Assessing QT prolongation in conscious dogs: Validation of a beat-to-beat method☆
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A model of sling-trained, conscious mongrel dogs instrumented with telemetric arterial pressure transmitters and ECG leads was validated for assessment of the QT–RR interval relationship at clinically used free and total plasma concentrations of positive and negative standards with known outcomes. The beat-to-beat technique for assessing the dynamic boundaries of the individual cardiac cycles was compared to the same data with typically used averaging techniques and corrections applied. Positive standards E-4031, cisapride, terodiline, and terfenadine showed increased sensitivity toward detection at clinically relevant levels when an outlier analysis of beats beyond the normal autonomic boundary is applied. Since methods to correct the QT interval for heart rate are often confounded with changes in autonomic state, a validation of the changes with reflex tachycardia induced by vasodilatation after nitroprusside and reflex bradycardia induced by sudden vasoconstriction with phenylephrine where shown to be differentiated from direct effects of repolarization with E-4031. These changes were also demonstrated to be identical to effects observed in humans after standing or challenged with a similar dose of phenylephrine. The conscious dog is also a sensitive model for studying the arrhythmia liability induced by beat-to-beat changes in cardiac ECG restitution (the relationship between QT and TQ intervals) and hysteresis. However, some caveats based on observations may need to be considered due to inherent differences in QT intervals and sinus arrhythmia between canines and humans.

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1. Introduction

The conscious dog is a standard animal model used for cardiovascular assessment and electrocardiogram (ECG) measures (i.e. to assess QT interval prolongation) of new drugs in the pharmaceutical industry and is recommended by regulators in the current International Conference of Harmonization guidelines (Anon, 2000, 2005a, 2005b). The most common methods for collection of canine data are either through an anesthetized preparation or conscious state in an ambulatory environment where cardiovascular parameters such as heart rate and blood pressure are monitored via telemetric implants. These preparations yield valuable information but, like any model, have both pros and cons that must be considered when asking a specific question for translation to humans.

The anesthetized dog model provides tremendous control over measurement of cardiovascular parameters in conjunction with the ability to determine the individual pharmacokinetics (PK) relationships. This latter point is extremely important because without individual PK relationships, comparisons to reported clinical outcomes and determinations of therapeutic indices generally cannot be reliably made from experiments with relatively few animals. ECG interval changes and even action potential durations can be precisely recorded through direct measures either at sinus rate or during pacing to alleviate the need for corrections of the QT interval. The caveat with this model is that normal autonomic mediated events will be blunted by anesthetics or overridden completely if pacing is used. The additional drugs used in this preparation...
may also interact with the test substance in a manner not predictable in humans.

The conscious ambulatory dog is most commonly advocated for toxicology or safety pharmacology studies. However, when trying to define narrow therapeutic indices for issues like cardiac conduction and repolarization, the variability in this model is often prohibitive. Individual PK is difficult to obtain because these dogs do not usually have indwelling catheters and collecting blood is likely to disrupt the cardiovascular or ECG measure for a significant portion of a critically important time period. The ambulatory environment also adds to the variability through behavioral or movement events that cannot be dissociated from drug effects. Therefore, in all the studies from our laboratory that will be presented in the validation of this model, we have used the conscious, isolated, chronically instrumented, sling-trained/restrained, mongrel dog. This model allows for stable baseline values with low resting heart rates of between 50 and 60 BPM. Indwelling catheters can be placed to allow minimal disruption for blood PK determinations, intravenous administration of test compound (in lieu of oral route if necessary) and intermittent pharmacology measures can continuously be obtained in sling-trained animals through bioimpedance we have previously described (Fossa & DePasquale, 1996; Knight, Fossa, & DePasquale, 1997).

The purpose of this review is to discuss data that has previously been reported from our lab using this model of the conscious dog (Raunig, DePasquale, Huang, Winslow, & Fossa, 2001; Fossa, DePasquale, Raunig, Avery, & Leishman, 2002; Fossa et al., 2005, Fossa, Wisialowski, & Crimin, 2006) and describe the evidence or lack thereof for validation in the preclinical assessment of cardiac liabilities in particular. First, a validation dataset of both positive and negative controls with reported clinical outcomes was compared at their respective PK concentrations adjusted for plasma protein binding differences between dog and humans. Second, the autonomic similarities and differences between dog and humans will be discussed and how this impacts the interpretation of the QT interval when compared to drugs that directly affect repolarization. Last, quantifying the effect of impaired repolarization in the dog and how this may differ in the extrapolation to arrhythmia vulnerability in man.

1.1. The QT–RR interval relationship in dog vs. man

The QT–RR interval relationship is highly dynamic from beat-to-beat and is non-linear in nature. Fig. 1 displays this relationship over a 10 min period in a conscious dog. This relationship at rest is dominated by the respiratory cycle causing a sinus arrhythmia of typically up to 1200 ms compared to healthy humans which are less than half that (Hariman, Hoffman, & Naylor, 1980; Fossa et al., 2005). During each exhalation cycle, vagal tone predominates and the QT interval changes very little at RR intervals greater than approximately 500 ms. As the animal inhales, the vagal tone is diminished and the sympathetic influence is more profound causing the QT interval to become more variable at shorter RR intervals and thus the QT–RR interval relationship changes from flat to steep. This oscillatory pattern between flat and steep relationships is very unique for each animal and is similar in this regard in humans (Batchvarov et al., 2002). The highly dynamic relationship does pose a problem though when correcting for the QT interval; particularly when heart rate is changing due to autonomic reflexes.

In traditional preclinical toxicology or in clinical studies for the mandated International Conference of Harmonization E-14 guidelines (Anon, 2005a, 2005b), the normal QT–RR physiological variation is collapsed by data averaging. In canine toxicology studies, the data are traditionally averaged over 1-minute periods and thus the RR variability is dramatically reduced to produce what would seem to be a new linear QT–RR interval relationship (Fig. 1). One potential problem is the averaged relationship does not even occur where the physiological relationship predominately exists. Furthermore, when fitting a correction line to this data, whether it be a Bazett (Bazett, 1920), Fridericia (Fridericia, 1920) or individual (Malik, Farbom, Batchvarov, Hnatkova, & Camm, 2002), extrapolation beyond the points to predict the QT interval at a given RR interval can produce an erroneous result outside of the normal physiological relationship. It is for this reason that the beat-to-beat method of quantification was developed (Raunig et al., 2001; Fossa et al., 2005).

The beat-to-beat method defines the upper (and lower) boundaries around the resting unstressed QT–RR interval relationship (Fig. 2). This relationship can also be determined during periods in which normal everyday events (i.e. standing, eating, sleeping) are occurring that would not reasonably influence the risk of arrhythmia liability. Upon challenge with a drug, if autonomic changes occur, such as through vasodilation, and the resulting QT interval relationship is within this bounds, it would be hypothesized that no increased risk of arrhythmia would be apparent. However, if the upper bounds are
exceeded at any given heart rate, then this may potentially pose some additional risk.

To validate this conceptual model, positive controls cisapride, terfenadine, terodiline and E-4031 were tested and compared to changes from drugs that are considered safe (negative controls) such as verapamil (Fossa et al., 2002) and nitroprusside (Fossa et al., 2005). The beat-to-beat patterns in the QT–RR interval relationship varied widely between drugs (Fig. 3). With cisapride, the QT–RR interval relationship at approximately 15 times the clinical concentration associated with arrhythmia (2–10 nM), showed a temporally heterogeneous pattern at shorter RR intervals (faster heart rates). Terfenadine and terodiline, drugs that are both associated with bradycardia, showed QT prolongation beyond the normal boundaries at the longer RR intervals but not necessarily at the shorter RR cycles. When fitting an individual correction line to these data for both drugs, frequently no differences were observed in individual dogs (Fig. 3) and detecting group statistical effects where clinically reported arrhythmias occur was difficult (Fig. 4A). However, analyses of the outlier beats showed robust effects at clinical concentrations for all positive controls in that there was an increase of beats beyond the 95% confidence bounds (Fig. 4B). Thus the sensitivity and accuracy of the analyses can be increased compared to correction factors, particularly for drugs like terfenadine and terodiline that tend to have undercorrected QT intervals at low heart rates. Conversely, verapamil, at concentrations near the clinical level, showed no prolongation at any heart rate nor an increase in the percentage of outlier beats above the upper normal QT–RR interval boundaries.

1.2. Influence of autonomic tone

To further validate this method under conditions that affect normal autonomic tone, reflex tachycardia was induced by the vasodilator...
nitroprusside and reflex bradycardia was induced on a separate day by the vasopressor agent, phenylephrine. Each response was compared to a class III antiarrhythmic, E-4031, that does not affect autonomic tone (Fossa et al., 2005) but is associated with an increased incidence of arrhythmia (Fujiki, Tani, Mizumaki, Shimono, & Inoue, 1994). During the maximum fall in blood pressure with nitroprusside, the effect of reflex tachycardia on the QT–RR interval relationship can be visualized in the beat-to-beat dynamics movie (canine nitroprusside.WMV and Fig. 5A). As the RR interval decreases with hypotension and the sinus arrhythmia diminishes, there is a lag in the QT adaptation (hysteresis) but at no time is the QT interval increased above the normal baseline confidence bounds. Analyses of these beats in the conscious dog during the acceleration phase showed an increase of the QT interval by Fridericia (QTcF) and Bazett (QTcB) corrections between 16 and 55 ms, respectively, while the beat-to-beat method (QTbtb), using a boot-strap sampling technique comparing beats across a comparable RR interval range, showed a 43 ms decrease in the QT interval (Table 1 during Period B). Upon return of the heart rate to normal, a clear hysteresis occurs in the opposite direction with QT again lagging behind the RR interval recovery to longer intervals. This response translates almost perfectly to humans under similar physiological conditions. In an unpublished study conducted at Pfizer in healthy volunteers standing quickly from a supine position, the QTcF and QTcB showed a similar dynamic pattern from Holter acquired beats and the QT interval was prolonged 16 to 40 ms, respectively, whereas the QTbtb was not changed (Fig. 5B; also see dynamic movie file human standing.WMV).

Reflex bradycardia in dogs, induced with a group mean 28 mm Hg increase in blood pressure by phenylephrine, caused the dynamic beat-to-beat relationship to clearly increase the QT interval above the baseline relationship by about 6 ms (Fig. 6A and Table 1 also see dynamic movie file canine phenylephrine.MWV). Again, these same beats corrected showed decreases of approximately −15 ms with QTcB and −7 ms with QTcF (Fossa et al., 2005). To demonstrate the

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**Fig. 4.** Concentration response relationship in the mean averages of (panel A) QT<sub>RR1000</sub> and (panel B) outlier responses from conscious dogs (n=4) each treated with terodiline, cisapride, terfenadine, E-4031 and verapamil on separate days. The mean vehicle response range and upper 95% confidence bounds ranges are displayed. Asterisks indicate significant differences (p<0.05) from mean vehicle response range or upper 95% confidence bounds. Arrows in respective treatment colors indicate mean free therapeutic concentrations used clinically (also see Fig. 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
transformation of this effect to humans, in the same unpublished study at Pfizer, phenylephrine was administered as a bolus dose to supine normal volunteers to produce a transient 20–30 mm Hg rise in blood pressure. The change in QTcB and QTcF was −11 and −4 ms respectively while again the QTbtb showed an 8 ms increase (Fig. 6B). Visualization of the dynamic relationship from the Holter acquired data looked exactly as that observed in the conscious dog (human phenylephrine.WMV).

E-4031 in conscious dogs produces no autonomic reflexes as evidenced by no change in arterial pressure but there was a slowing of

![Figure 5](image_url)  
**Fig. 5.** The QT–RR interval relationships on a beat-to-beat basis at resting baseline (blue dots) and during reflex tachycardia (red dots) after treatment with the non-arrhythmogenic vasodilator nitroprusside in canine or standing from supine position in human subject. Ten minutes of baseline and 4 min of nitroprusside or standing data are represented from each response. Both reflex tachycardia responses are interpreted as QT prolongation when blood pressure is maximally decreased and Bazett and Fridericia correction factors are applied to datasets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 1**  
Comparison of autonomic tone and delayed repolarization induced changes in the QT interval using different correction factors vs. beat-to-beat assessment. (Fossa et al. (2005) J Pharmacol Exp Ther 312: 1–11)

<table>
<thead>
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<td>B (−16 to −10)</td>
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**Fig. 6.** The QT–RR interval relationships on a beat-to-beat basis at resting baseline (blue dots) and during reflex bradycardia (red dots) after treatment with the vasopressor phenylephrine in canine (Panel A) and healthy normal human subject (Panel B) in supine position. Ten minutes of baseline and 5 min of phenylephrine data are represented from each response. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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(⁎ Represents statistical significance (P<0.05) from upper 95% confidence bounds established at baseline.)
heart rate throughout the infusion of 5–8 BPM (Fossa et al., 2005). Although QT prolongation was observed in the conscious dog with each method at the start of the infusion (QTb<sub>tb</sub>=27 ms, QTf<sub>f</sub>=23 ms and QTc<sub>b</sub>=20 ms), by the end when heart rate slowed there was no reduction in the QT interval from the dynamic visualization (canine E-4031.wmv) or QTb<sub>tb</sub> (25 ms prolonged) yet the QTc<sub>b</sub> and QTc<sub>f</sub> were decreased from baseline 12 and 16 ms, respectively (Fig. 7A; Table 1, Periods B–E). More importantly, when comparing the interpretation of corrected QT data between nitroprusside (a drug widely considered safe even in cardiac patients) and E-4031, a drug associated with a 1–4% incidence of torsades de pointes (canine and QTc<sub>b</sub>/20 ms), whereby the QTb<sub>tb</sub> accurately represented no prolongation of nitroprusside (Table 1). The response of the rapid potassium delayed rectifier current (IKr) blocker, E-4031, also translates to humans. Sotalol, another IKr blocker, given to normal healthy volunteers clearly prolongs the QT interval above the normal upper boundaries of the beat-to-beat QT–RR relationship (Fig. 7B; Fossa et al., 2007). These examples demonstrate that correction methods can produce both quantitative and qualitative errors leading to poor decision in drug development when safe autonomic influences occur (e.g. CNS or CV therapeutic agents).

1.3. Effect of impaired repolarization in conscious dog

Experiments were conducted to assess the effect of impaired repolarization through either inhibition of the slow (IKs) or fast (IKr) potassium delayed rectifier currents on the beat-to-beat QT–RR relationship (Fossa et al., 2006). Additionally a combination of IKr and IKs inhibition was examined to study the effect of minimal repolarization reserve. During each state of inhibition, the response to heart rate acceleration and deceleration was determined during a challenge with isoproterenol. We also studied this effect on ECG restitution (the relationship between the QT and TQ intervals) under normal sinus rhythm to determine if arrhythmia liability could be quantified. Restitution is the ability of the heart to recover from one beat to the next. The working hypothesis is that as the restitution relationship increases (either QT increases or TQ decreases or both), the likelihood of reentry arrhythmias increases. Only during severely impaired repolarization with both IKs and IKr inhibitors, does temporal heterogeneity increase and the minimum TQ boundary decrease (Fig. 8). The minimum TQ boundary, normally at about 200 ms between beats was reduced to only 28 ms. When challenged with isoproterenol (Fig. 9), the temporal heterogeneity of heart rate acceleration but not necessarily deceleration increased about 2-fold. These increases in heterogeneity and reduction in TQ boundary were reduced or not apparent when repolarization was only impaired by one mechanism, either IKs or IKr inhibition alone. Thus the dog has a tremendous capacity to prolong the QT interval on each beat without diminishing the recovery period between beats even during heart rate acceleration. In unreported studies, our lab has found that the healthy conscious dog can have approximately 150 to 200 ms of QT prolongation with increasing heart rate using isoproterenol before a torsades de pointes type of arrhythmia will be triggered. This then raises the question, is the normal dog an appropriate model for prediction of arrhythmia in humans?

2. Limitations of the canine model when translating data to humans

The question of whether the dog is a good model for predicting effects in humans must be taken into context with the differences in physiology like any other model. The above data shows that normal
conscious dog responds similar to humans with respect to the QT–RR interval relationship and has proven to be a good predictor of response based on PK/PD relationships for most compounds reported (see exception below). The dog also has a much more profound sinus arrhythmia than humans which is beneficial when using the beat-to-beat technique for comparison of QT intervals at comparable RR intervals. However, for predicting the actual concentration of arrhythmia onset, comparisons between dog and humans may be more difficult. The dog has a QT interval approximately 200 ms shorter than humans at a comparable RR interval (230 vs. 430 ms at 60 BPM). If restitution principles apply to the prediction of arrhythmia liability, this would give the dog an additional 200 ms of rest between beats to recover and thus mitigate the risk of arrhythmia making it more resistant than man. Additionally, the dog’s profound sinus arrhythmia predetermines the dog to be exquisitely adept to coping with sudden heart rate accelerations and decelerations that could trigger arrhythmias through impaired hysteresis of the QT interval. This is possible why humans with much higher vagal tone as measured through high heart rate variability on Holter analyses are also less prone toward triggering liability resulting in a rightward shift in this dose–response relationship.

In summary, the data above validates the dog as a highly useful model for predicting outcomes in humans. Like any model, knowing the limitations and using the model to its full benefit in a controlled environment can provide a wealth of underlying information for understanding both preclinical and clinical observations.

References


