Dynamic Beat-to-Beat Modeling of the QT-RR Interval Relationship: Analysis of QT Prolongation during Alterations of Autonomic State versus Human Ether a-go-go-Related Gene Inhibition

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ABSTRACT

Methods to correct the QT interval for heart rate are often in disagreement and may be further confounded by changes in autonomic state. This can be problematic when trying to distinguish the changes in QT interval by either drug-induced delayed repolarization or from autonomic-mediated physiological responses. Assessment of the canine dynamic QT-RR interval relationship was visualized by novel programming of the dynamic beat-to-beat confluence of data or “clouds”. To represent the nonuniformity of the clouds, a bootstrap sampling method that computes the mathematical center of the uncorrected beat-to-beat QT value (QTb) with upper 95% confidence bounds was adopted and compared with corrected QT (QTc) using standard correction factors. Nitroprusside-induced reflex tachycardia reduced QTb by 43 ms, whereas an increase of 55 and 16 ms was obtained using the Bazett (QTcb) and Fridericia (QTcf) formulae, respectively. Phenylephrine-induced reflex bradycardia increased QTb by 3 ms but decreased QTcb by 20 ms and QTcf by 12 ms. Delayed repolarization with E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-methylsulfonylaminobenzoyl-piperidine), an inhibitor of rectifier potassium current, increased QTb by 26 ms but QT prolongation calculations using QTcb and QTcf were between 12 and 52% less, respectively, when small decreases in heart rate (5–8 beats per minute) were apparent. Dynamic assessment of beat-to-beat data, using the bootstrap method, allows quantification of QT interval changes under varying conditions of heart rate, autonomic tone, and direct repolarization that may not be distinguishable with use of standard correction factors.

Ventricular repolarization, as reflected in the surface QT interval, can be affected by drugs as well as autonomic state, both directly and via their effects on heart rate (HR). Methods to normalize the QT interval for changes in heart rate have been described since early last century (Bazett, 1920; Fridericia, 1920). Bazett noted in his original proposal that the K from his correction factor of K/HR varied when the vagus nerve was sectioned or stimulated. Simonsen et al. (1962) demonstrated discrepancies in eight different correction factors when modeling the HR-QT interval relationship on the same dataset. Browne et al. (1982) later showed that a 75-ms shortening of the absolute QT interval with atropine during fixed rate atrial pacing was interpreted as a 43-ms prolongation using the Bazett correction. This 118-ms difference suggests that Bazett’s formula “undercorrected” the QT interval for the increase in HR observed during vagal inhibition. Similarly, patients receiving propranolol or the atropine/propranolol combination had differences in the corrected QT (QTc) interpretation of approximately 30 and 60 ms, respectively.

Prolongation of the QT interval has been associated with an increased incidence of the fatal ventricular tachyarrhythmia, Torsade de Pointe. Since many drugs that have been removed from the marketplace due to an increased occurrence of Torsade de Pointe prolong the QTc interval, regulatory agencies have increased their surveillance of potentially new and already marketed drugs for QT prolongation. Although less common, sudden cardiac death has been associated with shortening of the QT interval as well (Brugada et
al., 2004). Many drugs cause QT interval changes by either directly affecting repolarization through inhibition of cardiac ion channels (i.e., hERG), endogenously through changes in autonomic tone, or even a combination of both. It is critical that the interpretation of the QT interval is accurately assessed so that beneficial medications are not prohibited from development, or more importantly that pathophysiological QT lengthening or shortening are not underestimated. Two recent examples of drugs that do not affect hERG at their therapeutic concentrations but cause physiologically relevant vasodilatation and reflex tachycardia are vardenafil and alfuzosin. Both have been shown to produce QTc prolongation using standard correction factors. Given this uncertainty for using HR corrections during changing autonomic states, alternative methods to properly compare QT intervals are now being considered in drug safety assessment (CRDAC, 2003).

Figure 1 depicts our conceptualized hypothesis for the QT-RR interval relationship. This highly dynamic state that occurs from beat-to-beat allows humans to live within their own unique QT-RR boundary influenced by many different conditions of autonomic-mediated change such as eating (Nagy et al., 1997), sleeping (Roche et al., 2003), and exercise (Magnano et al., 2002) or by disease states that alter QT-RR heterogeneity (Berger et al., 1997; Faber et al., 2003). Holter acquired data plotted from long-term monitoring produces large QT-RR "clouds" that can easily span over 1000 ms in the RR interval range and 70 ms in QT magnitude in any given individual (Batchvarov et al., 2002). However, when assessing specific autonomic-mediated changes beat-to-beat within these large clouds, divergence from correction factors occurs, leading to misinterpretation of the actual QT interval when expressed as QTc (see shaded areas in Fig. 1 above and below Fridericia curve). Therefore, we propose that when the normal (unstressed) autonomic-mediated QT-RR boundary is established as the upper confidence bounds (or lower bounds for QT shortening) before drug administration, this should represent a safe cardiac physiological limit for QT change. QT prolongation beyond this limit may represent drug-induced delayed repolarization associated with some degree of as yet undefined arrhythmogenic risk.

The purpose of this study is to first demonstrate the utility of “dynamically” examining the continuous confluent beat-to-beat QT-RR interval relationship under varying conditions of autonomic baroreflex versus delayed repolarization changes. The commonly used vasodilator nitroprusside and the vasoconstrictor phenylephrine were chosen to induce reflex tachycardia and bradycardia changes, respectively (Glick and Braunwald, 1965). These changes were compared with the direct repolarization effects induced by the class III antiarrhythmic E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-methyl-sulfonylaminobenzoyl)-pipеридин) (Fujiki et al., 1994). Video links (Video Data Supplements) to this publication have been provided to observe the differential QT-RR dynamics of these changes. Second, we wanted to describe a new technique of analyzing and quantifying the uncorrected dynamic beat-to-beat data. Finally, we propose the concept to define the upper confidence bounds allowing the accurate assessment of the beat-to-beat dynamics of the QT-RR relationship during autonomic changes, providing a flexible methodology for the analysis of raw data without the limitations inherent in standard QT interval correction as demonstrated using the Bazett and Fridericia formulae.

Materials and Methods

Surgical and Experimental Protocol. All surgeries and experimental procedures were performed in agreement with an Animal Care and Usage Protocol approved by the Institutional Animal Care and Use Committee, and similar to those described elsewhere (Raunig et al., 2001; Fossa et al., 2002). Briefly, dogs (male and females between 10 and 13 kg) were trained to lie quietly in slings in isolation rooms and monitored to ensure no gross cardiac arrhythmias or behavioral irregularities. The surgical and experimental protocol had a telemetry device (Data Sciences International, St. Paul, MN) surgically implanted to measure arterial blood pressure (BP) via a femoral artery, and to record the Lead II electrocardiogram (ECG) from internal recording electrodes. In some studies, it was necessary to record the ECG from surface limb leads. All studies were performed in the morning to minimize circadian rhythm effects. On the day of each study, dogs were placed individually in an isolation room and a cephalic venous catheter was put in place for intravenous administration of vehicle or test compound via a remote-controlled infusion pump (Harvard Apparatus, Cambridge, MA). The digitized signals from the telemetry device were converted back to analog and sampled and saved to a computer at 1000 Hz using a Po-Ne-Mah data acquisition and analysis system (Gould Instrument Systems, Valley View, OH). Baseline data were recorded for at least 20 min before infusion of any test substance.

For human studies, a peripheral intravenous line was inserted. ECG electrodes were put in place and recorded on a Po-Ne-Mah data acquisition system. Baseline ECG data were recorded during a brief period of quiet rest in the supine position. The study protocol was approved by the Western Institutional Review Board.

Dosing Protocol. Sodium nitroprusside dihydrate was purchased from Sigma-Aldrich (St. Louis, MO), dissolved in saline, and given as a constant infusion of 6 μg/kg/min for 20-min or until the heart rate exceeded 180 bpm. 1-Phenylephrine hydrochloride was also purchased from Sigma-Aldrich, dissolved in saline, and given as a bolus dose of 10 μg/kg over 10 s. E-4031 was synthesized at Pfizer...
(Sandwich, UK) and administered as described previously (Fossa et al., 2002) to achieve a clinically relevant steady-state free plasma concentration of 5.3 nM free drug. Saline vehicle studies were conducted using the same bolus and infusion rates as drug treatment groups.

For human studies, isoproterenol was infused at 0.05 μg/min and increased in increments of 0.05 μg/min every 3 min until a peak HR of 100 bpm was achieved. Epinephrine was infused at 0.05 μg/kg/min and increased in 0.05 μg/kg/min increments until a peak HR of 100 bpm was achieved. A 30-min period was allowed between isoproterenol and epinephrine infusions to allow HR to return to baseline.

Review of Dynamic Beat-to-Beat Data. ECG and blood pressure waveforms were replayed on the Po-Ne-Mah system, and each cardiac cycle was analyzed for accurate detection of the beginning of the Q-wave and the end of the T-wave (Tend) by inspection of the computer-generated validation marks. The analysis software determines Tend by working forward in time from the peak (positive or negative) of the T-wave. The slope at which the T-wave approaches the previously established baseline (isoelectric line) is examined, and once the rate of change relative to the baseline drops below a specific threshold, Tend is marked. Several user-controlled attributes allow adjustment of thresholds when different T-wave morphologies occur. The signals were reviewed continuously for the last 10 min of the baseline period and during the drug infusion periods for nitroprusside, phenylephrine, E-4031, and saline. QT intervals that could not be measured due to motion artifact or electrical noise of the ECG were removed from the dataset, and if necessary the QT interval was manually measured using a digital measure command on the Po-Ne-Mah system. The replayed data files were imported into Excel (Microsoft, Redmond, WA) and transformed into a format that was read by programs written in MATLAB (MathWorks Inc., Natick, MA). These programs allow real-time visualization of the analyzed beat-to-beat QT-RR interval relationship and the simultaneously measured BP, as well as providing the ability to graphically assess the dynamic responses of these variables with various treatments. Each dog was given at least two of the treatments E-4031, phenylephrine, nitroprusside, and vehicle. A sufficient washout period of at least 1 week (>5 half-lives) insured that there was no carryover from previous treatments and carryover was not modeled in the analysis. Postbaseline time periods of data analyses (designated as periods A to E) were chosen based on representative changes in the QT-RR interval relationship during maximal hemodynamic responses for each treatment studied. Specifically, postbaseline periods for analyses of the median 100 beats were for nitroprusside: period A (0–2 min), period B (2–3 min), period C (3–4 min), period D (4–5 min), and period E (5–6 min); for E-4031, phenylephrine, and vehicle: period A (0–4 min), period B (4–8 min), period C (8–12 min), period D (12–16 min), and period E (16–20 min).

Statistical Analysis. The experiment was designed as a randomized block study with dog as the random block. The sample size for each set of 100 points representing each analysis period was based on an approximate statistical power of 0.80 and a significance level of 0.05 to detect a 5-ms change in QT at RR = 1000 ms.

Before administering each of the treatments, data were collected from each dog for a 10-min predose period. These data were used to establish baseline responses for each treatment-by-dog combination.

The baseline responses were then used as the reference for the analysis of significant QT prolongation.

The bootstrap method (Efron, 1979; Efron and Tibshirani, 1998) was used to estimate the uncorrected QT cloud of data (QTb0) at baseline and after dosing and then subsequently for representative sampled beats where Fridericia (QTcF) and Bazett (QTcB) corrections were applied to the same QT-RR dataset. The bootstrap technique uses high-frequency sampling (1000 random iterations with replacement) of heart beats from each median 100 beats during periods A–E to mathematically estimate both the center of a non-uniform cloud or “centroid”. This was then defined as the uncorrected QTb0 value and the centroid confidence bounds. During highly dynamic datasets, the QT-RR clouds were stretched over a wide range of RR values and often did not resemble a standard multivariate normal ellipse with an easily defined centroid. Consequently, even if a robust estimate of the centroid, such as a median, was used, the confidence bounds of the centroid, analogous to the standard error from normally distributed data, were not easily defined. If the data were represented by the average or median in an attempt to smooth out the noise (data filtering), then important variations such as physiologically significant outliers would be discounted or removed completely. Since smoothing is typically done over a small region of data, chronologically smoothing may average points over a wide range of RR values. Averaging over small QT-RR regions, however, may average data that are chronologically far removed from each other. The bootstrap method is a way to use the raw, unsmoothed data to find the true distribution of the QT-RR data and hence, the true centroid. Last, since the data were not evenly distributed about the Bazett and Fridericia curves, the bootstrap sampling method was also used to establish the confidence bounds of these correction factors. The Bazett and Fridericia corrections were chosen because of their widespread regulatory requirement in drug development studies for QT prolongation assessment as outlined in a recent draft from the International Committee for Harmonization and European Committee for Proprietary Medicinal Products guidelines (CPMP, 1997).

To analyze the long QT intervals (referred to as outliers), the data clouds were fitted to a three-parameter decaying exponential growth curve (Raufin et al., 2001), and the outlier beats were determined as those points that exceeded the single-sided 95% upper confidence bounds for the baseline data. Recognizing that the same concepts and concerns apply for assessment of QT shortening, a two-sided analyses could be incorporated to define the lower 95% confidence boundary if deemed necessary. All analyses were done using Matlab version 6.0 and R version 1.8. All significance measurements used a level of 0.05.

Results

Vehicle Response. Conscious dogs had a mean QTb0 value of 252 ms (Table 1), calculated by bootstrap sampling and determining the center of each individual animal’s RR interval data cloud during the baseline period. The same data points or individual beats corrected by QTcB or QTcF formulas gave values of 267 and 262 ms, respectively. After initiation of saline infusions, there were no statistically significant changes to the mean QT values calculated by all methods, but a significant decrease in HR of 4 bpm was measured during periods D and E (Table 2). As HR decreased with time, the QTb0 upper 95% confidence bounds of the clouds during periods D and E increased from 3 ms at period A to 9 ms at period E (Table 1). This resulted in an increase in the number of outlier beats above the 95% confidence bounds that was statistically significant in two of the five dogs (Table 3) during periods C–E. It should be noted that this effect was just 1 ms above the mean baseline QTb0 value and was probably a result of changes in animal behavior with time in the slings and/or the statistically significant effect on slowing of HR.

Nitroprusside-Induced Reflex Tachycardia. Before nitroprusside administration, the mean uncorrected QTb0 from the center of each individual dog’s RR interval data cloud was 239 ms (Table 1). The same beats corrected using QTcB or QTcF produced a difference of +25 or +16 ms, for mean respective baseline values of 264 and 255 ms. Nitroprusside infusion produced a drop in BP with a subsequent reflex tachycardia initiated in all dogs by 1 min and peaked
by 3 to 4 min (Table 2; see individual example in Fig. 2). After nitroprusside, there was no statistically significant increase in the number of outlier beats beyond the upper 95% confidence bounds of the QT-RR interval relationship established at baseline (Table 3). The sudden drop in BP caused a rapid reduction in RR and QT intervals that resulted in a dramatic divergence from the Bazett and Fridericia HR corrected relationships (Table 1). During period A (approximately 1–2 min after infusion), QT intervals measured on the cloud of 100 points had a mean value of 235 ms or a 4-ms reduction from baseline. By 2 to 3 min (period B), the QTbtb had decreased -43 ms from the original baseline (to view dynamic QT-RR movement, see Video Data Supplement 1). These same beats corrected using the Bazett or Fridericia formulas were interpreted as a prolongation of QTcB (+55 ms) and QTcF (+16 ms), a 59- to 98-ms difference and qualitatively opposing the interpretation from QTbtb. Hysteresis and heterogeneity were statistically evident during period E with an increase in outlier beats above the 95% confidence bounds for two of four dogs and a slight decrease in heart rate of 7 bpm (Tables 3 and 2, respectively).

**Phenylephrine-Induced Reflex Bradycardia.** Before the infusion of phenylephrine, the baseline QTbtb versus the QTcB and QTcF differed by 18 and 12 ms (250 versus 268 and

<table>
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<tr>
<th>Correction or Model</th>
<th>Model Corrected Baseline QT (95% CB)</th>
<th>Change in QTc interval (ms) and confidence bounds at time period after dosing</th>
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<tbody>
<tr>
<td></td>
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<td>A</td>
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<tr>
<td><strong>Vehicle</strong></td>
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<tr>
<td>Bazett (QTcB)</td>
<td>267 (253 to 290)</td>
<td>3</td>
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<tr>
<td>Fridericia (QTcF)</td>
<td>262 (242 to 281)</td>
<td>1</td>
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<tr>
<td>Beat-to-beat (QTbtb)</td>
<td>252 (221 to 265)</td>
<td>-3</td>
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<td><strong>Nitroprusside</strong></td>
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<tr>
<td>Bazett (QTcB)</td>
<td>264 (254 to 273)</td>
<td>15</td>
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<tr>
<td>Fridericia (QTcF)</td>
<td>255 (248 to 265)</td>
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<tr>
<td>Beat-to-beat (QTbtb)</td>
<td>239 (228 to 252)</td>
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<td><strong>Phenylephrine</strong></td>
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<td>Bazett (QTcB)</td>
<td>268 (256 to 279)</td>
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<td>Fridericia (QTcF)</td>
<td>262 (252 to 271)</td>
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<tr>
<td>Beat-to-beat (QTbtb)</td>
<td>250 (242 to 257)</td>
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<td><strong>E-4031</strong></td>
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<tr>
<td>Bazett (QTcB)</td>
<td>244 (234 to 255)</td>
<td>14</td>
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<tr>
<td>Fridericia (QTcF)</td>
<td>242 (236 to 247)</td>
<td>13</td>
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<tr>
<td>Beat-to-beat (QTbtb)</td>
<td>239 (229 to 262)</td>
<td>10</td>
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* Represents statistical significance (P < 0.05) from upper 95% confidence bounds established at baseline

TABLE 1.
262 ms, respectively; Table 1). Phenylephrine produced a reflex bradycardia of 12 bpm almost immediately after intravenous administration that temporally peaked with the maximal pressor response of 28 mm Hg (Table 2; see individual example in Fig. 3). Because HR slowed due to a sudden increase in BP, the QTtb interval increased quickly to a mean of 256 ms by period B, but this same dataset showed a decline of −7 ms in QTcF and −14 ms in QTcB (to view dynamic QT-RR movement, see Video Data Supplement 2). In addition, the beat-to-beat analysis showed three of the five dogs had a statistically significant increase in the number of outlier beats above the baseline 95% confidence bounds for the QT-RR interval relationship (Table 3). Differing interpretations in QT were also observed when BP was returning to normal over the last 15 min. The QTtb interval during this time (periods C–E) showed uncorrected QTtb values being above baseline, whereas those calculated using the Bazett and Fridericia formulae seemed lower compared with baseline (Tables 1 and 2).

**Delayed Repolarization Induced by E-4031.** E-4031 was used as a positive control to demonstrate the effect of an agent that causes direct QT prolongation by delayed cardiac repolarization. Before infusion, the baseline cloud of beats at the center of the RR intervals obtained had a QTtb interval of 239 ms, but was 244 or 242 ms when corrected by Bazett or Fridericia formulae, respectively (Table 1). As E-4031 was infused, mean decreases of 5 to 8 bpm in HR or 4 to 7 mm Hg in BP occurred (Table 2), and the QTtb interval was increased 27 ms by period B (see individual example in Fig. 4 and Video Data Supplement 3). All four dogs showed a ver-
tical shift of the QT-RR cloud as measured by an increase in the number of outlier beats above the upper confidence bounds of their normal QT-RR interval relationship (Table 3). Using the Bazett or Fridericia derived values from the same beats during period B, the maximum increase was 20 ms (QTcB) and 23 ms (QTcF). However, as HR continued to decline during periods C and D, QTbtb remained increased (25–26 ms) but the increases in QTcB (12–16 ms) and QTcF (16–19 ms) became smaller.

Applicability of Dynamic Beat-to-Beat Analysis in Humans: A Demonstration with Isoproterenol and Epinephrine Infusions. Dynamic changes are easily assessed in the resting dog because of a profound sinus arrhythmia (Hariman et al., 1980) that produces a wide range of RR intervals for facile comparison. Humans, on the other hand, do not normally possess this same degree of beat-to-beat RR interval variation at rest. Thus, dynamic differences may be more difficult to evaluate because the drug effect or autonomic tone may alter the RR interval beyond the baseline period. To determine whether this same technique could be applied to human digitized ECG data, the dynamic QT-RR profiles were examined after an infusion of isoproterenol (Fig. 5 or Video Data Supplement 4) or epinephrine (Fig. 6 or Video Data Supplement 5) into a normal healthy male subject after a 10-min baseline (HR = 48 bpm) was obtained. As the RR interval shortened to approximately 900 ms with isoproterenol, the QT interval decreased but less than predicted by the represented Fridericia relationship (Fig. 5, from 9 to 24 min). Thus, the QTcF is increased as opposed to decreased initially from baseline and is consistent to previously reported QTcF findings with isoproterenol (Lecocq et al., 1989; Magnano et al., 2002). These short RR intervals do not overlap the baseline RR intervals for QT comparison with the upper confidence bounds of what could be considered normal (i.e., no physiological reference standard). Upon cessation of the isoproterenol at an achieved HR of 100 bpm, the RR interval rapidly returned to baseline,
whereas the QT interval only slowly returned to baseline producing a marked hysteresis below the expected Fridericia relationship (Fig. 5, 24 to 27 min). Subtle differences in the QT-RR interval relationship can be distinguished between isoproterenol and epinephrine responses in the same individual using dynamic assessment. Figure 6 (from 6 to 23 min) shows a less gradual acceleration of the HR with epinephrine and a more abrupt shortening of the QT interval in relation to the shortening RR interval. Greater heterogeneity in QT interval is apparent compared with the response after isoproterenol. The beat-to-beat baseline clouds before and after isoproterenol and epinephrine (Figs. 5 and 6, see also Video Data Supplements 4 and 5, respectively) in this subject were very reproducible, but varied between individuals as well as dogs, (other subjects not shown) supporting findings previously reported by Malik et al. (2002) and Batchvarov et al. (2002).

**Discussion**

This study demonstrates that dynamic assessment of the beat-to-beat QT-RR interval relationship provides a means to differentiate QT interval prolongation effects incurred by a hERG blocking agent such as E-4031 from changes in the QT interval incurred through autonomic-mediated reflexes avoiding errors associated with the use of standard correction factors such as Bazett and Fridericia. This technique may prove useful to study QT interval heterogeneity, hysteresis, and beat-to-beat heart rate variability. Visualization of these data from continuous computer display of the confluence of individually plotted beats (see Video Data Supplements with figures) allows more precise evaluation of pharmacokinetic relationships and pharmacodynamic events such as behavioral or hemodynamic changes. However, to obtain an accurate statistical representation of the dynamic
QT-RR states, a common methodology used in other scientific fields dealing with highly variable relationships (Zhou and Tu, 2000), known as bootstrapping, was adopted. This technique of random, iterative sampling of the sequential cardiac cycles was necessary to mathematically reflect the varying density and nonuniform distribution bounds of each dataset as they are formed into clouds represented as the uncorrected QTbtb value. Traditional mathematical averaging, data smoothing and estimates of error should not be applied in this situation because they reduce outlier beats and total heterogeneity that may provide useful information to understanding arrhythmogenic liability. The bootstrap sampling technique, used across the continuum of RR intervals during a baseline period, allows the asymmetric upper 95% confidence bounds to accurately describe the normal QT limits (bounds) under varying physiological conditions and to permit quantification of outlier beats above this boundary.

Hysteresis of the QT interval occurred with the normal QT-RR relationship when autonomic tone was changed by classical hemodynamic maneuvers induced by nitroprusside and phenylephrine. Since these drugs are considered safe and not clinically reported for causing untoward cardiovascular arrhythmogenic events (www.qtdrugs.org) despite their ability to induce baroreflexes, they represent changes in the QT-RR relationship depicted in Fig. 1 as defining the area of reflex tachycardia and bradycardia. QT-RR clouds with hysteresis during acceleration of HR not following the Bazett and Fridericia correction relationships were interpreted as QTc prolongation (analogous to the hatched area above the Fridericia line in Fig. 1). This marked hysteresis present during autonomically mediated HR fluctuations makes it impossible for a single correction factor to accurately predict QT intervals in an individual, much less in populations (Malik, 2002). For instance, the “individual correction factor” is perhaps the most accurate correction method described to date (Batchvarov et al., 2002). However,
analysis using this method requires RR interval changes greater than 5 ms within a 10-s recording be eliminated from the dataset to derive the correction curve, thus diminishing the possibility of studying hysteresis. The dynamic movement of the QT-RR interval clouds in our studies clearly showed that this is a physiologically mediated autonomic reflex response in dogs due to hemodynamic changes. The assessment of the clouds at specific dynamic periods during reflex tachycardia induced by nitroprusside showed that the number and magnitude of beats above the 95% confidence bounds compared with the overlapping normal QT-RR interval relationship was not increased. However, this must be distinguished from the hysteresis that occurs during the slowing of HR under vehicle control conditions or during reflex bradycardia, where in an overshoot of the QT-RR relationship can occur that causes an increase number and magnitude of beats above the baseline 95% confidence bounds.

When the dynamics of the QT-RR data were examined after E-4031 that directly affects cardiac repolarization, a vertical shift of 25 to 27 ms in the QT-RR cloud and an increased number and magnitude of beats above the upper 95% confidence bounds of the normal relationship at all HR (RR intervals) without hysteresis was observed. Again referring back to Fig. 1, this effect with E-4031 would be analogous to the dark shaded area above the 95% confidence bounds.

Fig. 5. Time-course effect of an isoproterenol infusion on the beat-to-beat QT-RR interval relationship from a single human male (see also Video Data Supplement 4). The RR interval range from 300 to 1500 ms, corrected each 10 ms using the Fridericia formula, is depicted (pink line) for visual perspective from the beat-to-beat QT-RR interval relationship. Median 100 beats during each time periods (red triangles) were compared with beats obtained at baseline (blue squares) using upper 95% confidence bounds (CB) generated from bootstrapping (see Materials and Methods). The upper and lower 95% confidence bounds (ULCB) for QTcB and QTcF values were calculated from the QTcB values.
bounds. Using the Bazett correction during the same periods, the increase in QTcB was only 12 to 20 ms due to a decrease in HR from baseline. Thus, the use of Bazett or Fridericia correction factors in this case with E-4031 may have underestimated the QT prolongation as much as 50% when only subtle HR decreases of 5 to 8 bpm were apparent. It is interesting to point out that the magnitude of the QT prolongation interpretation for E-4031 with QTcB and QTcF was actually less in comparison with the effect of nitroprusside (i.e., QTcB = +20 ms for E-4031 versus QTcB = +55 ms for nitroprusside during period B), a drug with no direct effect on cardiac repolarization but reduced the QT interval through physiological reflex.

We have also observed these same vertical shifts of the QT interval cloud and increased outlier beats above the 95% confidence bounds with other hERG blocking agents with little or no observation of hysteresis. Cisapride showed a marked increase in heterogeneity of the QT interval (Raunig et al., 2001), and rate-dependent QT prolongation was caused by terfenadine and terodiline, despite no overall effect on the mean QT interval (Fossa et al., 2002). Much of the cause for discrepancy in correction factors is because no single mathematical transformation can describe the rapidly changing nonlinear dynamics of the QT-RR interval relationship. As individuals approach their unique negative inflection point of nonlinearity in their QT-RR in-

Fig. 6. Time-course effect of an epinephrine infusion on the beat-to-beat QT-RR interval relationship from a single human male (see also Video Data Supplement 5). The RR interval range from 300 to 1500 ms, corrected each 10 ms using the Fridericia formula, is depicted (pink line) for visual perspective from the beat-to-beat QT-RR interval relationship. Median 100 beats during each time periods (red triangles) were compared with beats obtained at baseline (blue squares) using upper 95% confidence bounds (CB) generated from bootstrapping (see Materials and Methods). The upper and lower 95% confidence bounds (ULCB) for QTcB and QTcF values were calculated from the QTbtb values.
terval relationship (where the QT interval decreases much more rapidly than RR interval), the variability in the QT interval will increase for a given RR interval change (Berger et al., 1997). Mechanistically, this may be due to the kinetics of some channel currents like the slowly activating potas-
sium delayed rectifier that do not inactivate at fast HR (Chinn, 1992). Additionally, the latency of calcium handling at fast HR (Chudin et al., 1999) can result in greater action potential alternans as a function of cardiac restitution (Franz et al., 1988). It seems that some cardiac disease states or drugs that are associated with a high risk of fatal ventricular arrhythmias also lower the threshold for alternans (Walker and Rosenbaum, 2003) and increase QT dynamicity (Cheva-
lier et al., 2003; Faber et al., 2003), thus changing the RR interval at which the negative inflection of the QT-RR interval of outlier beats, heterogeneity, and hysteresis above and below the confidence bounds of the QT-RR interval relationship.

**Current Limitations of This Technique.** Since T-wave morphology varies within subject and treatment, the algo-
rithms used for fiducial markings is still required until the algorithms can identify a wide range of T-wave morphology changes accurately. The use of the beat-to-beat QT assess-
ment in humans compared with data obtained in dogs is dependent on the range of individual RR intervals. In con-
test to dogs, human RR exceeding 100 to 120 bpm (RR intervals of 600 to 500 ms) may not be considered untested, and we recognize that all changes exhibited purely through autonomic tone may not imply cardiac safety. Therefore, drugs that move an individual's QT-RR cloud into a region incurred during extreme autonomic events (e.g., startle) may not be considered “safe”. In our human example, higher HR ranges still require nonlinear extrapolation of projected compa-
rator RR intervals and an assumption that heterogeneity will not significantly increase. This may be an invalid as-
sumption that will have to be modeled in future datasets. The ultimate standard to which QT prolongation risk assessment should be compared is the QT-RR interval relationship from 24-h Holter-acquired data (>500 Hz) in the same individual when known physiological events can be demarcated within the total cloud. This would allow a full range of QT-RR intervals to be collected during untested autonamically mediated changes (e.g., sleeping, eating and standing) to
determine the upper confidence bounds of uncorrected QTbtt that could be compared with those when given a test sub-
stance.

In summary, dynamic assessment of beat-to-beat data allows quantification of QT prolongation incurred through hERG inhibition beyond autonomic reflex changes in QT that is not distinguishable with use of Bazett and Fridericia cor-
rection factors.

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