysis were known to have minor or no intrinsic effect on heart rate, there were no separate steps to distinguish drug-induced changes in heart rate from drug effects on the QT interval. Eventually, a two-step approach can be considered in which predicted RR values in the absence of drug are used when characterising the QT interval prolongation.

In principle, the larger the value of the slope of the concentration-effect relationship, the stronger QTc interval prolonging effect of the drug is. This was observed for cisapride, sotalol, moxifloxacin, NCE03 and GSK6183343, for which a distinct QT interval prolonging effect was detected within the (putative) therapeutic concentration range of these compounds (table 6.3). On the other hand, if the slope estimates are around zero, there was no or borderline QTc-prolonging effect. This was observed for carabersat, SB237376 and GSK945237. In addition, we showed that a negative value of the slope indicates a shortening of the QT interval. This phenomenon was observed for NCE04 in dogs and humans. As most compounds have not been used in clinical practice, confirmatory data from clinical practice are not available to corroborate the predictive performance of the findings. We envisage therefore that the analysis of new compounds using the same methodology will provide further insight into the generalisability of the correlation as a scaling factor between dogs and humans.

4.3. Translational relevance: extrapolation from animals to humans

One of the main features of the approach proposed by our group was the identification of a measure that describes in a strictly quantitative manner the drug effect on QT interval. As shown in Figure 6.5, we were able to demonstrate a correlation between PKPD parameters in dogs and humans using data from 9 compounds with different mechanisms of action. The estimates of the slope of the PKPD relationship in dogs appeared to be linearly correlated with the parameter estimates in humans. Based on the linear regression defining the interspecies correlation, our findings indicate that at comparable drug levels, humans are on average > 11 fold more sensitive to the drug-induced effect on QT interval. Clearly, further investigation will be needed to assess whether this difference can be used as a scaling factor to predict drug-induced effects on QT interval n humans. However, the evidence of such a correlation emphasises the relevance of dogs as the species of choice for predicting drug-induced QTc interval prolongation in humans.

Most importantly, model predictions show that when QT prolongation occurs in dogs, drug effects may be observed at different (somewhat lower) exposure range in humans. It also reveals that without clear understanding of the expected pharmacokinetic profile and therapeutic exposure in humans, the absence of QT prolongation within a given concentration range in dogs does not allow one to conclude that QT prolongation will not occur in humans.

From a clinical perspective, another important point to consider is that our approach offers the flexibility to explore different thresholds for the QT prolonging effect, including a range from > 1 ms

to >10 ms. Interestingly, the curve describing the probability of QT interval prolongation in human shows a steeper increase across the therapeutic concentration range of each compound, indicating the higher sensitivity of human subjects to QT prolongation ≥ 10 ms at comparable levels in dogs [20]. Moreover, the maximum probability of QT prolongation is consistently observed at lower exposure in humans than in dogs.

Conceptually, our approach contrasts with previous efforts where PKPD relationships were characterised for drugs with known pro-arrhythmic activity [33,34,37,38]. Whereas other authors have also described interspecies differences in PKPD relationships [39-40], to date there are no clear examples of the translation of drug effects from animals to humans. In fact, a potential limitation of these earlier studies is that most models are too specific for the compound of interest or required different experimental data to allow the assessment of the underlying PKPD relationships. Instead, our results demonstrate the feasibility of utilising a single set of parameters and standardised experimental protocols as the basis for predicting the effect of new compounds in humans. In practice, this implies that those involved in the analysis and interpretation of preclinical data can re-use the same model every time a new compound is screened. Inferences about the magnitude of QT interval prolongation in the clinic can be extrapolated from the estimates of the slope of the PKPD relationship in dogs based on the interspecies correlation.

Although the pool of compounds used for the current analysis included only molecules for which the prodromic activity was directly linked to the levels of the parent drug, the same PKPD model may be applied to describe drug-induced effects when QT prolongation is caused by a different moiety or mechanism. In other words, the same model components can be used to assess the PKPD relationship even when delayed effects occur (e.g., presence of metabolites with QT prolonging effects). In these circumstances, a putative effect site compartment can be used to account for nonlinearities between drug exposure and the QT prolonging effect.

4.4. Limitations and recommendations

In traditional conscious *in vivo* cardiovascular safety studies PK sampling is often limited as it may interfere with the QT, RR and blood pressure measurements. Blood samples in these experiments are therefore only obtained after C_{max} and vary between 3-8 samples per animal per study arm. Such

a sampling scheme in dogs can lead to uncertainty in drug disposition parameters, yielding considerably large confidence intervals for the parameter estimates arising from PKPD modelling, as proposed here. It should become evident to the reader that uncertainty in drug levels represents an important confounding factor for the assessment of causality and most importantly for the accurate quantification and extrapolation of drug-induced QT effects. This uncertainty in preclinical pharmacokinetic data is much larger than what is commonly observed in humans. We recommend therefore that attention is paid to protocol design as to facilitate the collection of pharmacokinetic sampling in dogs. Appropriate estimation of pharmacokinetic variability will have considerable impact on the precision of the slope parameter of the PKPD relationships, i.e., high variability will result in large credible intervals, making the prediction of drug effects in humans rather difficult.

We would also like to emphasise that a sensitivity analysis may be required to further explore the implications of variability in parameter estimates as well as the impact of drug-induced effect on heart rate. Our findings suggest that the QT-RR relationship may vary across compounds, especially if chronotropic effects are observed at exposure levels which are relevant to humans. In other words, one needs to disentangle the chronotropic effect from the dromotropic effect to ensure accurate prediction of the magnitude of drug-induced changes in QT interval for drugs which have a direct effect on RR.

Lastly, we acknowledge the potential limitations of a linear pharmacodynamic model to describe the concentration-effect relationship across the therapeutic and supra-therapeutic exposure range. This choice is due to the fact that the maximal observable QT prolongation in clinical trials is likely to be censored by safety stopping criteria. Therefore, a linear concentration-effect relationship was required to avoid underestimation of the QT effect *in vivo*. Moreover, earlier publications that made an effort to fit drug induced QT(c) prolongation data did not see major differences in model performances between a linear and E_{max} model or showed good performance using a linear relationship [22,39,40].

5. Conclusions

In summary, the evidence of a correlation between the slope of the PKPD relationship in dogs and humans for compounds with no or varying pro-arrhythmic characteristics represents an opportunity for reducing attrition rates in the progression of compounds from preclinical to clinical development. Whereas further investigation is required to establish the generalisability of the correlation for a wider range of compounds, we anticipate the use of this correlation as a tool to predict the liability for QT interval prolongation in humans using clinical trial simulations. At this stage, it is unclear whether different correlations can be identified for compounds with distinct mechanisms of action and to what extent the affinity for other ion channels may result in false positive or false negative rates. Nevertheless, our approach provides the basis for further integration of and effective extrapolation of preclinical data, enabling prediction of the drug effects on QT interval before exposing humans to new chemical and biological entities.

6. References

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Appendix

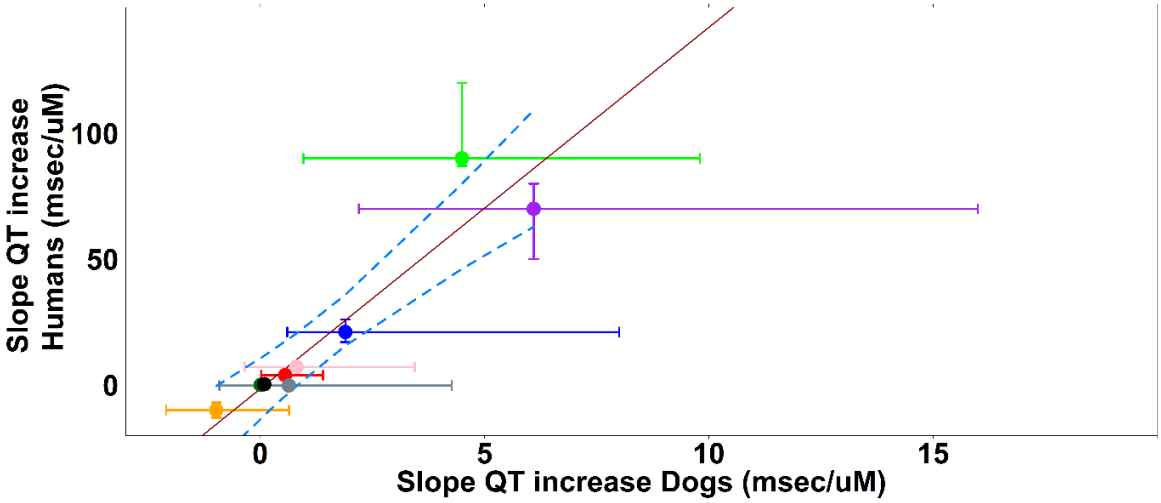

Figure S6.1. Unweighted linear correlation of the slope (ms/uM)in dogs and humans (including cisapride). *Compounds are shown with different colours: cisapride (green), moxifloxacin (red), sotalol (blue), NCE04 (orange), NCE03 (purple), carabersat (grey), GSK618334 (pink), GSK945237 (dark green), SB237376 (black). Dashed lines represents the 95% confidence interval around the mean, linear correlation (red line)= y=-1.5+14.5x, R^2=0.855*