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BRIEF REPORT

A "one-step" approach to heart rate correction and statistical analysis applied to conscious dog QTc studies

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Abstract

A "one-step" method which combined the heart rate correction and statistical analysis for conscious nonhuman primate (NHP) QTc assessment was recently published. The principles of this method are applicable to other species. In the current analysis, we demonstrate the utility of the technique in conscious dog QTc studies. Two studies in male dogs ($n = 8$ and $n = 7$) implanted with telemetry devices were used. In both studies, treatments were randomized and all animals received all treatments. In the primary study, the effect on QTc of moxifloxacin was compared with vehicle. Each treatment (vehicle and moxifloxacin) was given on two separate occasions. In the second study, dogs were given vehicle or dofetilide. Conventional QTc analysis was compared with the "one-step" method. The effect on QTc relative to vehicle was determined along with the median minimal detectable difference. As expected, both moxifloxacin and dofetilide gave QTc increases with a maximum of ~20 ms. There was a significant increase in the sensitivity to detect a QTc effect when using the "one-step" method. The minimal detectable difference was 1.6 ms for the "one-step" method compared with 6.2 ms for the conventional method. These analyses are consistent with the increased sensitivity described for the "one-step" method applied to studies in NHP. The increased sensitivity should enhance the ability to support an integrated assessment of the QTc prolongation liability for new drugs.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Conventional analysis uses two steps, rate correction and then statistical analysis, the sensitivity of this method is known.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study examined whether a "one-step" method which improved sensitivity in NHP could provide a similar advantage in dog studies.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The study demonstrates that the "one-step" method works across species. The "one-step" method increases the statistical sensitivity by 3- to 4-fold when

compared with conventional methods. The sensitivity, combined with the species pharmacological sensitivity, makes the conscious dog model a valuable tool in predicting the potential to prolong the QTc interval in man.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The sensitivity of animal studies can now match or exceed clinical studies. Furthermore, the method is likely to provide the same sensitivity boost in clinical studies if equivalent data density is achieved.

INTRODUCTION

The use of nonclinical data to discharge clinical QT risk has focused attention on the statistical and discriminatory performance of the nonclinical in vivo QTc assessment.[1–4] Critical to the use of the nonclinical in vivo data is the sensitivity of the assessment. Recently, an analysis was published which demonstrated the effect of a drug on the QT interval in nonhuman primates (NHP) could be evaluated in a single step.[5] This contrasted with more common methods which involve an arithmetic normalization of the QT interval prior to statistical evaluation. The "one-step" method had the advantages of making fewer assumptions in addressing the relationship between the QT interval and heart rate, and by using all the data in a single step, being 3–4-fold more sensitive than the more common methods. An earlier evaluation of the "one-step" method in dog[6] had shown similar advantages on a smaller scale owing to the sparser data set being collected at the time.

The present analyses return to evaluation of the "one-step" method in dog. In a modern rich telemetry data set, the advantages of the "one-step" method observed in NHP should be observable in dog. Two positive reference agents and a vehicle–vehicle comparison serve as positive and negative test scenarios in the current analyses.

METHODS

Two studies were available for re-analysis. These studies included positive reference agents, moxifloxacin (30 mg/kg p.o.), and dofetilide (30 μg/kg). These, or similar doses have been associated with QTc prolongation in dog[7,8] and achieve plasma concentrations in excess of the critical clinical concentration (CC) which produces 10 ms QTc prolongation in man.[9] One of the studies also included a vehicle–vehicle comparison which can serve as an example of a negative control or negative test agent.

Latin square cross-over designs were used in both studies. Each study had four treatments and included eight animals, these were therefore double 4×4 Latin squares. In the second study, one animal's data were lost due to signal failure restricting analysis to seven animals. The treatments in the primary study were vehicle, moxifloxacin, vehicle, moxifloxacin. Each treatment was therefore repeated twice. This study design was similar to that conducted in NHP to demonstrate the utility of the "one-step" method.[5,10] In the case of the dog study, the smaller number of animals ($n=8$) allowed full randomization of treatments. This had not been possible in the larger NHP study ($n=48$) which used a fixed sequence of treatments and four separate cohorts. Plasma samples were taken during electrocardiogram data acquisition to verify exposure to drug. Full pharmacokinetic profiles were determined on two separate occasions using the same animals. In the second study, the treatments included vehicle and dofetilide, as well as two further test articles which were considered out of scope for the current analyses. The treatments were randomized. Again, plasma samples were taken during pharmacodynamic data collection to verify exposure and full pharmacokinetic profiles were determined for these animals on a separate occasion.

Both studies were conducted in animals with surgically implanted telemetry devices (L21; Harvard Biosciences). The implantation, recovery, and study acclimation procedures, for this well-established model, have been described elsewhere.[11]

Statistical analysis

Conventional analyses

The conventional analyses involved correcting the QT interval for the influence of heart rate (HR) at the time of primary data aggregation using a linear QT-HR regression. Briefly, an individual correction factor for each animal (IACF) was derived from the individual's 1 min mean QT and RR data for the first (moxifloxacin study) or only (dofetilide study) vehicle treatment period (0–24 h). The corrected QT interval (QTc) was derived as follows:

$$QTc = QT + iacf \times (75 - HR),$$

where 75 bpm is the reference HR value for correction.

The subsequent statistical analyses involved an ANOVA model:

$$QTc \sim Treatment + ID,$$

where each animal serves as their own control.

The model was used to derive the effect of treatment relative to the vehicle. The analyses were conducted at hourly intervals.

"One-Step" model

The "one-step" method does not require a previous determination of the individual animal's QT-HR slope or arithmetically determining a corrected QT interval. The method addresses the relationship with HR within the statistical assessment of the treatment effect on the QT interval.

$$QT \sim (Treatment \times HR) + (ID \times HR)\ (@\ HR\ Reference),$$

where × signifies the interaction of Treatment or ID with the QT-HR slope.

Again, the model was used to derive the effect, at the reference HR value of 75 bpm of treatment relative to vehicle. The analysis was conducted at hourly intervals.

All analyses were conducted in R (v4.3.2, 2024), the estimated marginal means were determined using the package Emmeans.[12]

Ethics statement

All procedures in the studies complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (Code of Federal Regulations, Title 9), the Public Health Service Policy on Humane Care and Use of Laboratory Animals from the Office of Laboratory Animal Welfare, and the Guide for the Care and Use of Laboratory Animals from the National Research Council. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

RESULTS

Exposure to moxifloxacin and dofetilide exceeded the critical concentrations associated with 10 ms QTc prolongation in man both during pharmacodynamic data collection and in separate pharmacokinetic profiling. Moxifloxacin (C_{max} = 4275 ng/mL) achieved exposures exceeding the CC (1867 ng/mL) adjusted to 1445 ng/mL to account for species difference in plasma protein binding (PPB)[9,13,14] for at least 12 h post-dose. Dofetilide (C_{max} = 4.8 ng/mL) achieved concentrations exceeding the CC in man (0.37 ng/mL) adjusted to 0.29 ng/mL for species PPB[9,15] for 24 h. Full pharmacokinetic profiles and the exposures determined during pharmacodynamic data collection are shown in Figure 1.

The effects of moxifloxacin and dofetilide on the QTc interval relative to the vehicle are shown in Figure 2. In addition, the effects of vehicle in a vehicle–vehicle comparison are also illustrated. The mean effect and time–effect profile for moxifloxacin and dofetilide are similar for both the conventional and "one-step" methods. The initial rate of rise and initial peak effect appear slightly larger with the "one-step" method, and this is particularly evident for dofetilide treatment. The mean effect of moxifloxacin exceeds 10 ms throughout 24 h; that for dofetilide exceeds 10 ms for the first 6 h.

The common statistical hypothesis used in safety pharmacology is that there is no effect of treatment (H0: $\mu = 0$ ms). Consistent with this hypothesis, the confidence intervals for the conventional analysis vehicle–vehicle comparison include 0 ms throughout 24 h. In the case of conventional analysis, the lower 95% confidence interval excludes 0 ms for 1–24 h for moxifloxacin and 1–6 h for dofetilide. There is a statistically significant QTc effect when the lower confidence interval excludes 0 ms. Using the "one-step" method the lower 90% confidence interval again excludes 0 ms for 1–24 h for moxifloxacin. In the case of dofetilide, there is a statistically significant effect using this null hypothesis for 1–15 h post-dose. It is evident that using the "one-step" method with the narrower confidence intervals even in vehicle–vehicle comparisons, the confidence intervals can exclude 0 ms. This suggests statistically significant QTc prolongation and shortening, albeit of small magnitude, for this negative control. Using the "one-step" method with its increased sensitivity, it can be beneficial to use a statistical hypothesis more common in clinical thorough QT (TQT) studies. The null hypothesis in this case is that H0: $-X\ ms < \mu < X$ ms. In this case, it is acknowledged that there can be small QTc fluctuations $<\pm X$ ms. A threshold of 5 ms is illustrated in Figure 2 with a reference line. Where the upper 95% confidence interval exceeds 5 ms, there would be a statistically significant effect relative to a TQT-like null hypothesis. In the case of conventional analysis, there would be a statistically significant QTc change for 1–24 h for moxifloxacin, 1–15 h for dofetilide (plus 17 h), and most of the 24 h for the vehicle. The "one-step" method has a significant QTc prolongation for 1–24 h for moxifloxacin, 1–8 h for dofetilide, and only at 1 h for the vehicle. Raising the threshold to 10 ms would have no impact on the results

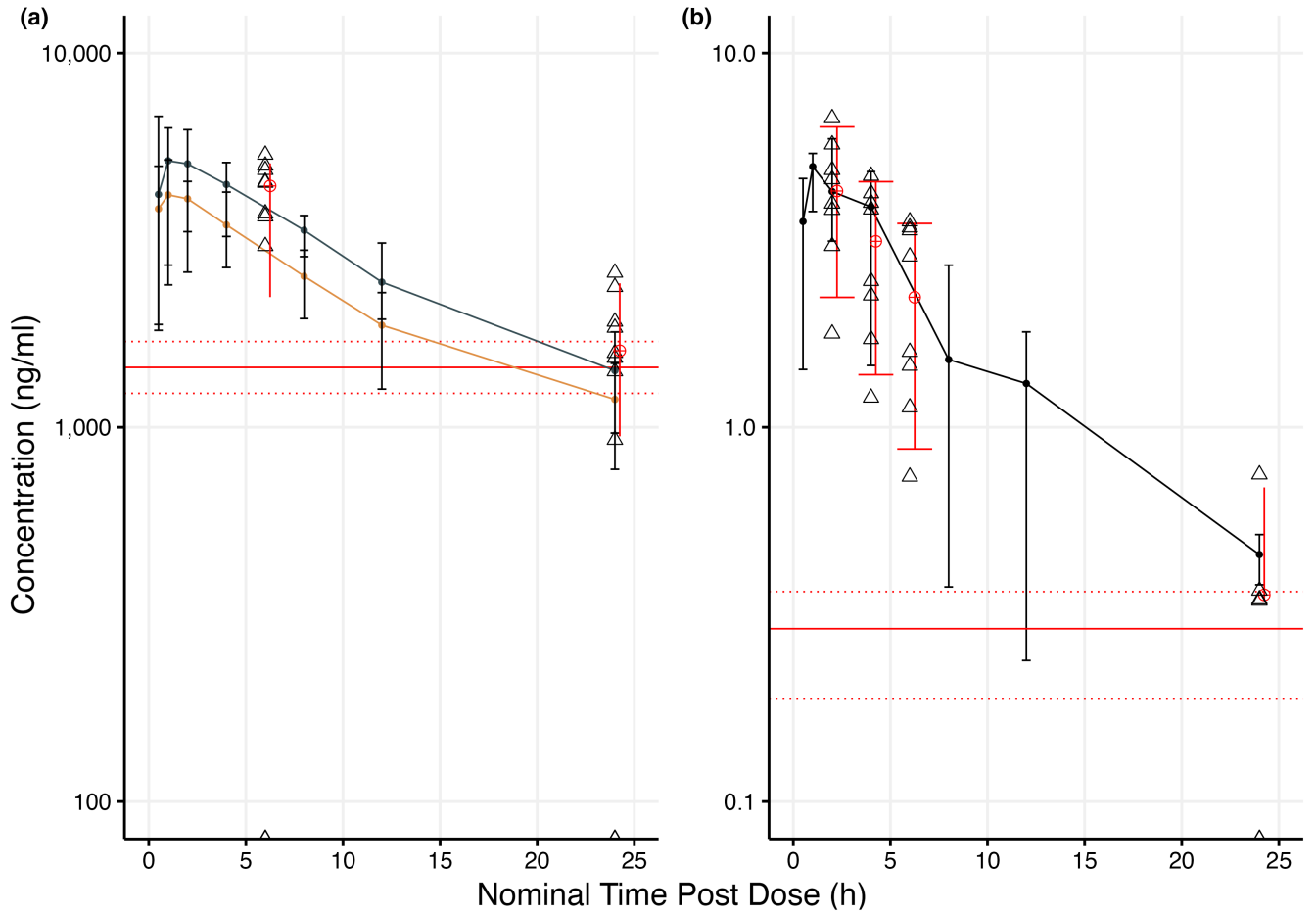

FIGURE 1 The plasma concentrations of moxifloxacin (a) and dofetilide (b) are shown as the median (90% confidence intervals). There were two full pharmacokinetic profiles collected for moxifloxacin; the second collection is shown in the lighter colored line and symbols. Individual sparse samples collected during pharmacodynamic collections are shown by the open triangles. Data were collected at 6 and 24 h during moxifloxacin pharmacodynamic data collection (open triangles). Samples were taken at 2, 4, 6, and 24 h during dofetilide pharmacodynamic data collection (open triangles). Some emesis was noted in moxifloxacin-treated animals and low values were observed at 6 and 24 h for one animal on one occasion. The median exposure (90% confidence intervals) is also illustrated (open circles plus error bars). No pharmacodynamic data were excluded from the QTc analysis. The solid horizontal lines represent the mean critical concentrations in man. The dotted lines represent the 90% confidence intervals. The critical concentrations (dofetilide 0.37 ng/mL (0.24, 0.55); moxifloxacin 1866 ng/mL (1591, 2188)) were adjusted for the small plasma protein binding differences between species (moxifloxacin: Dog—29%, man—45% [Siefert et al.[13]; Gotta et al.[14]]; dofetilide: Dog—54%, man—64% [Smith et al.[15]]).

for moxifloxacin, would restrict statistical significance with dofetilide to 1–4 h and there would be no significant effect of vehicle.

The Fisher's least significant differences for each hourly comparison are equivalent to the halfwidth of the confidence intervals. The median minimal detectable difference (MDD; 80% power at $p<0.05$, $n=8$) for all the hourly comparisons was 6.2 ms for conventional analysis and 1.6 ms for the "one-step" method.

DISCUSSION

The MDD of 1.6 ms for the "one-step" method in dogs is comparable to that described for NHP (2 ms).[5] The 6.2 ms MDD for $n=8$ and hourly comparisons is consistent with the published sensitivities for this number of dogs and using conventional methods in the same laboratory.[11] The approximately 3- to 4-fold increase in sensitivity noted in NHP was evident in analysis of the dog QTc data using the "one-step" method. In addition to the increase in sensitivity, it was also evident that a more TQT-like statistical null hypothesis would be reasonable when using the "one-step" method and this would improve the sensitivity and specificity relative to conventional analysis (conventional analysis and a safety pharmacology-like null hypothesis has high specificity but has more modest sensitivity). A threshold of 5–6 ms would be consistent with the 10 ms threshold used in man when accounting for the difference in baseline QTc values for dogs and

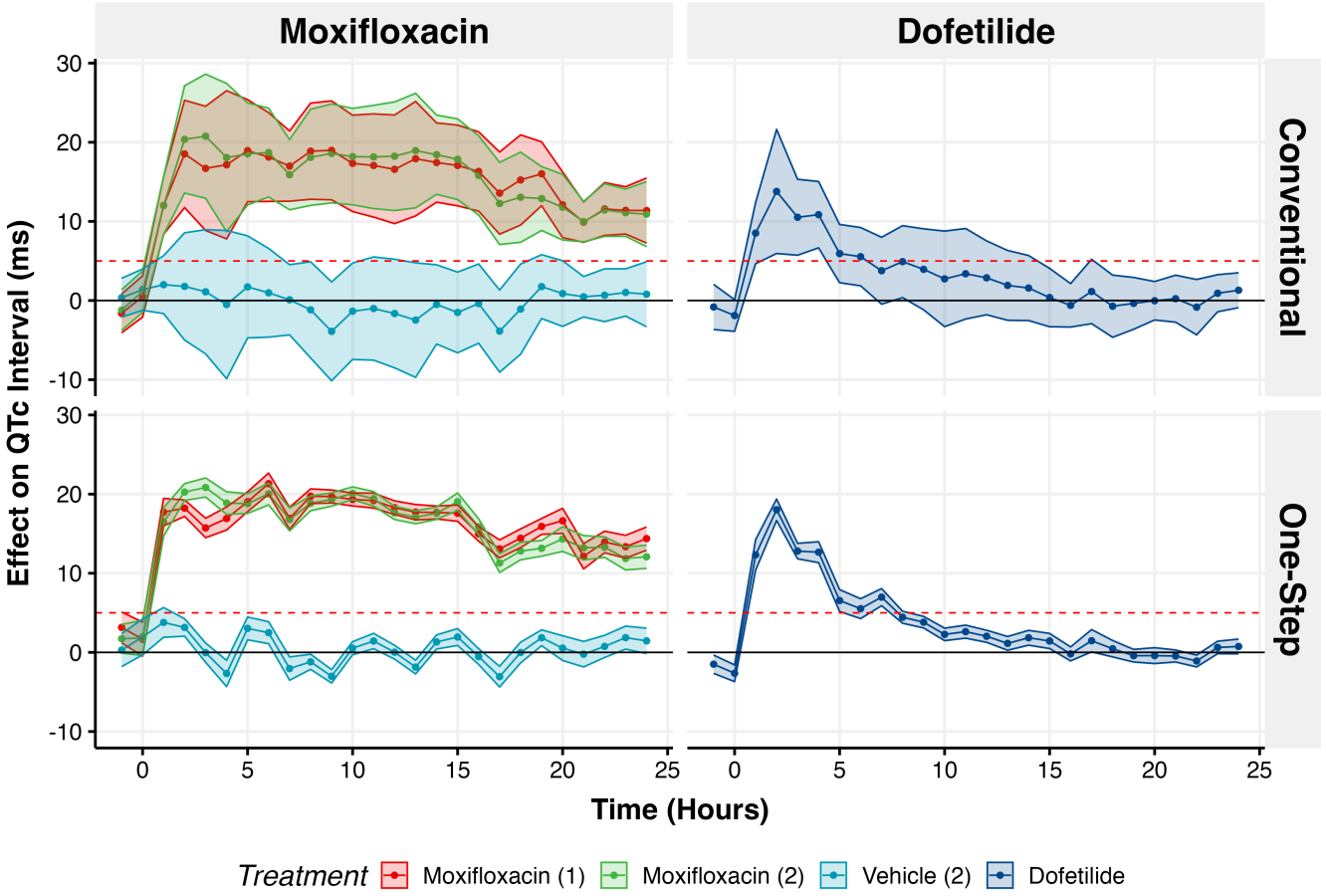

FIGURE 2 The effect of treatment on the QTc interval relative to the vehicle. The lines and symbols represent the mean QTc effect. The ribbons indicate the 90% confidence intervals. The panels on the left represent data from the moxifloxacin study, and the panels on the right represent data from the dofetilide study. The upper panels show the QTc effect determined using conventional methods. The lower panel illustrates the effect determined using the "one-step" method. The 3–4-fold increase with the "one-step" method is evident in the reduction in the width between the 90% confidence intervals.

man. The number of animals used in the current studies is higher than that used by many laboratories ($n=4$ is the most common design). The MDD for $n=4$ based on the current analyses was 11.4 and 3 ms for the conventional and "one-step" method, respectively. This demonstrates that the "one-step" method could be particularly useful in the more common smaller studies.

When considering the concentration–time profile and the time above the CC there were differences between dofetilide and moxifloxacin. Moxifloxacin had a prolonged QTc effect where the effect was evident and significant for the 24 h of analysis even after concentrations had fallen below the critical levels. The reverse was true for dofetilide where the QTc effect returned to baseline despite concentrations above the critical level. The "one-step" method gave statistically significant effects at exposures (<1-fold and >2-fold the CC for moxifloxacin and dofetilide, respectively). These modest differences in pharmacological sensitivity have been confirmed in a recent concentration–QTc analysis in dog.[16] There were some other subtle differences in the QTc-time profile between analysis methods with a faster initial rise in QTc effect for both drugs and a higher mean maximal effect for dofetilide. Similar differences were observed in NHP.[5,17] Concentration–QTc (C–QTc) analysis using a one-step method has been demonstrated recently for super-interval data,[18] smaller time averages can be used in C–QTc analysis by first correcting for HR using an hourly, on treatment correction. This correction is most similar to the "one-step" method, alternatively, we have found the "one-step" method useful at 20–30 min intervals rather than hourly. Given the small MDD possible with the "one-step" method in conscious dogs the sensitivity of these studies can be similar to a TQT study in man.

Overall, these analyses have shown that the early promise of the "one-step" method in dog,[6] when used with modern dense continuous data sets, can be realized. The "one-step" method makes small studies in dogs comparable to human TQT studies in terms of statistical and pharmacological sensitivity.

AUTHOR CONTRIBUTIONS

M.M.A. and D.J.L. wrote the manuscript. All authors designed the research. D.J.L. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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