ARTICLE

Evaluation of moxifloxacin in canine and non-human primate telemetry assays: Comparison of QTc interval prolongation by timepoint and concentration-QTc analysis

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Abstract

The in vivo correct QT (QTc) assay is used by the pharmaceutical industry to characterize the potential for delayed ventricular repolarization and is a core safety assay mentioned in International Conference on Harmonization (ICH) S7B guideline. The typical telemetry study involves a dose-response analysis of QTc intervals over time using a crossover (CO) design. This method has proven utility but does not include direct integration of pharmacokinetic (PK) data. An alternative approach has been validated and is used routinely in the clinical setting that pairs pharmacodynamic (PD) responses with PK exposure (e.g., concentration-QTc (C-QTc) analysis. The goal of our paper was to compare the QTc sensitivity of two experimental approaches in the conscious dog and non-human primate (NHP) QTc assays. For timepoint analysis, a conventional design using eight animals (8×4 CO) to detect moxifloxacin-induced QTc prolongation was compared to a PK/PD design in a subset ($N = 4$) of the same animals. The findings demonstrate that both approaches are equally sensitive in detecting threshold QTc prolongation on the order of 10 ms. Both QTc models demonstrated linearity in the QTc prolongation response to moxifloxacin dose escalation (6 to 46 ms). Further, comparison with human QTc findings with moxifloxacin showed agreement and consistent translation across the three species: C-QTc slope values were 0.7- (dog) and 1.2- (NHP) fold of the composite human value. In conclusion, our data show that dog and NHP QTc telemetry with an integrated PK arm (C-QTc) has the potential to supplement clinical evaluation and improve integrated QTc risk assessment.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Typical cardiovascular studies usually employ timepoint analysis. Published in vivo corrected QT (QTc) assay data has exhibited variability in QTc sensitivity that results in challenges in nonclinical-clinical assessment of translation.

WHAT QUESTION DID THIS STUDY ADDRESS?
Comparison of nonclinical timepoint and concentration QTc (C-QTc) analyses and how it relates to clinical moxifloxacin data.
WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
Dog and non-human primate (NHP) QTc timepoint and C-QTc analyses detect QTc internal prolongation, have equivalent sensitivity, and improve confidence in these models for proarrhythmic risk mitigation.
HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
Risk assessment in nonclinical models translates well to human thorough QT (TQT) data for moxifloxacin. The new data highlights the value of a high-quality dog or NHP QTc assay to support clinical risk assessment and regulatory decision making.

INTRODUCTION

The nonclinical in vivo correct QT (QTc) assay is used by the pharmaceutical industry to characterize the potential of a new drug to cause delayed ventricular repolarization (e.g., QTc interval prolongation). The need for this cardiovascular safety core assay is described in the International Conference on Harmonization (ICH) S7B guideline.[1] The in vivo QTc assay has demonstrated high translational value for predicting QTc interval prolongation in humans.[2–7] In conjunction with the in vitro human ether-a-go-go K+ current assay, a negative signal in the in vivo QTc assay lowers the probability of clinical ventricular arrhythmia risk for a new drug product.[8] Various animal species have been used to conduct the QTc assay, notably the dog, non-human primate (NHP), and minipig, based on their electrophysiology and pharmacology. All have established sensitivity to detect drug-induced QTc prolongation caused by direct blockade of cardiac delayed rectifier K+ current (I_{Kr}).[5,9–11]

For small molecules, the typical cardiovascular (CV) telemetry experiment utilizes a crossover (CO) design to determine QTc interval changes at specified timepoints following treatment.[12,13] In a 4×4 CO design, the effect of a test article (at 3 doses) is compared to vehicle and the treatments are administered in randomized fashion with each animal ($N = 4$) receiving each treatment. A statistical analysis is then used to determine the magnitude of QTc effect, and its statistical significance. Other experimental designs have been used, such as an escalating dose paradigm in the same animals[14,15] or separate groups of animals dosed in parallel.[16] The design choice is influenced primarily by the pharmacokinetic (PK) profile of the agent, or the modality type (e.g., new chemical entity vs. a biotherapeutic). Each of these study designs have demonstrated the capability to detect drug-induced QTc interval prolongation following treatment with known I_{Kr} blockers (e.g., moxifloxacin), but the sensitivity to detect a small effect size (e.g., 10 ms), is variable. Some of the factors that affect QTc study sensitivity include group size, data binning

approaches, heart rate (HR) range in each species, the formula used for QT correction, and statistical approach.[13,17–19] Using a larger group size ($N = 6–8$) is a proven way to improve statistical power for the detection of small QTc effects (5–7 ms) in conscious dogs and NHPs.[3,18,20,21]

An alternative approach to evaluate the drug-induced QTc prolongation is concentration-QTc (C-QTc) analysis. This method is used routinely in the clinical setting to differentiate new drugs with low and high QTc prolongation risk and is a valid substitute for a statistical timepoint analysis as used in the thorough QT (TQT) study.[22] The C-QTc analysis is valuable in early clinical development because all the human exposure data can be integrated across multiple phase I dose cohorts to develop the C-QTc relationship, which has greater QTc detection sensitivity compared to time-response analysis in individual dose cohorts with low sample size (6–10 subjects) and therefore insufficient power.[22–24] The utility of C-QTc analysis has been explored in a few conscious dog[25–28] and NHP[2,7,26] QTc assays, and the results indicate that nonclinical C-QTc analysis has translational value to human findings. The C-QTc method requires that a separate PK study be conducted in the same animals after the QTc assessment is completed to enable blood sampling at multiple timepoints after dosing to facilitate analysis, but this is not routine practice across the industry. A separate PK phase is needed to avoid the confounds of physiological stress and sympathetic activation on arterial pressure, HR, cardiac contractility, and QTc intervals associated with manual restraint of animals for blood sampling.[29] Ideally, a limited number of PK samples, timed to avoid potential effects, are also collected during the telemetry phase to verify that exposures are consistent during the two phases.

A collaboration of multiple pharmaceutical companies[7] evaluated six clinical reference compounds in the NHP QTc assay and demonstrated that C-QTc could accurately identify the five positive drugs and single negative in a manner consistent with the human C-QTc data.[22] The current study was undertaken to compare QTc prolongation sensitivity of

two QTc experimental assay approaches in the conscious dog and NHP. In the first approach, a conventional CO design with high statistical power ($N = 8$/dose group) was used to detect QTc prolongation following moxifloxacin treatment (i.e., a time-response analysis). For the second approach, a PK-pharmacodynamic (PK/PD) analysis approach was used, which combined QTc telemetry evaluation and drug exposure from a separate PK phase in the same animals. The findings in both nonclinical species were compared to clinical QTc studies with moxifloxacin to assess cross-species translation quantitatively.

METHODS

Animals

Animals were cared for in accordance to the Guide for the Care and Use of Laboratory Animals, eighth edition (2011). Animals were housed individually at an indoor, Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), international-accredited facility in species-specific housing. Telemetry studies were conducted at Covance, Inc. (Madison, WI). All procedures in this protocol complied with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. All studies were reviewed and approved by study site institutional animal care and use committees.

These studies were completed in non-naïve beagle dogs (Covance Research Products Inc., Kalamazoo, MI) and NHPs (cynomolgus monkeys; Covance Research Products Inc., Alice, TX), as these are commonly used species for nonclinical cardiovascular assessments.[30] Male beagle dogs ($N = 8$, 0.9–1.8 years old; 9.8–12.3 kg) and NHPs ($N = 8$, 4.3–5.1 years old; 4.0–6.5 kg) were housed individually in stainless steel cages. Animals were offered certified canine diet (#5007C; PMI Nutrition International) or primate diet (#5048; PMI Nutrition International) and water ad libitum. Water samples were routinely analyzed for specified microorganisms and environmental contaminants. Environmental controls for the animal room were set to maintain 18–26°C, a relative humidity of 30–70%, a minimum of 10 room air changes/hour (h), and a 12 h light/12 h dark cycle. Animals were returned to the colony following the completion of the study.

Telemetry preparation

Animals were surgically implanted with telemetry devices at least 2 weeks prior to study initiation. A PCT or PCTP transmitter (Data Sciences International, St. Paul, MN) was implanted into the abdomen and sutured to the abdominal wall for collection of electrocardiograms (ECGs), blood pressure, and temperature. The ECG leads of the implanted transmitter were arranged in an approximate Lead II configuration. The pressure catheter was advanced toward the abdominal aorta via the femoral artery. Detailed surgical procedures have been published previously.[31,32]

All animals were acclimated to the study room and dosing procedure for 2 weeks prior to dosing. Moxifloxacin hydrochloride (Bayer Healthcare Pharmaceuticals Inc.) was suspended in 0.5% methylcellulose in reverse osmosis water for oral dosing.

Telemetry study design

A double Latin square dosing design ($N = 8$/dose group; 8×4 CO) was used to evaluate moxifloxacin (Table S1). This study design has been used routinely (since 2009) for QTc interval assessment because of its consistent high sensitivity to detect small degrees of QTc prolongation.[21] Moxifloxacin was dosed at 10, 30, and 100 mg/kg in dogs and 30, 80, and 175 mg/kg in NHPs. Animals were not fasted before dosing. Telemetry data were collected for at least 90 min prior to dosing and continuously for at least 48 h postdose. Telemetry timepoints were analyzed based on a nominal dose time (e.g., 10 a.m. = 0 h). These studies were conducted (2011) to establish QTc sensitivity of this design as part of our regular practice to validate the pharmacological sensitivity of dog and NHP cardiovascular models with human therapeutics.[32–35]

Plasma collection and exposure analysis

Blood samples (1 ml) were collected into K_2EDTA tubes, following administration of moxifloxacin (nonfasted), from dogs via the jugular vein and NHPs via the cephalic vein. Blood was collected from a subset of telemetry animals during the PK phase of the study ($N = 4$/dose level, Table S1). Samples were collected predose and at ~ 2, 4, 8, 24, and 48 h postdose. All samples were frozen and stored at −80°C prior to analysis using liquid chromatography tandem mass spectrometry.

Data acquisition and analysis

ECG signals were collected at a sampling rate of 500 Hz. All signals were captured and analyzed with a Data Sciences International (DSI) Ponemah system (St. Paul, MN).

Cardiovascular parameters (HR; QT, and QTcI intervals) were analyzed using SAS System software. The QT interval was corrected for heart rate changes for each animal using a

linear model, dog QTcI = QT − Individual Animal Correction Factor (IACF) × (HR − 75) or NHP QTcI = QT + IACF × (750 − interval between successive R waves (RR)) where the variables and linear regression slope were established on an species/individual animal-specific basis using QT and HR or RR (used to derive HR) data points from 1-min means collected during the vehicle collection.[2,18,36] Vehicle-corrected QTc (ΔQTc) were calculated by subtracting vehicle QTc values from each animal at each timepoint and dose level. ΔQTc values were further corrected by subtracting baseline values from each animal to obtain ΔΔQTc values used for C-QTc analysis.

QTc by timepoint analysis

Prolongation of QTc intervals in the moxifloxacin treatment groups were compared to time-matched vehicle in the same conscious animals using a repeated-measures analysis of covariance model. QTcI data was averaged into 1 h data bins starting at 0 h. The statistical model comprised multiple factors including dosing day (period), animal, treatment group (treatment), time after dose (time), and the following interaction terms: treatment*time, period*time, and animal*time.[13] Baseline was included in the model as the covariate. The variance-covariance structure in the analysis was selected by evaluating the Akaike Information Criterion. The 24 h data was separated into four separate time blocks for statistical analysis: 0 to 7 h (block 1); end of block 1 to 19 h (block 2); end of block 2–25 h (block 3), and 46 to 49 h (block 4). When the treatment times time interaction was significant, covariate-adjusted means were compared at each time point. When the treatment times time interaction was not significant, but the treatment effect was significant, a post hoc analysis was conducted, and the grand covariate-adjusted means within each block were compared to the vehicle group. Post hoc group comparisons against the vehicle group were evaluated by Dunnett-Hsu adjusted *t*-test at the 5.0%, two-tailed probability level.

Concentration-QTc analysis

This pharmacometric analysis of moxifloxacin was conducted in conscious beagles and NHPs (N = 4/dose level; Table S1) according to prior methods.[7] Regression analysis used either total or unbound plasma concentrations matched with the corresponding vehicle- and baseline-adjusted QTcI intervals (ΔΔQTcI), with a total of 72 matched pairs for each species. QTcI data was reported as the median value of 1-min data bins for the 30 min period prior to the plasma collection timepoint (as detailed in Komatsu et al.[7]). An alternative approach using 1-h data bins is reported in the

Supplementary Data. No significant differences were noted between the two approaches. The concentration necessary to produce a 10 ms increase in ΔΔQTcI was determined based on the derived regression line and 90% confidence intervals (CIs) around the regression parameters. The 10 ms effect size is an arbitrary nonclinical threshold, as there is no stated sensitivity target described in the ICH S7B guideline, but 10 ms has been used as a threshold effect level in nonclinical[3,7,26] and clinical[22] C-QTc studies. Linear regression analysis values (slope; intercept), projected values (predicted change in QTc at maximum plasma concentration [C_{max}]; plasma concentration needed to produce a 10 ms increase in QTc) and CIs (90%) were also calculated. Predictions based on published C-QTc data were performed according to calculation methods used by Gotta et al.[17] Unbound concentrations were calculated based on a fraction unbound values of 0.71 in dogs, 0.82 in NHPs, and 0.55 in humans.[7,17,37]

Hysteresis

Time delays that are not accounted for can increase the probability of a false negative in the C-QTc analysis and in the absence of active metabolites, a PK/PD model is advised.[38] Although there are recent publications proposing a quantitative threshold for meaningful hysteresis,[39] the most common approach is a visual inspection of the C-QTc plot, connected by temporal order (for individual animal or mean per dose cohort). A counterclockwise loop is indicative of a time lag/hysteresis between drug concentration and QTc prolongation effect.

To address the issue of hysteresis, we developed a PK/PD model consisting of a two-compartment oral PK model with linear absorption and an effect compartment. The ΔΔQTcI values are assumed to be driven by the concentration in the effect compartment in a linear function. Individual animal PKs was estimated using NONMEM version 7.4.3 and ΔΔQTcI was modeled as:

$$Q = \text{SLOPE} \bullet E + \text{INT},$$

Where E is the concentration in the effect compartment and

$$\frac{\mathrm{d}E}{\mathrm{d}t} = K_{EF} \bullet C - K_{EF} \bullet E,$$

Where C is the concentration in the central compartment and K_{EF} is the rate of transfer between the central compartment to the hypothetical effect compartment. We then calculated the slope between the predicted effect compartment concentration and ΔΔQTcI values.

RESULTS

Time-response relationships

Baseline (predose) values for QT/QTcI and HR parameters collected from conscious ($N = 8$) beagle dogs and NHPs ($N = 8$) are shown in Table S2. Figure 1 illustrates the effect of moxifloxacin on QTcI intervals and $\Delta\Delta$QTcI in conscious dogs (Figure 1a and b, respectively). The QTcI interval increased in a dose-dependent manner (Figure 1a; Table 1) and was elevated over 24 h. Vehicle- and baseline-adjusted QTcI ($\Delta\Delta$QTcI) values (Figure 1b) demonstrated dose-dependent prolongation at all dose levels tested. Plasma levels of moxifloxacin increased dose-dependently in dogs (Figure 1c; Table 1). Figure 2 illustrates QTcI intervals and $\Delta\Delta$QTcI following moxifloxacin treatment in conscious NHPs. QTcI intervals increased in a dose-dependent manner (Figure 2a; Table 1) and were sustained. The $\Delta\Delta$QTcI values (Figure 2b) were prolonged at all doses (Table 1). Moxifloxacin

demonstrated dose-related plasma exposure with a C_{max} at 4 h (Figure 2c, Table 1).

Concentration-QTc relationship

Linear regression and slope analysis of the total C-QTc slope and the predicted change in $\Delta\Delta$QTcI for C_{max} values are shown for each in vivo QTc assay (Figure 1d, Figure 2d; Table 1). Positive slopes were noted for conscious dogs ($p < 0.0001$) and NHPs ($p < 0.0001$). The predicted exposure that resulted in a 10 ms prolongation of $\Delta\Delta$QTcI was 4627 ng/ml (90% CI: 3774–5586 ng/ml) in dogs and 3357 ng/ml (90% CI: 2764–3951 ng/ml) in NHPs (Table 1). The predicted values and overlapping confidence intervals indicated that both species had similar QTc sensitivity following moxifloxacin. The C-QTc relationships based on unbound or free moxifloxacin exposures are presented in Figure 3 and Table 1. Individual C-QTc plots, connected by temporal order (Figures S4 and S5), showed counter-clockwise loops in some of the animals
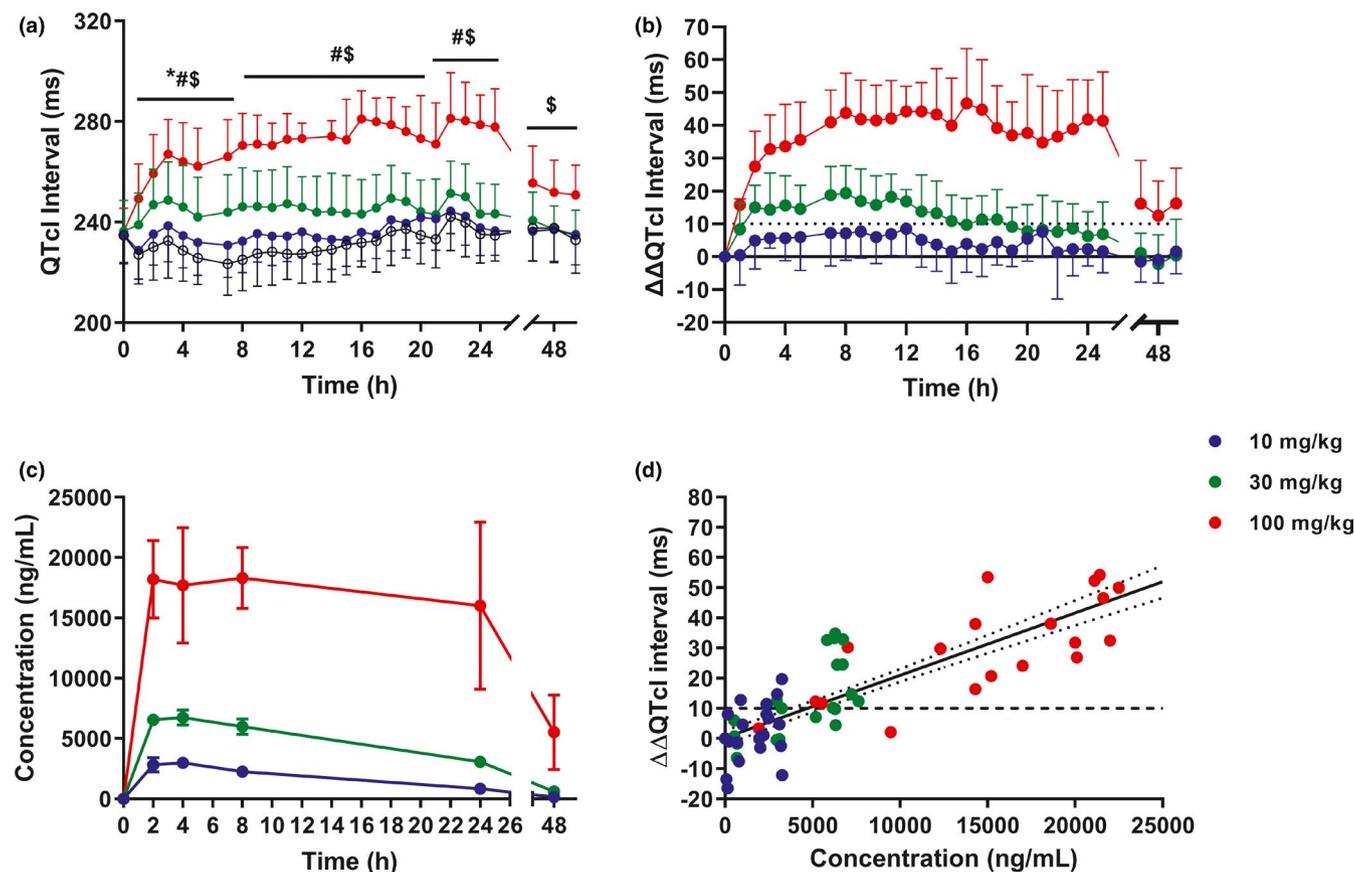

FIGURE 1 Time-response and concentration-QTc (C-QTc) relationship evaluation of moxifloxacin-induced QTc prolongation in conscious beagle dogs. Vehicle (○) and moxifloxacin (10, 30, and 100 mg/kg) were administered at 0 h. The plots represent timepoint analysis of absolute QTcI (a) and baseline- and vehicle-corrected QTcI effects ($\Delta\Delta$QTcI) (b) following treatment. The moxifloxacin pharmacokinetic curve (c) and C-QTc relationship for moxifloxacin (d) are also shown. Group sizes were eight (a/b) or four (c/d) and values are mean ± SD. *Indicates significance ($p < 0.05$) for control versus low dose. The # indicates significance ($p < 0.05$) for control versus mid dose. The $ indicates significance ($p < 0.05$) for control versus high dose (repeated measures analysis of covariance followed by Dunnett's pairwise comparisons). For panel d, data were fitted by linear regression (solid line) and dotted lines represent 90% confidence interval of the model-predicted mean $\Delta\Delta$QTcI

TABLE 1 Evaluation of QTc prolongation by timepoint and C-QTc analysis in conscious beagle dogs and NHPs following moxifloxacin

Dose (mg/kg)	Timepoint analysis (N = 8)			C-QTc analysis (N = 4)			Predicted change in ΔΔQTcI (ms) at C_{max}	Predicted concentration (ng/ml) for 10 ms increase
	ΔQTcI (ms) from vehicle	C_{max} total exposure (ng/ml)	T_{max} (h)	Slope (ms/ng/ml)	Intercept (ms)	Slope of baseline-normalized (% of baseline)		
Dog								
10	5.9 ± 3.6*	2980 ± 405	4	0.0021 (0.0018–0.0023) [1.184 free]	0.2832	0.0009	6.5	4627 (3774–5586) [8.3 µM free]
30	17.4 ± 7.9*	6730 ± 640	4				14.4	
100	45.5 ± 10.6*	18300 ± 2520	8				38.1	
NHP								
30	11.3 ± 5.3*	3110 ± 1150	4	0.0038 (0.0032–0.0044) [1.794 free]	−2.755	0.0012	9.1	3357 (2764–3951) [6.9 µM free]
80	27.8 ± 10.5*	7710 ± 864	4				26.5	
175	40.5 ± 13.9*	11500 ± 1520	4				40.9	

Timepoint analysis values are mean ± SD.

Abbreviations: Cmax, maximum plasma concentration; C-QTc, concentration QTc; NHPs, non-human primates; QTc, correct QT; Tmax, time to maximum concentration.

*indicates significance ($p < 0.05$) when compared to control.

at higher dose levels, suggesting the potential for hysteresis. To address this, the data was corrected and the hysteresis-adjusted predicted exposure that resulted in a 10 ms prolongation of ΔΔQTcI was 4331 ng/ml (90% CI: 3527–5278 ng/ml) in dogs and 3181 ng/ml (90% CI: 2635–3702 ng/ml) in NHPs (Table 2). In a couple of NHP cases, a clockwise loop was observed instead, and these data were not able to be estimated using the PK/PD model. In these cases, the observed plasma concentrations were retained for analysis. Extrapolated multiples of the ΔΔQTcI values and their corresponding predicted threshold concentrations are shown in Table S3.

Comparison of QTc sensitivity: Timepoint versus C-QTc analysis

Correlation plots of actual QTc prolongation for timepoint analysis and C-QTc modeling are shown in Figure 4. Published human QTc data with 400 mg moxifloxacin shows that the magnitude of QTc prolongation determined by timepoint and C-QTc analysis is highly correlated and equivalent statistically (Figure 4a; adapted from Florian et al.[40]). The timepoint and C-QTc effect sizes were linear, highly correlated, and overlaid over the human QTc effect range in the dog and NHP (Figure 4b) QTc assays.

DISCUSSION

The main goal of this study was to define the quantitative relationship between moxifloxacin exposure and QTc prolongation in two higher species assays that are used to assess the ventricular repolarization risk of new chemical entities. This study demonstrated that both timepoint and C-QTc approaches can detect moxifloxacin-induced QTc prolongation in a sensitive and equivalent manner. In conscious dogs and NHPs, sensitivity to detect small QTc effect sizes (5.9–11.3 ms) was observed by timepoint analysis using an 8×4 CO experimental design. As an alternative analysis based on the "totality of data" approach used for clinical QTc evaluation,[23,41] C-QTc regression confirmed that QTc sensitivity (10 ms) in dogs and NHPs was achievable. Hysteresis analysis showed limited impact, resulting in minor adjustments to both slope and 10 ms threshold exposure predictions (<10% for variables and species). This is consistent with nonclinical[7] and clinical[22,40,42] data, where moxifloxacin was shown to have no/minimal hysteresis effects, although a recent clinical study suggested that diurnal factors may result in more pronounced hysteresis.[43] Therefore, both methods are effective ways to optimize the sensitivity of the telemetry assay for detecting QTc effects (e.g., increase the group size [N = 8] or including a separate PK phase to facilitate the C-QTc analysis in a smaller group of animals [N = 4]). This
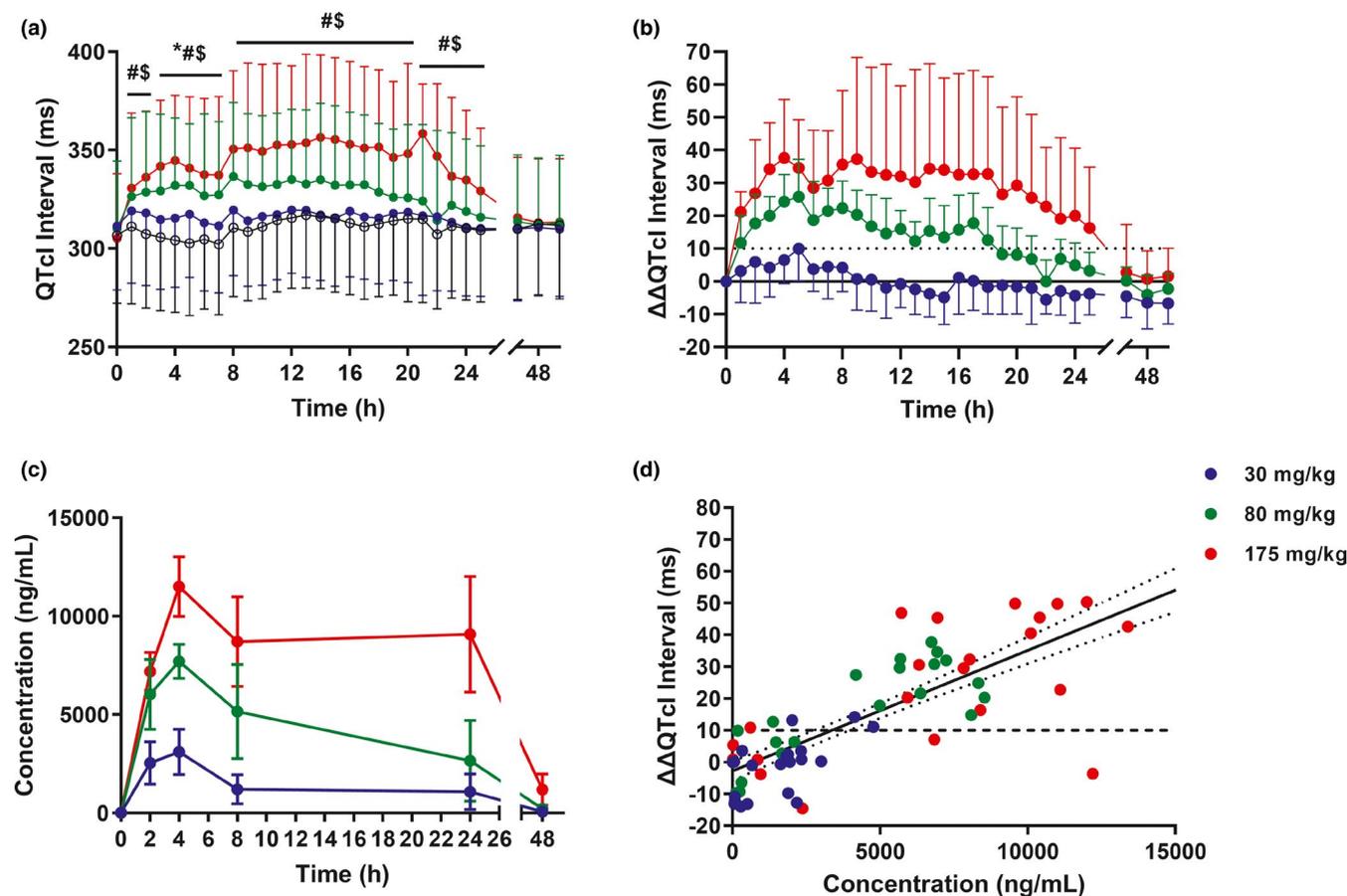
FIGURE 2 Time-response and concentration-QTc (C-QTc) relationship evaluation of moxifloxacin-induced QTc prolongation in conscious non-human primates (NHPs). Vehicle (○) and moxifloxacin (30, 80, and 175 mg/kg) were administered at 0 h. The plots represent timepoint analysis of absolute QTcI (a) and baseline- and vehicle-corrected QTcI effects (ΔΔQTcI) (b) following treatment. The moxifloxacin pharmacokinetic curve (c) and C-QTc relationship for moxifloxacin (d) are also shown. Group sizes were eight (a/b) or four (c/d) and values are mean ± SD. *Indicates significance ($p < 0.05$) for control versus low dose. The # indicates significance ($p < 0.05$) for control versus mid dose. The $ indicates significance ($p < 0.05$) for control versus high dose (repeated measures analysis of covariance followed by Dunnett's pairwise comparisons). For panel d, data were fitted by linear regression (solid line) and dotted lines represent 90% confidence interval of the model-predicted mean ΔΔQTcI.

latter observation suggests that the C-QTc approach may have a positive benefit on animal use (3Rs). Overall, the dog and NHP QTc assay findings with moxifloxacin highlight the assay's value for identifying drug-induced repolarization risk, supporting human safety assessment with good clinical translation.

Translation and clinical implications

The requirement for undertaking a nonclinical-clinical QTc translation is the need to utilize high quality animal and human data sets generated under common or standard conditions to minimize QTc variability (per species) to optimize the comparison of QTc signals and evaluate effects at similar drug exposure across species. For the clinical component, an average of moxifloxacin effect sizes from 20 TQT studies was used to represent the typical human QTc response as a

reference point.[40] Likewise, we tested moxifloxacin in the dog and NHP to generate QTc data using identical experimental designs, group sizes, QTc data analysis approaches, and PK evaluation, and used one laboratory site and study team (Covance-Madison) to minimize potential sources of QTc variability and overall study error. Another consideration in the design of cardiovascular safety pharmacology telemetry studies is that new agents are tested at dose levels that exceed the anticipated primary PD or therapeutic exposure range in humans to establish a clinical safety margin.

In this validation exercise, the moxifloxacin exposures attained in the in vivo QTc assay satisfied these conditions, and achieved high exposure multiples in the dog (6×) and the NHP (3.5×) over the human C_{max} level (3000 ng/ml; average) following a 400 mg oral dose.[40,44] The QTc by time-point analyses in the nonrodent assays were highly powered to detect a small degree of QTc prolongation (5.9 ms – dog; 11.3 ms – NHP) at the therapeutic exposure. The sensitivity
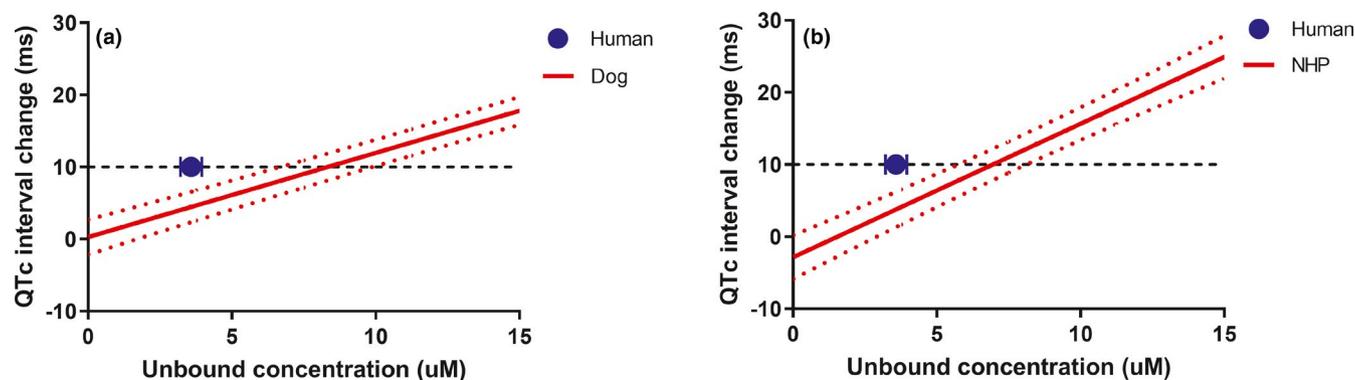
FIGURE 3 Comparison of unbound concentration-QTc (C-QTc) relationship following moxifloxacin administration in: dog (a) and non-human primate (NHP) (b) models. Human reference values were adapted from a meta-analysis of 20 thorough QT (TQT) studies.[40] Unbound concentrations were calculated using species specific plasma protein binding, as specified in the Methods section. Dog and NHP data were fitted by linear regression (solid line) and dotted lines represent 90% confidence interval CI) of the model-predicted mean $\Delta\Delta$QTcI. Human data is mean value, with error bars representing 90% CI

TABLE 2 Evaluation of hysteresis-adjusted C-QTc analysis in conscious beagle dogs and NHPs following moxifloxacin

Dose	Slope (ms/ng/ml)	Intercept (ms)	Predicted change in $\Delta\Delta$QTcI (ms) at C_{max}	Predicted concentration (ng/ml) for 10 ms increase
Dog				
10	0.0022 (0.0019–0.0024)	0.4713	6.1	4331 (3527–5278)
30			14.3	[7.8 µM free]
100			39.8	
NHP				
30	0.0040 (0.0034–0.0046)	−2.7244	9.7	3181 (2635–3702)
80			28.1	[6.5 µM free]
175			43.3	

Abbreviations: Cmax, maximum plasma concentration; C-QTc, concentration QTc; NHPs, non-human primates; QTc, correct QT.

of the dog and monkey assays was similar to the QTc prolongation signal observed in healthy humans exposed to moxifloxacin (i.e., 6.4–14.9 ms).[40] Clinical observations of excessive QTc prolongation (>60 ms) and Torsade de Pointes have been observed with moxifloxacin, but only in the presence of predisposing risk factors, including cardiac disorder/disease, age, or comedication.[45] The largest QTc changes observed in normal healthy dogs and NHPs treated with higher doses of moxifloxacin ranged between 40 and 50 ms in this study. A prior conscious dog telemetry study of moxifloxacin infused intravenously demonstrated profound QTc prolongation of 75 ms at a plasma exposure of 39,200 ng/ml, which is 13 times higher than the human C_{max} level.[46]

In regard to nonclinical-clinical QTc translation, a comparison of the dog and NHP C-QTc findings (e.g., sensitivity) to the meta-analysis of 20 TQT studies showed a negligible difference (0.7-fold and 1.2-fold, respectively) with human moxifloxacin C-QTc data.[40] Slope analysis of the NHP data was consistent with prior evidence, and identical to recently published data in the same species.[7] Threshold concentrations predicting a 10 ms QTc prolongation (Tables 1–3) are

also very close (1.1- to 2.5-fold) to prior dog and NHP estimates.[7,26] Clinical slope comparisons are also similar, as our data are within 0.4- to 1.2-fold of human estimates.[22,25,40] Consortium data (Japanese Safety Pharmacology Society and the Japanese Society for Biopharmaceutical Statistics) suggested that an interspecies C-QTc slope factor of 10 ($N = 5$ QT-positive; $N = 1$ QT-negative) was an acceptable threshold for translational agreement.[7] Evaluation of the free fraction allowed comparison of direct exposure responses among dogs, NHPs, and humans, demonstrating very similar predicted 10 ms threshold concentrations (Figure 3 and Tables 1–3). Florian et al.[40] performed a correlation analysis between $\Delta\Delta$QTc derived from human timepoint and C-QTc analyses and showed statistical equivalence using these approaches, and similar analyses with our dog and NHP data demonstrated overlap with the $\Delta\Delta$QTc ranges observed in human studies (Figure 4). The correlations between nonrodent and human QTc data emphasize the similar sensitivity of timepoint (8×4 CO) and C-QTc ($N = 4$) evaluations. Our findings are also consistent with nonclinical C-QTc simulations which indicated that C-QTc methodology had greater
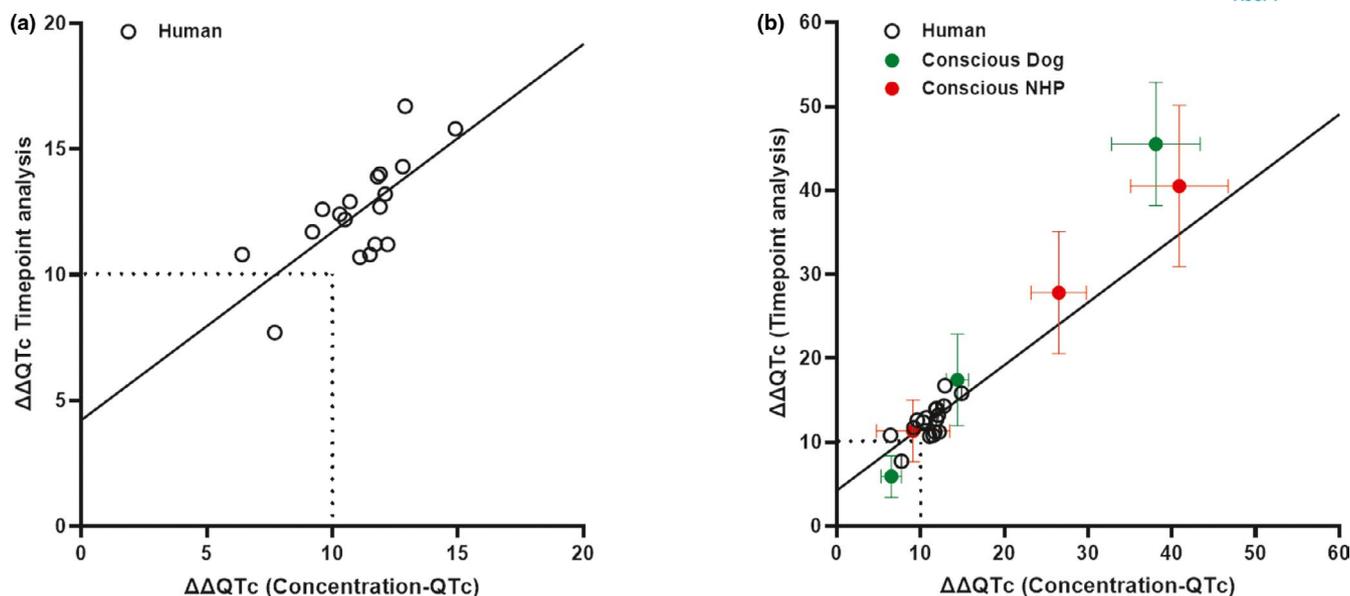
FIGURE 4 Correlation plots of ΔΔQTc values for timepoint analysis and concentration-QTc (C-QTc) modeling from human, dog, and non-human primate (NHP) QTc assays. Human TQT data (adapted ref. 40; $N = 18$ studies; $R = 0.7093$, $R^2 = 0.5031$) are presented in panel a. Dog and NHP data are overlaid with human data (from panel a) in panel b. Each nonclinical data point represents mean data ($n = 8$ for timepoint analysis and $n = 4$ for concentration-QTc) at maximum plasma concentration for each dose level, with error bars representing SD

TABLE 3 Comparison of moxifloxacin C-QTc relationships in dogs, NHPs, and humans

In vivo QTc assay	C-QTc slope (ms/ng/ml)	Intercept	C_{max} (ng/ml)	Predicted change in ΔΔQTcI (ms)	Predicted concentration (ng/ml) needed to produce a 10 ms increase in ΔΔQTcI
NHP[b]	0.0015	2.08	8418	14.7	4181 (8.5 μM free)
NHP[a,c]	0.0040	ND	12,591	50.4[a]	2500[a] (5.1 μM free)
Dog[a,c]	0.0014	ND	45,285	63.4[a]	7143[a] (12.6 μM free)
Human[d]	0.0031	1.9	ND	ND	2613 (3.6 μM free)

Abbreviations: Cmax, maximum plasma concentration; C-QTc, concentration QTc; ND, no data available; NHPs, non-human primates; QTc, correct QT.

[a]Calculations performed based on methods used by Gotta et al.[47]

[b]Komatsu et al.[7]

[c]Dubois et al.[26]

[d]Florian et al.[40]

power to detect a 10 ms change when compared to timepoint statistical analysis.[47]

The challenge of translation: Establishing consistent in vivo QTc assay sensitivity

A comparison of our moxifloxacin responses in the dog QTc timepoint assay to prior studies highlight that QTc sensitivity is dependent on the nonclinical study design. For example, a dog QTc telemetry study that was very similar to our design ($N = 8$; 10, 30, and 90 mg/kg moxifloxacin PO) achieved similar PK exposure to our study but had much lower QTc sensitivity; potentially related to the inclusion of both male and female dogs in the study.[48] In contrast, it is possible to get exquisite QTc detection sensitivity in the dog (3–6 ms) with

smaller group sizes ($N = 4$) or by incorporating super-interval analysis,[15,19] but other dog studies (also $N = 4$) have reported lower sensitivity for QTc detection (17–20 ms),[4,12,49] which likely reflects inter-laboratory differences in animals, study environment, or data analysis methods. Published findings on NHP moxifloxacin-induced QTc effects are less prevalent relative to the dog, however, our NHP data compares favorably with available reports. Timepoint analysis showed close agreement with NHP data ($N = 4$) derived from a dose-escalation study, which had a minimum detectable change of 10 ms (compared to 11.3 ms in this study).[14] Other studies have shown similar findings.[50,51] C-QTc slope comparisons in the same species ranged from 1.0- to 2.5-fold of published evidence (Table 3).[7,26]

The challenges associated with QTc translation across species were previously highlighted by Ewart et al.[4] in a

cross-company collaboration that sought to compare dog QTc responses with human QTc effects (phase I trials) for 113 novel drugs. Their analysis clearly demonstrated that the dog telemetry QTc assay had excellent negative predictive value (high specificity) for phase I findings. However, the translation was hampered by a few limitations, which might have masked the true performance characteristics of the dog QTc assay. For example, the dog timepoint analysis data was sourced from seven pharma companies with different nonclinical study protocols, statistical power, analysis approaches, and QTc sensitivities, which were uncontrolled factors that introduced variability into the nonclinical dataset. In addition, the PK data used to estimate exposure-windows (and safety margins) was sourced from different animals (e.g., toxicokinetic data), which was an additional variable. Last, positive control data was not available or assessed to compare QTc sensitivity across the seven companies. These various nonclinical factors and sources of error were purposefully minimized in the current study to optimize the cross-species comparison of moxifloxacin-induced QTc prolongation.

In summary, the experimental design modifications used in the dog and NHP QTc assays demonstrated that timepoint analysis and C-QTc evaluations improve our confidence in the use of these nonclinical models for proarrhythmic risk assessment of new drug candidates. These improvements in QTc interval evaluation best practice represent an opportunity to use optimized in vivo C-QTc assays to support a fully integrated nonclinical-clinical QTc risk assessment and the value of nonclinical data for clinical safety regulatory decisions.[8,52]

Limitations

These studies were designed and executed in 2011, and we recognize some limitations of the study in retrospect. For example, each animal was dosed twice with moxifloxacin, but drug exposure was confirmed only in the PK phase (i.e., a blood sample was not taken to determine moxifloxacin exposure in the telemetry phase). This is a minor point for this study with a known QTc prolonging agent, but confirmation of exposure during the telemetry phase is very important when testing new molecules, especially when no QTc effect is observed. Another gap was that PK profiles for all three moxifloxacin doses was assessed in a subset of animals, and not the entire group (Table S1). Inaccessibility of some study animals during the PK phase was a factor, but it would have been ideal to have C-QTc data for the entire cohort to create a larger dataset. A key learning was that the inclusion of a separate PK arm on the back-end of a telemetry study is needed for optimal C-QTc data-pairing in the same animals and is feasible for agents with reasonably short half-lives (e.g., small molecules). The development of new methods that enable noninvasive collection of blood samples during

the CV telemetry phase could mitigate the need to conduct two separate dosing sessions, and the potential to have inconsistent drug exposure in each session.[53] For agents with long half-lives, PK sampling can be integrated into the telemetry phase, but taken on a nonrecording day to minimize disruptions in QTc data acquisition which would be preferred for PK/PD modeling.

CONFLICT OF INTEREST
All authors are employees and shareholders of Amgen Inc.

AUTHOR CONTRIBUTIONS
R.W.C., J.B., F.A.C., and Z.W.J. wrote manuscript, analyzed data, and designed research. M.J.E. and H.M.V. wrote manuscript and designed the research.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Chui RW, Baublits J, Chandra FA, Jones ZW, Engwall MJ, Vargas HM. Evaluation of moxifloxacin in canine and non-human primate telemetry assays: Comparison of QTc interval prolongation by timepoint and concentration-QTc analysis. *Clin Transl Sci*. 2021;14:2379–2390. https://doi.org/10.1111/cts.13103