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RESEARCH PAPER

Inter-study variability of preclinical *in vivo* safety studies and translational exposure–QTc relationships – a PKPD meta-analysis

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BACKGROUND AND PURPOSE

Preclinical cardiovascular safety studies (CVS) have been compared between facilities with respect to their *sensitivity* to detect drug-induced QTc prolongation (ΔQTc). Little is known about the *consistency of quantitative* ΔQTc predictions that are relevant for translation to humans.

EXPERIMENTAL APPROACH

We derived typical ΔQTc predictions at therapeutic exposure (ΔQTc_{THER}) with 95% confidence intervals (95%CI) for 3 K_v11.1 (hERG) channel blockers (moxifloxacin, dofetilide and sotalol) from a total of 14 CVS with variable designs in the conscious dog. Population pharmacokinetic-pharmacodynamic (PKPD) analysis of each study was followed by a meta-analysis (pooling 2–6 studies including 10–32 dogs per compound) to derive meta-predictions of typical ΔQTc_{THER}. Meta-predictions were used as a reference to evaluate the consistency of study predictions and to relate results to those found in the clinical literature.

KEY RESULTS

The 95%CIs of study-predicted ΔQTc_{THER} comprised in 13 out of 14 cases the meta-prediction. Overall inter-study variability (mean deviation from meta-prediction at upper level of therapeutic exposure) was 30% (range: 1–69%). Meta-ΔQTc_{THER} predictions for moxifloxacin, dofetilide and sotalol overlapped with reported clinical QTc prolongation when expressed as %-prolongation from baseline.

CONCLUSIONS AND IMPLICATIONS

Consistent exposure-ΔQTc predictions were obtained from single preclinical dog studies of highly variable designs by systematic PKPD analysis, which is suitable for translational purposes. The good preclinical–clinical pharmacodynamic correlations obtained suggest that such an analysis should be more routinely applied to increase the informative and predictive value of results obtained from animal experiments.

Abbreviations

ANCOVA, analysis of covariance; BSV, between-subject variability; CI, confidence interval; C_u, unbound plasma concentration; $Cu_{5ms}/Cu_{10ms}/Cu_{20ms}$, C_u leading to a 5/10/20 ms QTc prolongation; C_{tot}, total plasma concentration; CV, cardiovascular; hERG, human Ether-à-go-go-Related Gene; IOV, inter-occasion variability; ISV, inter-study variability; NONMEM, non-linear mixed effect modelling; PI, prediction interval; PKPD, pharmacokinetic-pharmacodynamic; QTc, QT interval corrected for heart rate and circadian variation; ΔQTc, drug-induced QTc prolongation; ΔQTc_{THER}, drug-induced QTc prolongation at therapeutic exposure; TQT, clinical thorough QT study

Tables of Links

TARGETS
K_v11.1 channel (hERG)

LIGANDS	
Dofetilide	Sotalol

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013).

Introduction

Drug effects on the heart rate-corrected QT interval (QTc) are systematically evaluated in preclinical cardiovascular (CV) safety studies as a surrogate biomarker for pro-arrhythmic risk in humans (ICH S7B Guideline, 2005; Valentin, 2010). Respective *in vivo* experiments, including usually four to eight animals (e.g. conscious dog), should allow a reproducible quantification of drug-dependent QTc (or other CV) effects – that is independent of varying study designs, which may be used across different laboratories. Furthermore, good evidence should be available for translational relationships with human to be of predictive value for clinical risk assessment. With regard to QTc evaluation, such evidence may not only contribute to appropriate candidate and dose selection, but also assist clinical settings where a thorough QT (TQT) study cannot be performed (e.g. oncology), or where the ultimate goal would be to circumvent the conduction of a TQT study (Darpo *et al.*, 2015).

Little, however, is known about the quantitative consistency of preclinical predictions: Inter-facility variability has only been assessed with respect to the sensitivity of standard positive control protocols to detect an 8–10% (≈25 ms) QTc prolongation in preclinical species (Sasaki *et al.*, 2005; Ewart *et al.*, 2013) – while in humans, a 10 ms prolongation is already of regulatory concern (ICH E14 Guideline, 2005). In another review, some evidence was found for consistent preclinical QTc prolongation after moxifloxacin administration across companies (Holzgrefe *et al.*, 2014). However, a quantitative comparison was difficult due to the fact that varying dose levels were used in the studies included, and that temporal changes in drug exposure (pharmacokinetics, PK) were not taken into account. Furthermore, studies that assessed the translational value of non-clinical effects frequently lacked the characterization of concentration–effect relationships (Wallis, 2010).

The difficulty in comparing heterogeneous studies quantitatively can be overcome by comparing *concentration*–effect relationships over time [i.e. by pharmacokinetic-pharmacodynamic (PKPD) analysis], instead of *dose*–effect relationships at pre-defined time points (as assessed by conventional analysis of covariance, ANCOVA; Aylott *et al.*, 2010). Therefore, PKPD modelling is also a method advocated for translating drug effects from preclinical species to humans (Leishman *et al.*, 2012; Caruso *et al.*, 2014; Parkinson *et al.*, 2014). In CV safety, several isolated examples are published that compare the magnitude of QTc interval prolongation in the conscious telemetered dog (Ollerstam *et al.*, 2006; Chain *et al.*, 2013; Parkinson *et al.*, 2013; Sparve *et al.*, 2014) and other species (Watson *et al.*, 2011; Caruso *et al.*, 2014) with humans. A possible limitation to generalize respective findings is that the translational relationships derived were based on rather a small number of animals (n = 4–8) and a single clinical study only, thus making the accuracy of typical translational predictions uncertain, and ignoring inter-study variability (ISV).

The objectives of the following analysis were thus (i) to assess quantitatively the consistency of typical exposure–QTc relationships predicted from different CV safety studies in the conscious telemetered dog after administration of three well-known reference compounds; (ii) to characterize both the typical (mean population) concentration–effect relationship and between-subject variability (BSV) for each compound in a PKPD meta-analysis, which is with more confidence than from a single preclinical study; and finally (iii) to contrast meta-predictions derived in the dog with those reported in the literature for dogs and humans, to update and increase the evidence for translational pharmacodynamic (PD) relationships between dogs and humans.

Methods

Figure 1 illustrates the schedule used for this PKPD meta-analysis. Briefly, 14 CV studies were analysed both individually (an independent concentration–ΔQTc relationship prediction was derived from each) and in a meta-analysis after pooling studies from each compound (to derive meta-predictions of typical concentration–ΔQTc relationships). Study predictions of QTc prolongation at therapeutic expo-

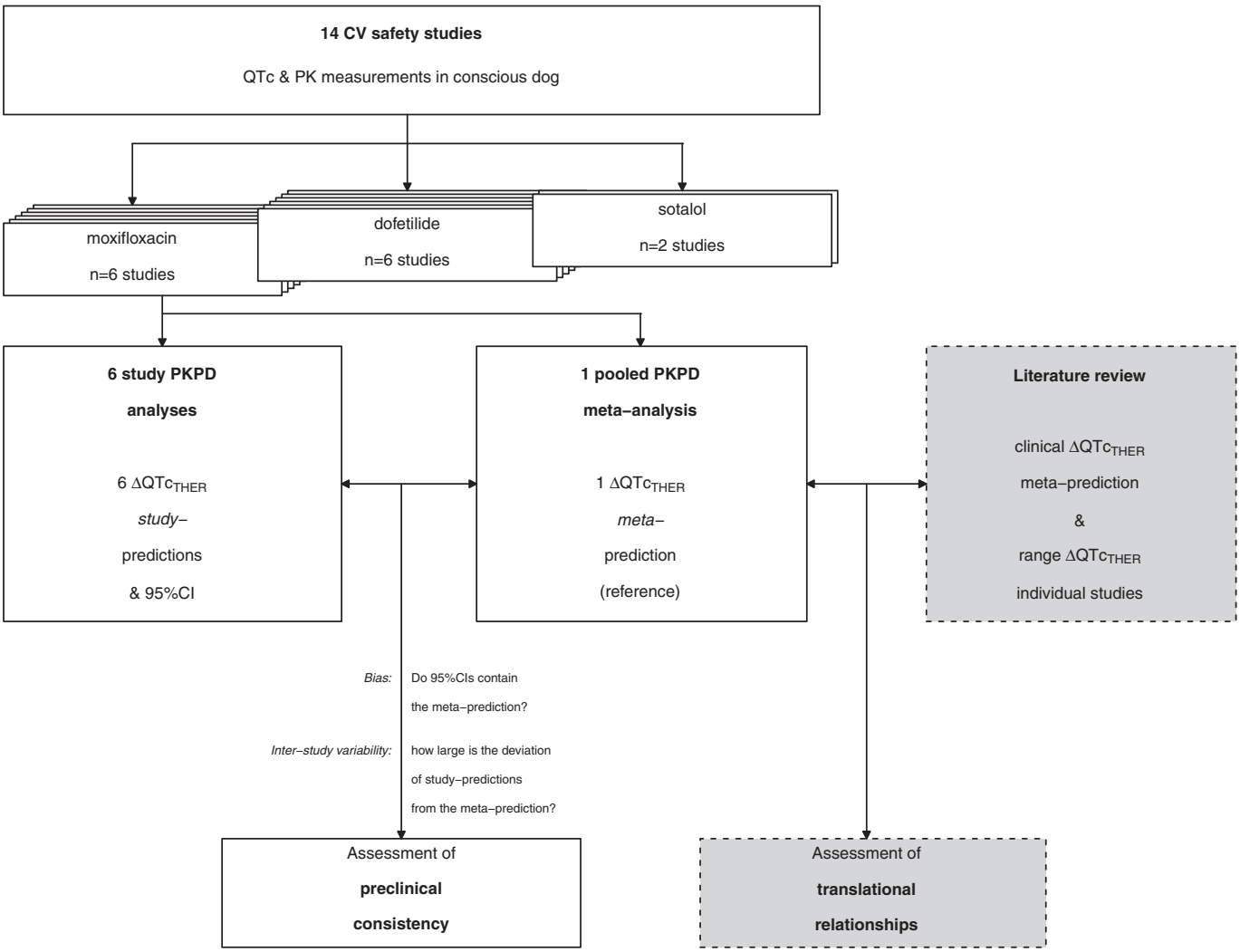

Figure 1

Illustration of the workflow of this PKPD meta-analysis (fully illustrated for moxifloxacin but also applies equally well to dofetilide and sotalol). An ISV in ΔQTc_{THER} predictions was also calculated from all 14 preclinical studies. The main results are summarized in Figure 5.

sure (ΔQTc_{THER}) were compared with the meta-prediction to assess bias and ISV (assessment of consistency). A literature review was performed to relate the preclinical meta-predictions with clinical ΔQTc_{THER} (assessment of translational relationships).

Animals and experimental procedures

All animal experiments were conducted in accordance with Good Laboratory Practice Regulations [in particular with 'the Provision of the European Convention on the protection of vertebrate animals, Appendices A and B', made at Strasbourg on 18 March 1986 (Belgian Act of 18 October 1991), and with guidelines provided by the National Research Council Institute for Laboratory Animal Research (NRC ILAR)]. All studies were approved by an appropriate ethics committee, and reported in accordance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

QT/RR-interval measurements (PD) data and, if possible, drug plasma concentration (PK) data in the conscious telemetered dog (freely moving Beagle dogs) were obtained after administration of three reference compounds (moxifloxacin, dofetilide, *d,l*-sotalol) from a total of six different companies in a consortium (2–6 studies per compound; see Tables 1–3). Studies differed with respect to their design in various factors, such as number of animals, number of doses and dose levels studied, route of administration, strategy of PK sampling (in the same animals or in a satellite group) and ECG data processing (automatic dense data extraction, automatic/manual data validation at fewer time points, data averaging). Four studies had already been analysed and reported before: moxifloxacin study 3 (Chain *et al.*, 2013), sotalol study 1 (Chain *et al.*, 2013; Gotta *et al.*, 2015), and moxifloxacin study 4 and dofetilide study 6 (Ollerstam *et al.*, 2007). PK data were not available for moxifloxacin study 3.

Table 1

Moxifloxacin studies (freely moving Beagle dogs)

	Study 1	Study 2	Study 3	Study 4	Study 5	Study 6
Company	A	B	C	D	E	F
Reference			(Chain *et al.*, 2013)	(Ollerstam *et al.*, 2007)		
Administration route	Oral	Oral	Oral	i.v.	Oral	Oral
Dose levels	Vehicle, 3, 10, 30 mg·kg^{-1}	Vehicle, 3, 10, 30 mg·kg^{-1}	Vehicle, 10, 30, 100 mg·kg^{-1}	Vehicle, 12 mg·kg^{-1} (given over 3 h)[a]	Vehicle, 10, 30, 100 mg·kg^{-1}	Vehicle, 10, 30, 100 mg·kg^{-1}
Animals [*n*] per dose level	4 (only PD)	4 (PK and PD)	8 (only PD)	6 (PK and PD)	6 (PD) 2 (PK)	4 (PD) 4 (PK)
Study duration	24 h	24 h	48 h	24 h	22 h	28 h (vehicle: 56 h)
Gender	Male	Male	Male/female	Male/female	Female	Male/female
Body weight (kg)	12–16	15.5–17.9	9–12.6	15.7–19.8	12	8.7–12.4
ECG sampling	Every 2 min (averaged from every 20 s data)	Every 15 min	Every 1 min	Every 5 min (averaged from every 20 s data)	Every 30 min	Every 1 min
	60 min[b]	60 min[b]	60 min[b]	15 min (to 6 h) 60 min[b]	30 min (to 4 h) 60 min[b]	60 min[b]
(*n* samples/dose and individual)	*n* = 26	*n* = 25	*n* = 48	*n* = 43	*n* = 27	*n* = 28
PK sampling (*n* samples/dose and individual)	–	1–6	–	14	6 (satellite group)	7 (satellite group)

[a]Given in two subsequent infusions of 8 and 22 µmol·kg^{-1} = 3.2 and 8.8 mg·kg^{-1} over each 90 min.
[b]Data were averaged over longer periods in the pooled analysis.

Table 2

Dofetilide studies (freely moving Beagle dogs, unless otherwise stated)

	Study 1	Study 2	Study 3[a]	Study 4a/b	Study 5a/b	Study 6
Company	B	B	B	E	E	D
Reference						(Ollerstam *et al.*, 2007)
Administration route	i.v. (slinged)	Oral	Oral	Oral	Oral	i.v.
Dose levels	Vehicle, 25.1 μg·kg^{-1} (given over 2 h)[b]	Vehicle, 5, 15 μg·kg^{-1}	Vehicle, 50 μg·kg^{-1}	Vehicle, 10, 20, 30 μg·kg^{-1}	Vehicle, 5, 10, 20 μg·kg^{-1}	Vehicle, 110 μg·kg^{-1} (given over 3 h)
Animals [*n*] per dose level	4 (PK and PD)	7 (PK and PD): 3 (5 μg·kg^{-1}) 4 (15 μg·kg^{-1}) 7 (all, vehicle)	4 (PK and PD)	8 (PK and PD)	8 (PK and PD)	7 (PK and PD)
Study duration	2.4 h	24 h	24 h	4–6 h	4–6 h	24 h
Gender	Male	Male	Male	Female	Female	Male/female
Body weight (kg)	13.7–15.7	14.0–17.6	13.3–16.7	9.7–13.8	9.7–13.8	13–18
ECG sampling	Every 1 min	Every 15–30 min	Every 15 min	Every 30 min	Every 30 min	Every 5 min (averaged, raw data: every 20 s)
(*n* samples/dose and individual)	*n* = 154	*n* = 96	*n* = 92	*n* = 16–18	*n* = 16	*n* = 254
PK sampling (*n* samples/dose and individual)	8	8 (different day)	10 (different day)	1–6	1–6	11

[a]Although three of the individuals in this study 3 were included also in study 1 and/or 2, they were treated as independent subjects in the pooled PKPD analysis. PKPD profiles were very different (see Results/Discussion), and study 3 was performed ≈2 years before study 1 and study 2.
[b]Loading dose (over 5 min): 0.27, 0.63, 1.8, 6.3 μg·kg^{-1} + maintenance dose (over 25 min): 0.81, 2.7, 8.1, 27 μg·kg^{-1}·h^{-1}.

Table 3
Sotalol studies (freely moving Beagle dogs)

	Study 1	Study 2
Company	E	F
Reference	(Chain *et al.*, 2013; Gotta *et al.*, 2015)	
Administration route	Oral	Oral
Dose levels	Vehicle, 4, 8, 36 mg·kg^{-1}	Vehicle, 3, 15, 40 mg·kg^{-1}
Animals [*n*] per dose level	6 (PK and PD)	4 (PD) 9 (PK): 3 (3 mg·kg^{-1}) 3 (15 mg·kg^{-1}) 3 (40 mg·kg^{-1})
Study duration	24 h	96 h
Gender	Female	Male/female
Body weight (kg)	11.2–13.5	11–13.4
ECG sampling	Every 30 min (averaged from 5 min raw data)	Every 60 min (averaged from 1 min raw data)
(*n* samples/dose and individual)	*n* = 48	*n* = 96
PK sampling (*n* samples/dose and individual)	8	7 (satellite group)

Population PKPD modelling

Both the individual study and meta-models were built in a stepwise procedure: first, PK models were developed, then respective typical parameters (if PK was studied in a satellite group) or individual parameters (if PK was acquired simultaneously with ECG measurements) were fixed to their final estimates to obtain continuous concentration predictions for PKPD analysis. In the moxifloxacin studies in which no PK measurements were available at all, typical predictions from a literature PK model (for individual study analysis; Chain *et al.*, 2013) or from the final PK meta-model (for PKPD meta-analysis) were used. To simplify the pooled study analysis (avoid computational problems) and evaluate observations from different studies more equally (e.g. individual samples up to 4000 per dosing level were available in one study vs. only 16 in a different study), the number of samples per individual was standardized by averaging data over 15–60 min (moxifloxacin, PK and PD were mainly measured in separate studies) and 1–30 min (dofetilide, PK and PD were measured simultaneously in all studies giving more confidence in the predicted individual PK profiles; a fuller extraction was made in the case of shorter study durations). In a sensitivity analysis, model parameter estimates resulting from shorter and longer averaging periods were compared.

The QT variation over time was modelled as a linear function of heart rate, periodic circadian variation (for studies over ≥24 h, and if visually plausible), and total drug plasma or effect-site concentration. To describe the concentration–effect relationship (PD), linear, power and (sigmoidal) E_{max} models were tested. Direct and link models (in the case of observed hysteresis) were tested to characterize temporal PK–PD relationships. For more details, see Supporting Information Appendix S1.

Sources of ISV. Two approaches were used to investigate sources and effect of ISV in the final PKPD meta-models: (i) Estimation of ISV with nested BSV (only moxifloxacin). This approach is expected to result in unchanged typical parameter estimates, but a better distinction between BSV and ISV (Laporte-Simitsidis *et al.*, 2000). Because of the strictly nested structure, this hierarchical model is not applicable to the pooled dofetilide studies, since individual dogs from the same company were included in several studies. (ii) Estimation of study-covariate effects on typical parameter estimates. This method does not allow the derivation of an overall mean concentration-effect estimate, but illustrates a study effect on BSV estimates. ISV was not investigated in the sotalol meta-analysis because only two studies were included.

Model-derived PD predictions: preclinical exposure–QTc relationships

From the final PKPD model parameter estimates, typical concentration–effect profiles were derived from each study individually and the meta-analyses. The 95% confidence intervals (95%CI, measure for uncertainty) were calculated from 5000 multivariate parameter simulations of the estimate's (NONMEM R-) covariance matrix. Predicted QTc prolongation over unbound concentration (C_u) was derived from total concentrations (C_{tot}) by the fraction unbound (f_u): $C_u = C_{tot} \cdot f_u(dog)$. Unbound concentrations leading to 5–20 ms prolongation were derived (Cu_{5ms}, Cu_{10ms}, Cu_{20ms}) as a measure to assess safety margins.

The 90% prediction intervals (90%PI, measure of total variability) were derived only from the meta-models through 5000 Monte Carlo simulations over the concentration range studied. This reflects expected variability composed of BSV, inter-occasion variability (IOV, only estimated on baseline QTc values) and residual variability.

Assessment of consistency of study predictions. Predictions of typical QTc prolongation at lower and upper limits of thera-

peutic unbound exposure ($\Delta QTc_{THER(LL)}$ and $\Delta QTc_{THER(UL)}$) were derived from each study. Those study predictions were compared with the meta-prediction using the 95%CI to assess if study predictions are biased. The mean deviation from the meta-prediction (in [ms] and [%]) was calculated as an estimate of ISV.

Literature review: clinical exposure–QTc relationships

A literature search was performed for previously reported concentration–effect relationships in both humans and dogs (details: see Supporting Information Appendix S2). From each reference, typical $\Delta QTc_{THER(LL)}$ and $\Delta QTc_{THER(UL)}$ were predicted. To facilitate dose–effect comparisons, the human dose kg^{-1} was also calculated and used to derive the corresponding dose kg^{-1} in the dog by body-size-based (allometric) scaling: equivalent dog [$mg \cdot kg^{-1}$] = therapeutic dose humans [mg $(70\ kg)^{-1}$] · 37 [$k_{m,human}$]/20 [$k_{m,dog}$] (Reagan-Shaw *et al.*, 2008); k_m = body weight [kg]/body surface area [m^2].

Assessment of translational PD relationships. Preclinical and clinical predicted exposure-dependent QTc prolongation and $\Delta QTc_{THER(LL/UL)}$ (i.e. from both the present PKPD analyses and literature review) were summarized numerically and visually compared in terms of [ms] and [%] prolongation from baseline.

Results

PKPD modelling

Individual study analyses. The final population PK and PKPD models are summarized in Supporting Information Appendix S1, where each study data set is additionally illustrated with model predictions. Both one- and two-compartment models were, dependent on the study, appropriate for describing PK profiles of the same drug. Furthermore, for the same drug and depending on the study, linear, sigmoid and hyperbolic models were appropriate for describing PKPD relationships.

Meta-analyses. Figure 2 illustrates data and model predictions from the pooled study analyses. Corresponding meta-model parameters are summarized in Table 4.

PK meta-models. A two-compartment model with linear elimination best described the typical concentration-time profile of all three drugs in the pooled analysis. Concerning moxifloxacin, concentrations were over-predicted after oral high dose administration (100 $mg \cdot kg^{-1}$, p.o.) when assuming dose proportionality of exposure. This prediction bias could be corrected by estimating a separate typical absorption rate (lower k_a) and fraction absorbed (reduced bioavailability F), which yielded improved description of the rather flat and low PK profile after high dose. Complete bioavailability was in contrast estimated for the lower dose levels. Variability in the rate of absorption between dose occasions (IOV_{ka} of 19%) was higher than between individuals (BSV_{ka} close to 0%).

PD meta-models. A sigmoidal E_{max} model best described the pooled PD data of moxifloxacin, dofetilide and sotalol [lowest objective function value (OFV) and residual error, best individual fits] with similar maximal estimated QTc prolongation (E_{max}) of ≈50 ms. A proportional drug effect model (with maximal drug effects of ≈22% for all drugs) described the data similarly well as an additive drug effect model.

Temporal PK–PD relationship. While hysteresis was observed in some dofetilide, moxifloxacin and sotalol studies to a more or less significant extent, a distribution delay was only retained in the pooled sotalol analysis, with a respective distribution half-life of 38 min (k_{e0} = 1.1 h^{-1}). It should be noted that respective individual random effects were not normally distributed (BSV estimate: 209%), as hysteresis was only observed in individuals from one of the two studies (see Supporting Information Appendix S1).

For dofetilide, estimated PD parameters from a direct or link model were almost identical, as well as residual variability, and the estimated distribution half-life was short (2 min).

For moxifloxacin, it seemed plausible that observed hysteresis in some studies was an artefact of IOV and dose dependency of absorption (no hysteresis was observed in the i.v. study; all oral PK studies were performed in a satellite group with few data during the absorption phase; proteresis – a steeper initial increase in the effect than in concentrations – was partly also observed in oral studies). Allowing an IOV in k_a (or accounting for a distribution delay with an estimated distribution half-life of 45–60 min, yielding almost identical 'effect' concentration predictions) led to E_{max} and EC_{50} estimates that were approximately 2× higher than in the direct effect model, and the residual error decreased from 7.7 to 6.8 ms. Sources of this IOV_{ka} (estimate: 55%, Table 4) seemed incompletely understood (IOV_{ka} estimate larger than expected from PK model) and respective random effects were not normally distributed. An underlying bimodal mixture distribution (Carlsson *et al.*, 2009) with 40% of occasions classified as 'normal' absorption (k_a = 0.47–0.77 h^{-1}, Table 4) and 60% as very slow absorption (k_a = 0.05 h^{-1}) may explain this, which reduced IOV_{ka} to 28%. Using a transformed distribution (e.g. box-cox or logit; Petersson *et al.*, 2009) was less successful to correct the mispredicted distribution of IOV_{ka}. Because of the lack of physiological explanations for this bimodal distribution, and because the resulting estimated PD relationships were unchanged, a mixed distribution was not kept in the final model.

In a sensitivity analysis, averaging ECG data over 5–30 min (dofetilide), and up to 60 min (moxifloxacin and sotalol), did not significantly affect PD parameter estimates (especially in direct effect models), while unexplained residual variability decreased. In the link models, hysteresis became more clear after data averaging (especially in one dofetilide study), and in the pooled analysis of the moxifloxacin studies visually more plausible estimates of E_{max} (and of EC_{50}) were obtained, which were more consistent with estimates from individual study analysis.

Inter-study variability. For moxifloxacin, nested BSV in ISV could be estimated, ISV though with relative standard errors of 140–550%. As expected, typical parameter estimates were unchanged; however, a 10–20% decrease of BSV in PD

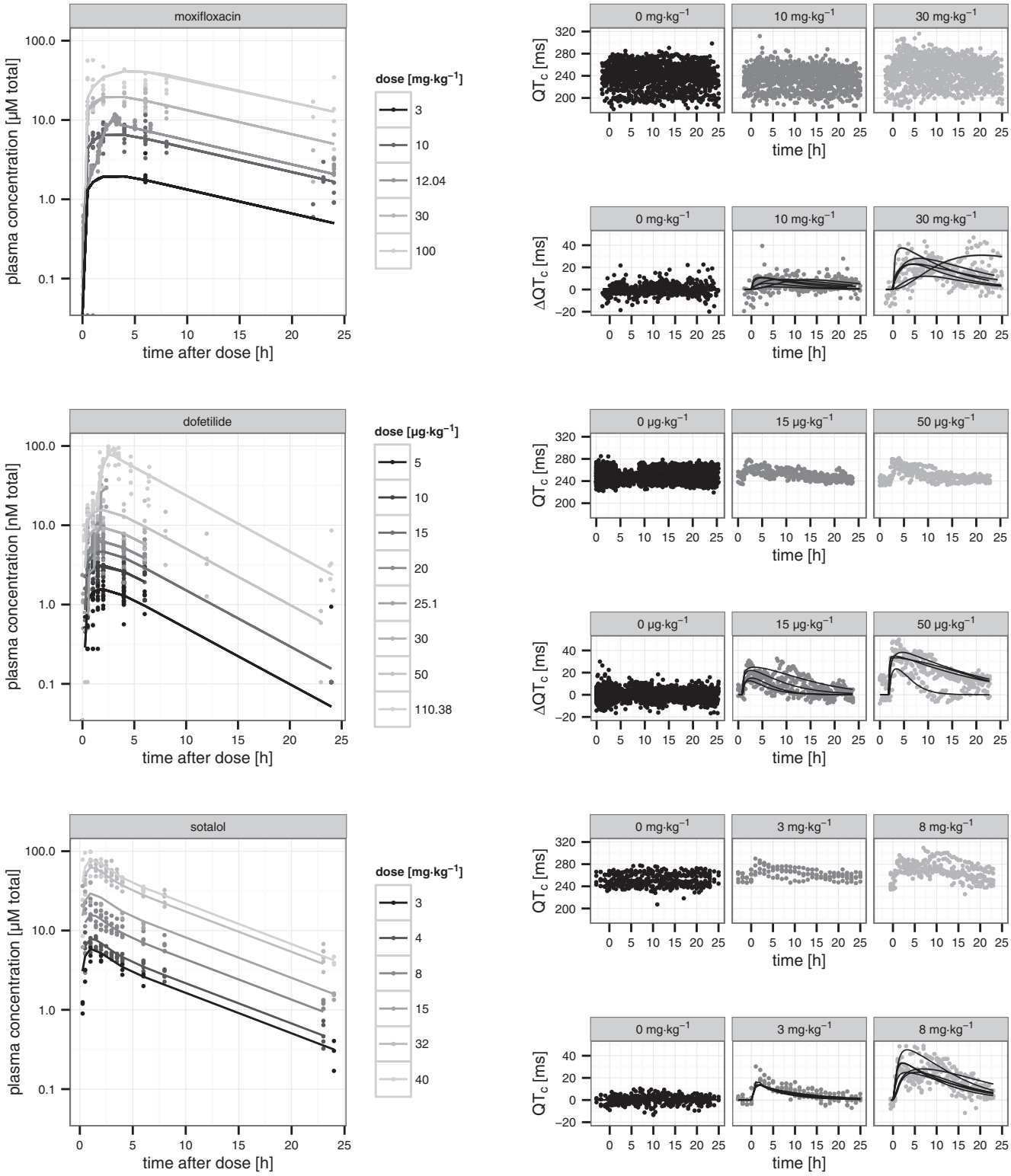

Figure 2

Illustration of pooled study data and meta-model predictions (parameters: see Table 4). Left: *PK*: plasma concentration over time. Drugs were mainly given orally, only moxifloxacin 12.04 mg·kg^{-1} and dofetilide 25.1 and 110 µg·kg^{-1} were given as i.v. infusion. *Dots*: measured plasma concentrations. Lines: typical concentration prediction from meta-model. Right: *PK-PD*: Individual QTc (upper panel; all individuals, correction was made for heart rate and circadian variation) and drug-induced QTc prolongation (ΔQTc, lower panel) over time after vehicle administration, ≈human equivalent dose and ≈3× human equivalent dose. Dots: observations. Lines*:* individual model predictions. For moxifloxacin, only one individual per study is represented in the lower panels.

Table 4

Summary of final population PK and PKPD meta-models (pooled study analysis)

	Moxifloxacin	Dofetilide	Sotalol
Parameter	Estimate (RSE%)	Estimate (RSE%)	Estimate (RSE%)
PK meta-models			
Individuals [*n*]	16	25	15
PK samples [*n*]	241	527	250
Typical value			
k_a (h^{-1})	0.77 (<100 mg·kg^{-1}) (12%) 0.43 (100 mg·kg^{-1}) (31%)	0.91 (19%)	1.6 (21%)
CL ($L·h^{-1}·kg^{-1}$)	0.21 (7%)	0.43 (10%)	0.24 (4%)
V_c ($L·kg^{-1}$)	0.75 (14%)	0.35 (31%)	1.1 (10%)
V_p ($L·kg^{-1}$)	2.2 (8%)	2.1 (6%)	0.73 (18%)
Q ($L·h^{-1}·kg^{-1}$)	4.1 (22%)	4.4 (20%)	0.40 (11%)
F [–]	1 (<100 mg·kg^{-1}) (fixed[a]) 0.7 (100 mg·kg^{-1}) (9%)	0.52 (7%)	Not estimated (*fixed to 1*)
Lag_1 (h)	–	0.23 (5%)	–
Variability			
BSV k_a	–	–	78% (23%)
BSV CL	23% (23%)	40% (18%)	12% (23%)
BSV V_c	–	95% (33%)	18% (31%)
BSV V_p	22% (32%)	–	–
BSV Q	–	33% (38%)	–
BSV F1	–	–	–
IOV k_a	19% (20%)	40% (16%)	63% (35%)
Residual			
Proportional	23% (10%)	24% (10%)	19% (5%)
Additive	–	0.2 nM (40%)	–
PKPD meta-models			
Individuals [*n*]	32	27	10
ECG samples [*n*]	4084	7548	2754
Typical value			
QTc_{BL60} (ms)	239 (1%)	248 (1%)	249 (1%)
E_{max} (ms)	54.6 ms (10.6%)/22.8%	50.2 (20%)	55 (9%)
EC_{50} (nM or µM) total	16.3 µM (9%)	9.5 nM (39%)	10.1 µM (10%)
EC_{50} (nM of µM free)	≈11.6 µM free	≈4.2 nM free	≈10.1 µM free
Hill coefficient [–]	1.8 (9%)	1.4 (10%)	1.1 (7%)
K_{e0} (1 h^{-1})			1.1 (45%)
Variability			
BSV QT_{BL60}	8% (13%)	4% (16%)	4% (23%)
BSV E_{max}	51% (22%)	53% (22%)	24% (25%)
BSV EC_{50}	42% (23%)	105% (21%)	19% (39%)
BSV Hill coefficient	50% (16%)	74% (15%)	16% (33%)
BSV k_{e0}		–	209%[b] (32%)
Correlation (ρ) between individual random effects	(Not estimated)		(Not estimated)
ρ E_{max}-EC_{50}		0.78	
ρ E_{max}-Hill coefficient		–0.51	
ρ EC_{50}-Hill coefficient		–0.17	
IOV QT_{BL60}	0.7% (10%)	2.2% (7%)	1.6% (19%)
IOV k_a	55%[b] (10%)	–	–
Residual			
Additive (ms)	6.8 (2%)	6.2 (2%)	5.8 (3%)

RSE%: relative standard error.
k_a: first-order absorption rate constant. CL: clearance. V_c: central volume of distribution. V_p: peripheral volume of distribution. Q: inter-compartmental clearance. F: bioavailability. QTc_{BL60}: baseline QTc interval [at a heart rate of 60 bpm, QTc was corrected for both heart rate (linear correction) and circadian variation]. E_{max}: maximal drug-induced QTc prolongation. EC_{50}: plasma or effect concentration leading to a half-maximal drug-induced QTc prolongation. Hill coefficient: parameter improving description of sigmoid (if >1) or hyperbolic (if <1) relationships. K_{e0}: distribution rate constant to effect side.
[a]Estimating F at lower doses yielded estimates of 1 – because this is the highest value that bioavailability can take, the value was fixed.
[b]Respective individual random effects were not normally distributed (see Results).

parameters was observed, but not in baseline QTc values. Estimated ISV was of a similar magnitude to that of BSV. Estimating a study effect on different PKPD parameters indicated that the sigmoidicity parameter was possibly the most study-specific parameter [biggest decrease in NONMEM OFV by –25, corresponding to a significant likelihood ratio test ($P < 0.001$) for six additional parameters], with a decrease in BSV on the Hill coefficient from 50% to almost 0 (6% with relative standard error of >150%). BSV did not seem to be affected by a study-covariate effect on other parameters.

It was not possible to estimate a nested hierarchical model for dofetilide. Significant 'study covariate effects' could be estimated for all PD parameters and baseline QTc (OFV: –240 to –86; $P < 0.0001$), while BSV decreased by 5–15%. Also here, BSV decreased most (–15%) when assuming a study effect on the sigmoidicity parameter.

Predicted preclinical exposure–QTc relationships and consistency

Figure 3 illustrates observed and model-predicted exposure–ΔQTc relationships from the pooled and individual study PKPD analyses, respectively, along with the 90%PI and 95%CI. A numerical summary of respective predictions at therapeutic exposure ($\Delta QTc_{THER(LL/UL)}$), deviations from the pooled analysis and safety margin measures (Cu_{5ms}, Cu_{10ms}, Cu_{20ms}) is given in Table 5.

The 95%CIs for each study prediction are additionally summarized and illustrated in Supporting Information Appendix S3. The 95%CIs of $\Delta QTc_{THER(LL/UL)}$-predictions of 13 out of 14 (93%) studies included the meta-prediction and were thus not biased. The overall mean deviation from the meta-prediction was ±30% at upper exposure levels (Figure 5), while for each drug this ISV estimate ranged between 14 and 44% (Table 5).

Clinical QTc prolongation and translational PD relationships

Figure 4 shows a comparison of exposure–effect relationships ($\Delta QTc_{THER(LL/UL)}$) in the dog with literature-derived relationships in humans within the unbound human therapeutic concentration range in terms of [ms] and [%] prolongation from baseline. A numerical summary is given in Table 5; for details of the literature values see Supporting Information Appendix S2. Figure 5 summarizes the main study findings (consistency and translational value of preclinical studies).

Discussion

This study presents for the first time a systematic population PKPD analysis of drug-induced QTc effects in 14 CV safety studies in the conscious telemetered dog. Characterizing concentration–effect instead of dose–effect relationships allowed the consistency of QTc predictions to be assessed quantitatively among all studies, and the comparison of preclinical and clinical PD. Studies for each compound [moxifloxacin, sotalol and dofetilide, all $K_v11.1$ channel (hERG) blockers] were furthermore pooled in a meta-analysis (included 10–32 dogs per compound) to characterize typical concentration–effect relationships with more confidence than from individual studies.

Results showed that 93% of QTc predictions derived from single dog studies (included four to eight dogs) were unbiased with respect to the mean estimated from the meta-analysis. An average deviation of 30% from this dog-specific mean can however be expected in future studies (ISV estimate at upper therapeutic concentrations; meta-predictions of $\Delta QTc_{THER(UL)}$ were >10 ms). Comparing meta-predictions with systematically reviewed clinical ΔQTc_{THER} effects showed increased evidence for overlapping PD relationships in the conscious dog and human. Assuming equal %QTc prolongation from baseline in dog and human seems thus a good approximation for translation of QTc effects, although a slightly higher effect in human might be expected for predicted ΔQTc >> 10 ms (see dofetilide and sotalol at upper therapeutic exposure). If such a simple translation approach is also valid for non-hERG or mixed ion-channel blockers should be further investigated.

Consistency of QTc predictions

Quantitative consistency of preclinical QTc predictions, despite heterogeneous study designs, is a first prerequisite for appropriate translation of preclinical effects to human.

Similar to previous inter-facility comparisons (Sasaki *et al.*, 2005; Ewart *et al.*, 2013), we confirmed that the positive control drugs showed significant QTc prolongation in *all* studies. In addition we showed that PKPD analysis was sensitive enough to detect this: concentration–effect relationships were estimated from all studies with good confidence, while the highest dose exceeded human equivalent doses by 2- to 10-fold. More importantly, significant QTc prolongation at therapeutic exposure was quantified in all but one study (13/14 studies, = 93%), and 95%CI of predicted Cu_{10ms} were in all but one study clearly overlapping with therapeutic exposure (possible reasons for divergent predictions are discussed under 'limitations'). This is consistent with our previous sensitivity estimate of ≥90% to detect QTc prolongation of ≥10 ms using PKPD analysis, superior to traditional dose-effect analysis (Gotta *et al.*, 2015).

Considering the meta-predictions as reference, individual study predictions varied on average by 30% (range: 1–69%) from this value at highest therapeutic exposure (Figure 5). This ISV estimate is close to a previous estimate from a simulation study (24% for QTc ≥10 ms; Gotta *et al.*, 2015), while the exact magnitude of ISV may be drug dependent (Table 5). No significant bias was observed in predictions from individual studies within therapeutic concentrations (ΔQTc_{THER}): 95%CI of 13 out of 14 studies (93%) comprised the meta-prediction.

PKPD-derived predictions at therapeutic exposure were similar to the mean ΔQTc around time of C_{max} calculated from conventional analysis (ANCOVA) under human equivalent therapeutic dose (data not shown). For example, for moxifloxacin (10 $mg \cdot kg^{-1}$ p.o. to 12 $mg \cdot kg^{-1}$ i.v. ≈human equivalent dose), a 4.7–20 ms QTc prolongation was estimated by ANCOVA from different studies (vs. 4–12 ms by PKPD). The added value of PKPD analysis is that an exposure dependency *within* therapeutic doses could be seen, which is suggesting that the risk of QTc prolongation can significantly increase above therapeutic exposure and/or that a patient's individual risk of QT prolongation can be guided by plasma levels.

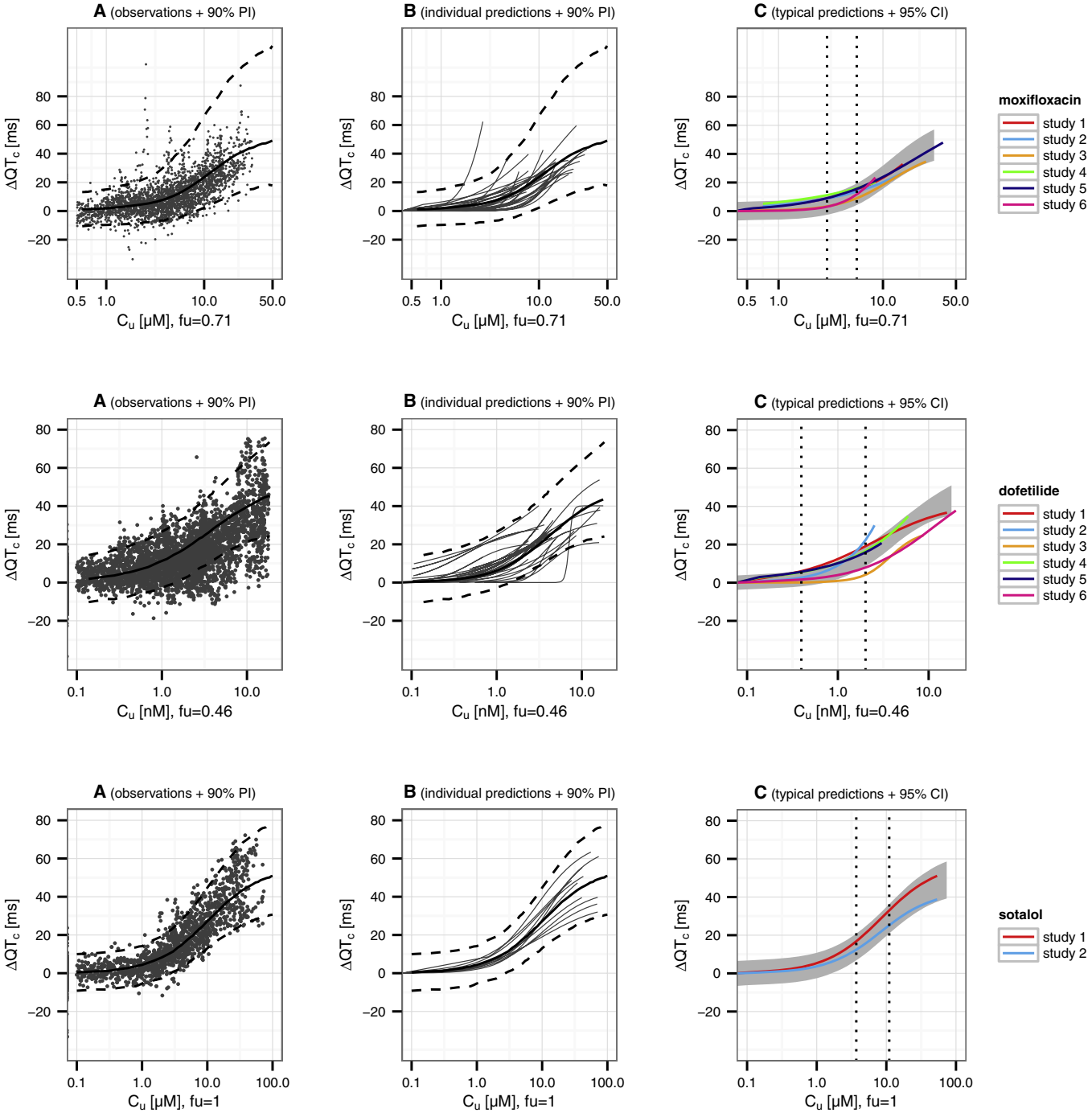

Figure 3

PD: Observed and predicted PD relationships from meta-analyses (upper panel*:* moxifloxacin, mid panel: dofetilide, lower panel*:* sotalol). (A) Observed ΔQTc (dots) with 90%PI (dashed lines*:* 5th and 95th percentiles) and typical meta-prediction (solid black line: 50th percentile). (B) Model-predicted PD relationships of individual dogs (thin grey lines) with 90%PI (dashed lines) and typical meta-prediction (solid black line). (C) Typical PD predictions from each study (coloured lines) and 95%CI of the typical prediction from the meta-analysis (shaded curve). Dotted lines*:* human therapeutic interval.

Despite the fact that typical ΔQTc predictions can be considered consistent (non-biased with variability of 30%) between studies according to their 95%CI, it should be noted that BSV in PKPD relationships was significant (Figures 2 and 3B). Similar variability was observed in QTc predictions from clinical literature, especially at upper therapeutic concentrations [e.g. moxifloxacin: 7–42 ms; dofetilide: 22–76 ms; sotalol: 48–120 (and up to 230 ms; Chain *et al.*, 2013)].

Table 5

Predictions from individual study PKPD analyses, PKPD meta-analyses and literature review

	Moxifloxacin	Dofetilide	Sotalol
Clinical therapeutic dose (mg and mg·kg^{-1}) (normal renal function)	400 mg/24 h oral/i.v. 6 mg·kg/24 h	0.5 mg/12 h 7 µg·kg/12 h	80–120 mg/12 h 1–2 mg·kg/12 h
Corresponding dose in dogs (mg·kg^{-1})	12 mg·kg/24 h	13 µg·kg/12 h	2–3 mg·kg/12 h
Fraction unbound (f_u) in plasma [–]	0.55 (human) 0.71 (dog)	0.36 (human) 0.46 (dog)	1 (human) 1 (dog)
Clinical unbound therapeutic concentrations (lower to upper level, LL to UL)	2.9–5.6 µM free	0.4–2 nM free	3.7–11 µM free
Predicted ΔQTc at therapeutic exposure (ms)			
$\Delta QTc_{THER(LL)}$ to $\Delta QTc_{THER(UL)}$			
Meta-ΔQTc_{THER} prediction conscious dog [95%CI]	4.4–11.9 [–2.6 to 11.3] to [4.3–19.6]	2–13.4 [–1.9 to 6.2] to [8.3–18.9]	13.8–28.5 [6.9–20.8] to [20.9–36.4]
Expected total variability:			
90%PI $\Delta QTc_{THER,LL}$	–7 to 25	–7 to 19	3–27
90%PI $\Delta QTc_{THER,UL}$	–4 to 42	3–35	13–45
Study-ΔQTc_{THER} predictions[a] *conscious dog, range*	3.1–11.2 to 9.0–16.1	0.1–6.5 to 3.9–22.8	12.4–16.6 to 25.2–32.8
(Number of studies analysed)	(*n* = 6)	(*n* = 6)	(*n* = 2)
Inter-study variability, LL	±3.8 ms (86%)	±2.4 ms (117%)	±2.2 ms (16%)
|range|	1.1–6.8 ms (25–155%)	0.5–4.7 ms/26–200%	1.5–2.9 ms (10–21%)
Inter-study variability, UL	±2.7 ms (22%)	±5.9 ms (44%)	±3.9 ms (14%)
|range|	0.1–4.3 ms (1–36%)	2.6–9.4 ms (19–69%)	3.4–4.4 ms (12–15%)
Clinical reported ΔQTc (literature)[b,c] range (number of studies) reviewed	3.5–10.2 to 6.7–19.6 (*n* = 20, Florian *et al.*, 2011) 3.5–22 to 6.7–42 (*n* = 28)	2–46 (*n* = 5, Jonker *et al.*, 2005) 2–10 to 22–75 (*n* = 9)	16–40 to 48–120 (*n* = 8) 78–231 (*n* = 1, Chain *et al.*, 2013)
Preclinical reported ΔQTc (literature)[b] conscious dog, range	2.3–19 to 4.4–27	2.8–5 to 14–25	3–7 to 9–22
(Number of studies) reviewed	(*n* = 5)	(*n* = 3)	(*n* = 2)
Predicted safety measures [µM or nM free]			
Typical Cu_{5ms}			
Meta-analysis [95%CI]	3.2 [0–5.8] µM free	0.8 [0.2–1.5] nM free	1.2 [0–3.2] µM free
Range, *individual study* analyses[a]	0.7–3.9 µM free	0.3–2.3	1.0–1.3
Typical Cu_{10ms}			
Meta-analysis [95%CI]	5 [2.5–7.6] µM free	1.5 [0.5–1.6] nM free	2.5 [0.8–5.0] µM free
Range, *individual study* analyses[a]	2.3–6.1	0.8–3.9	2.0–2.8
Typical Cu_{20ms}			
Meta-analysis [95%CI]	8.5 [5.9–12.2] µM free	3.1 [2.2–4.4] nM free	6.1 [3.4–10.7] µM free
Range, *individual study* analyses[a]	7.9–11.0	1.9–7.6	4.7–7.1
Expected total variability (PKPD meta-analysis)			
90%PI, Cu_{5ms}	0–14 µM free	0–2.3 nM free	0–5 µM free
90%PI, Cu_{10ms}	0–21 µM free	0–3.7 nM free	0–8 µM free
90%PI, Cu_{20ms}	2–72 µM free	0.7–9.3 nM free	2.5–20 µM free

[a]Details of individual study predictions: see Supporting Information Appendix S3.
[b]Details of literature review: see Supporting Information Appendix S2.
[c]References including a meta-analysis of several clinical studies are cited extra (Florian *et al.*, 2011 and Jonker *et al.*, 2005), as well as one study predicting ~2× higher QT values than the other references (Chain *et al.*, 2013).

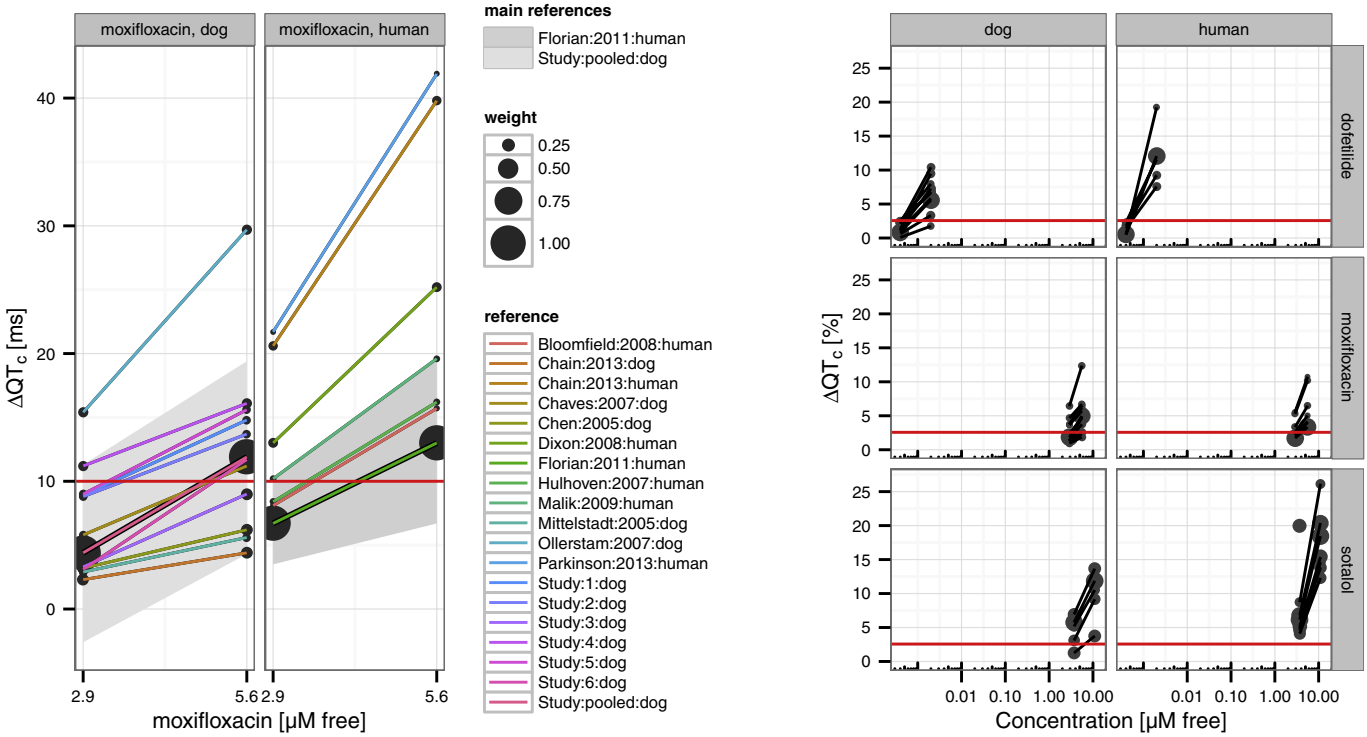

Figure 4

Summary and comparison of PD relationships in dogs and humans within unbound human therapeutic concentration range (predicted $\Delta QTc_{THER(LL)}$ and $\Delta QTc_{THER(UL)}$, from both the presented PKPD analyses and literature review). Left: QTc prolongation in [ms] illustrated for moxifloxacin in detail (for dofetilide and sotalol: see Supporting Information Appendix S2). Red horizontal line: 10 ms prolongation. Right: Corresponding QTc prolongation from baseline in [%] summarized for all three drugs. Red horizontal line: 2.5% prolongation (corresponding to ≈10 ms in human and 6 ms in the conscious dog). Dots: the point size of predictions is illustrated relative to the number of individuals included in the studies ('weight'). Lines: the lines connect the predictions from different studies. Shaded areas: 95%CI (from pooled dog studies, i.e. preclinical meta-analysis) and range of study predictions from clinical meta-analysis (Florian *et al.*, 2011) respectively.

Translational relationships

Here, we would like to relate our results from the meta-analysis with clinical PKPD meta-analyses [moxifloxacin: including 20 studies (Florian *et al.*, 2011), dofetilide: including 5 studies (Jonker *et al.*, 2005)]. For sotalol, we report the range of QTc prolongation in adults (Kimura *et al.*, 1996; Padrini *et al.*, 1997; Barbey *et al.*, 1999; Somberg *et al.*, 2010; Darpo *et al.*, 2014), while excluding one study (Chain *et al.*, 2013). This reference predicted a typical prolongation of 80–230 ms at therapeutic exposure, while such ΔQTc was not visible in published figures and would be associated with unacceptable safety concerns (ICH E14 Guideline, 2005; Trinkley *et al.*, 2013). Considering different baseline QTc values in dogs (≈240 ms) and humans (≈390 ms; Jonker *et al.*, 2005), the following QT increase within therapeutic unbound exposure can be cited (Figure 5).

Moxifloxacin induced a typical 4–12 ms prolongation in the dog (2–5%, 2.9–5.6 μM), with a corresponding typical ≈7–19 ms (2–5%) prolongation in human. For dofetilide, a 4–18 ms prolongation in the dog (2–8%, 0.4–2 μM) translated to a 2–46 ms (1–12%) prolongation in human. For sotalol, a 14–29 ms prolongation in the dog (6–12%, 3.7–11 μM) corresponded to a ≈23–100 ms (6–26%) prolongation in human adults. On a relative scale (% ΔQTc from baseline), $K_v11.1$ channel (hERG)-induced QTc prolongation seems thus consistent between dogs and humans, similar to recent reports (Holzgrefe *et al.*, 2014; Sparve *et al.*, 2014). This confirms with increased evidence that the conscious dog is an appropriate model to detect clinically relevant QTc prolongation and highlights the importance of adequate sensitivity – of both the study design (Leishman *et al.*, 2012) and the statistical analysis (Aylott *et al.*, 2010; Gotta *et al.*, 2015) – for detecting clinically relevant QTc prolongation of 10 ms (≈2.6% from baseline in human) in the preclinical setting. Usually supra-therapeutic doses are studied preclinically making the detection of small effects at therapeutic levels possible (Gotta *et al.*, 2015).

PKPD model variability

Different PKPD models were developed for the same compound from different studies, while typical ΔQTc_{THER} can be considered consistent as discussed above. This is reassuring and suggests that QTc prolongation predicted from systematically evaluated though heterogeneous final structural PKPD models is robust to changes in study designs, and that reproducible estimates suitable for translation of effects to human can be derived.

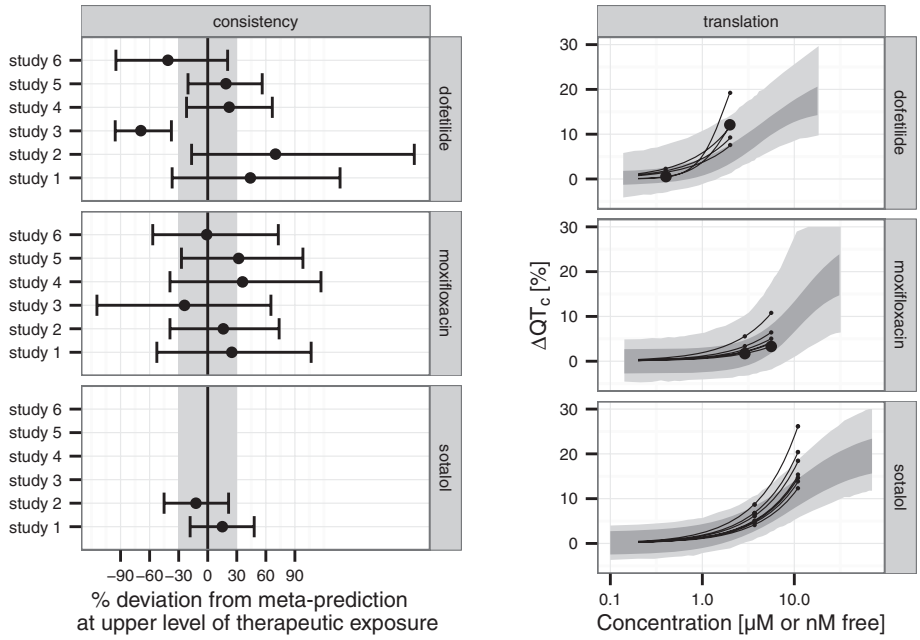

Figure 5

Summary of main findings. Consistency: % deviation of individual study predictions from meta-predictions at upper level of therapeutic exposure ($\Delta QTc_{THER(UL)}$; $\Delta QTc_{THER(LL)}$ is illustrated in Supporting Information Appendix S3). Error bars: 95%CI. Vertical shaded area: ±30% = overall mean ISV estimate. Translation: PD relationships in the conscious dog [shaded area: 95%CI of meta-predictions (dark grey) and 90%PI (light grey)] are overlapping with clinical QTc prolongation (thin lines) at the lower and upper levels of therapeutic exposure (dots; clinical meta-predictions are indicated with larger dots) when expressed as %QTc prolongation from baseline (%ΔQTc).

The different model choices for the same drug are probably explained by the varying designs under which studies were performed and reported. Differences included: (i) linear and non-linear PD models, depending on the range of drug exposure studied; (ii) direct and delayed PKPD models, depending mainly on the time to distribution equilibrium of a compound, while not accounted circadian variation and over-predicted absorption rates may also be a reason for observed hysteresis (e.g. our explanation for moxifloxacin since both hysteresis and proteresis were observed); (iii) one- and two-compartment models to characterize PK, since PK samples were not always taken during the initial distribution/elimination phase after peak concentrations; and (iv) PKPD models based on typical concentration predictions from a satellite group or individual concentration predictions.

Based on the investigation of ISV in the final PKPD models, it seems that sigmoidicity or 'curve shape' parameters (here in the range of 0.5–2.7) should be interpreted with caution. This parameter allows a more flexible model that may better describe a particular data set. However, it might also lead to an over-parameterization and loss of predictive performance of the model on a new dataset: estimated variability between individuals in this parameter appeared to a great part attributed to variability between studies. In general, however, variability between studies and individuals seemed to be of similar magnitude as expected from the small number of subjects included per study.

Limitations and suggestions for study designs

While our PKPD analysis allowed a quantitative comparison of ΔQTc predictions between studies, it did not allow us to assess or classify study designs qualitatively. The studies varied in so many ways that it is difficult to speculate about the reasons that may cause divergent predictions. In particular, the dofetilide study 3 predicted lower ΔQTc_{THER} than the other studies, while a clear increase in QTc prolongation was observed above therapeutic exposure. A reason for that under-prediction may be that only one supra-therapeutic dose was studied over 24 h, thus with incomplete drug elimination at study end. This might have impeded the ability to characterize the concentration–effect relationship at lower therapeutic exposure.

Best characterization of PK (and partly PKPD) in terms of lowest residual error (intra-individual variability) was achieved in i.v. studies. Oral PK may indeed be more difficult to characterize because of between- and within-subject variability in drug absorption. Thus, also the temporal relationship between PK and PD, and in turn PD, may be described with more confidence from a well-designed i.v. study (e.g. slow infusion with different infusion rates; Gabrielsson *et al.*, 2010) than from an oral PK study. If taking a temporal delay between PK and PD profiles into account has an influence on ΔQTc predictions should be evaluated in each study: the presence of delayed PD effects had in some but not all studies an effect on PD parameter estimates. Additionally, their relevance for QTc predictions was more study- than drug-dependent in this analysis. It should also be noted here that most of the data were automatically extracted and used as such for modelling and were thus not validated previously. It could therefore be expected that precision of predictions, and thus sensitivity and consistency, can be improved by using validated data.

Furthermore, to compare relationships in dogs and humans, we used a fixed fraction unbound in both dogs and humans. Variability between studies or individuals might thus partly also be explained by differences in f_u, especially for drugs with high protein binding (low f_u).

Finally, it should be mentioned that some differences (although statistically not significant when considering 95%CI) could be observed in the predictions from the present and previous PKPD analyses of the same study data (Ollerstam *et al.*, 2007; Chain *et al.*, 2013). Modelling procedure (data preparation, analysis plan, model building and evaluations) and final model choice may indeed depend on the modellers' evaluation criteria and/or experience (Bonate, 2011). This highlights the necessity to define best practice analysis standards (e.g. Snelder *et al.*, 2009), similar to those proposed for dose-effect analysis (Aylott *et al.*, 2010), especially if such models are used for regulatory decisions. An integration of PKPD models to assess drug effects on the QTc interval and other CV biomarkers (Snelder *et al.*, 2013) simultaneously would also be desirable.

Our work demonstrates the potential utility and practical application of modelling and simulation approaches in cardiovascular safety testing (Collins *et al.*, 2015). However, it has been recognized that communication of such approaches to a non-technical audience is a challenge, which to date has limited implementation and acceptance in drug discovery and development (Leinfuss, 2015). We hope that the current paper with contributors from various organizations and disciplines also helps in the advancement of the field in that respect.

Conclusion

This first preclinical meta-analysis showed that consistent (non-biased, reasonably precise) exposure-ΔQTc predictions can be obtained from single dog QT studies of variable designs by systematic PKPD analysis, that is suitable for translational purposes. Evidence for overlapping QTc prolongation in dogs and humans was generated for all three K_v11.1 channel (hERG) blockers, especially when %QTc prolongation from baseline was considered. This highlights the usefulness of preclinical population PKPD analysis to increase the predictive value of results from studies conducted in conscious dogs, and to possibly help assess the need to conduct a TQT study.

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Author contributions

V. G., M. D., D. J. G. and P. H. G. designed the research study. F. C., K. A., S. A. G. V., P. M. and F. S. contributed essentially to data acquisition. V. G. analysed the data and wrote the manuscript. All authors were involved in project and results discussion and interpretation, as well as in the manuscript review and approval.

Conflict of interest

The authors state no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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Appendix S1 Individual study illustration and PKPD models.
Appendix S2 Literature review.
Appendix S3 Details of individual study predictions (typical ΔQTc_{THER} and Cu_{5-20ms}).