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REVIEW

The impact of varying autonomic states on the dynamic beat-to-beat QT–RR and QT–TQ interval relationships

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The beat-to-beat dynamicity of the QT–RR interval relationship is difficult to assess with the use of traditional correction factors (QTc) and changes in QTc do not accurately reflect or quantify arrhythmogenic risk. Further, the interpretation of arrhythmogenic risk is influenced by autonomic state. To visualize the QT–RR interval dynamics under varying conditions of autonomic state from impaired repolarization, we have developed a system to sequentially plot the beat-to-beat confluence of ECG data or 'clouds' obtained from conscious dogs and humans. To represent the non-uniformity of the clouds, a bootstrap sampling method that computes the mathematical centre of the uncorrected beat-to-beat QT value (QTbtb) and defines the upper and lower 95% confidence bounds is used. The same method can also be used to examine heterogeneity, hysteresis (both acceleration and deceleration) and restitution (beat-to-beat QT–TQ interval relationship). Impaired repolarization with the combination of E-4031 and L-768,673 (inhibitor of IKs current) increased heterogeneity of restitution at rest 55–91%; increased hysteresis during heart rate acceleration after isoproterenol challenge by approximately 40–60%; and dramatically diminished the minimum TQ boundary by 72% to only 28 ms. Impaired repolarization alters restitution during normal sinus rhythm and increases hysteresis/heterogeneity during heart rate acceleration following sympathetic stimulation. These findings are supported by similar clinical observations in LQT1 and LQT2 syndromes. Therefore, the assessment of the dynamic QT–RR and QT–TQ interval relationships through quantification of heterogeneity, hysteresis and restitution may allow a more accurate non-invasive evaluation of the conditions leading to cardiac arrhythmia.
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Abbreviations: APD, action potential duration; DI, diastolic interval; LQT, congenital long QT syndrome

The QT interval has become an important surrogate for assessment of drugs for liability of the ventricular arrhythmia, torsades de pointes (TdP). Historically, as the QT interval varies with heart rate, correction factors have been applied to provide a QTc value to normalize the interpretation. However, with the increased scrutiny of this method by the implementation of the International Conference of Harmonization (ICH) clinical (E14 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005) and preclinical (S7B International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005) guidelines on compounds with established safety records, it is apparent that improvements are needed to accurately assess the arrhythmogenic risks with unknown agents (Shah, 2005). As scientists in the pharmaceutical industry are affected by these guidelines, we must ask ourselves the following questions related to the data we produce and the disparity that exists. Are we collecting data in the proper manner? Is correcting the QT interval accurately differentiating normal physiological changes (that is, autonomic effects) from changes leading to a pro-arrhythmic event? Have we properly incorporated our understanding of cardiac physiology into our most state-of-the-art ECG systems? Are we examining the right biomarker(s) for arrhythmia liability? The following discussion is not meant to provide definitive answers towards measuring the QT interval and its translation for assessing arrhythmia liability, but rather to provide another perspective to engage meaningful discussion towards new solutions.

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Are we collecting the proper ECG data?

The QT–RR interval relationship is highly dynamic from one cardiac cycle to the next or beat to beat. The RR interval in humans can vary approximately 500 ms (Batchvarov *et al.*, 2002), and in dogs, with their profound sinus arrhythmia

(Hariman *et al.*, 1980), can vary 1200 ms within a few cardiac cycles (Fossa *et al.*, 2002). In normal healthy patients, greater RR interval variability is associated with the high-frequency component of heart rate variability from a 24-h Holter analyses and is primarily influenced by the vagus. In disease states associated with sudden cardiac death, the heart rate variability decreases (Kleiger *et al.*, 1987) as sympathetic influences tend to dominate and QT variability increases (Saul *et al.*, 1988; Berger *et al.*, 1997; Smetana *et al.*, 2004). The autonomic control of the QT variability appears to be much more complex with possibly rate-dependent mechanisms (Gilmour *et al.*, 1997), influences by membrane ion potentials (Watanabe *et al.*, 2001), intracellular calcium handling (Chudin *et al.*, 1999; Kaufman *et al.*, 2000; Walker and Rosenbaum, 2003) and electrotonic effects (Pastore and Rosenbaum, 2000; Cherry and Fenton, 2004).

Figure 1 illustrates 30 min of successive beat-to-beat QT–RR cardiac cycles from a normal resting dog. The change in QT interval for a given RR interval is not linear but has a relatively flat relationship at RR intervals of approximately 600 ms or more (100 BPM (beats per minute) or slower), with a strongly diverging inflection range and steep relationship below RR intervals of 400 ms (150 BPM or faster). The density of beats occurring at each heart rate throughout the entire RR interval range also varies due to the sinus arrhythmia. Vagal activation on each expiratory respiration cycle concentrates cardiac cycles at high RR intervals, whereas sympathetic influences dominate as vagal tone diminishes during inhalation to concentrate the cardiac cycles at lower RR intervals (Berne and Levy, 1981). This pattern is unique to an individual dog and has also been described in humans, although with a somewhat lesser degree of variability (Batchvarov *et al.*, 2002). Averaging of the beat-to-beat cycles (1 min means shown in Figure 1), as is commonly carried out in toxicology studies or clinical trials (by choosing a mean of three beats or signal averaging across leads and beats), collapses the relationship thereby reducing the variability. This, combined with transforming the RR interval into heart rate that is also commonly carried out, tends to linearize the relationship. A correction factor simply rotates this transformed line of collapsed data to zero, from which we extrapolate the QT interval from a given RR interval to, perhaps, inappropriately consider the resulting QTc value. This is troublesome because the averaged data express values for that subject that do not actually represent the normal physiology of how the cardiac cycles occurred. Often the mean value represents the inflection range where the flat and steep relationships intersect, and the relationship is most prone to error when extrapolated. Thus, one must ask oneself, if one only saw the mean data, could the true QT–RR interval relationship be constructed, and if not could the right interpretation of QT be made? This fundamental question must be resolved before prediction of arrhythmia liability can occur using the QT–RR interval relationship.

Do correction factors differentiate autonomic effects properly?

The highly dynamic state of the beat-to-beat QT–RR interval relationship as depicted in Figure 2 (from Fossa *et al.*, 2005) may describe how humans live within their own 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 disease states that alter QT–RR heterogeneity (Berger *et al.*, 1997; Faber *et al.*, 2003). If the normal (unstressed) autonomic-mediated QT–RR boundary can be established through high-quality 24-h Holter-acquired ECGs as the upper confidence bounds (or lower bounds for QT

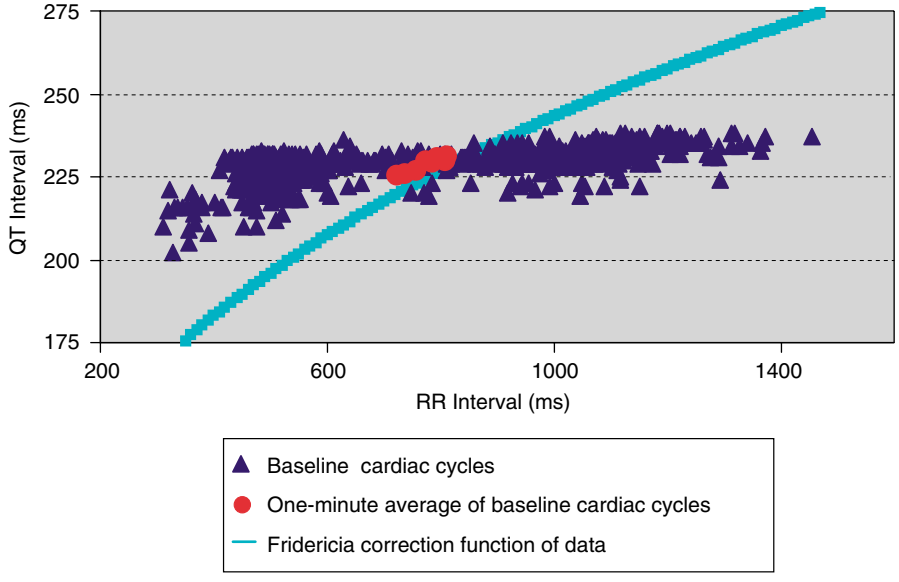

Figure 1 The normal beat-to-beat QT–RR interval relationship in a conscious dog at rest. Ten minutes of sequential cardiac cycles were plotted as the QT interval vs the preceding RR interval. The same beat-to-beat cardiac cycles were averaged for 1-min intervals. The resultant average beats do not lie with the predominance of data, nor would a linear correction (Fridericia best fit to the beat-to-beat data) accurately reflect the QT interval beyond the mean RR boundary from which it was derived.

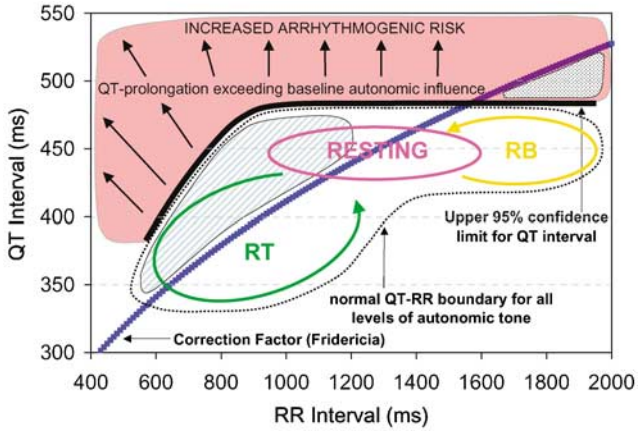

Figure 2 The normal dynamic QT–RR interval relationship encompasses autonomic reflex responses such as tachycardia (RT) and bradycardia (RB) with hysteresis. The statistical outer boundary of the normal cloud is defined as the upper 95% confidence bounds. The Fridericia correction factor applied to the resting QT–RR interval relationship overcorrects dynamic responses in the normal range (striped area above correction line and below 95% confidence bounds) or underestimates QT prolongation at slow heart rates (shaded area above 95% confidence bounds but below Fridericia correction). QT prolongation of undefined arrhythmogenic risk (dark-shaded area) occurs when exceeding the 95% confidence bounds of QT intervals during unstressed autonomic influence. From Fossa *et al.* (2005).

shortening) before drug administration, this should represent a safe cardiac physiological limit for QT change. QT prolongation, outlier heterogeneity or hysteresis (the lag between QT adaptations for a given RR interval change) beyond this limit may represent drug-induced delayed repolarization associated with some degree of as yet undefined arrhythmogenic risk.

To examine the impact of autonomic change on the interpretation of dynamic events, a method was developed to visualize sequentially the beat-to-beat QT-RR interval relationship by novel programming of the confluence of data or 'clouds' (Fossa *et al.*, 2005). Each QT and RR fiducial interval was automatically calculated for Lead II by the ECG data acquisition system then checked for accuracy by retrospective manual overreading of the computer-generated validation marks for the entire data set and changed when necessary. Premature beats were included as separate beats and measured from the onset of the Q wave of the QRS interval wave complex. As sling-trained dogs were used for all studies, very few beats were excluded for transient movement common in ambulatory environments. A bootstrap sampling technique was applied to iteratively sample each cloud 1000 times for every 100 beats and mathematically describe the non-uniform centre and 95% confidence boundaries of each cloud. Benign autonomic reflexes were induced in conscious dogs either given the vasodilator nitroprusside to produce reflex tachycardia or the vasopressor phenylephrine to cause reflex bradycardia. These responses were compared to delayed repolarization by the class III antiarrhythmic E-4031 (a class of drugs associated with approximately 1–4% incidence of TdP). Data sets of the exact same beats or clouds during changes in autonomic tone were analysed by the beat-to-beat method and using correction factors. E-4031 produced vertical shifts of the QT interval cloud and increased outlier beats above the 95% confidence bounds with little or no observation of hysteresis. The increase in QT interval, irrespective of the method, was initially interpreted as between 20 and 27 ms. However, even though no autonomic reflex occurred because of a lack in blood pressure change with E-4031, there was a slight heart rate decrease of 5–8 BPM over the latter part of the infusion period. A display of the beat-to-beat cloud pattern at this time showed no substantial decrease in the magnitude of the QT interval but simply more beats occurred at longer RR intervals. When assessing the data from this latter time period, the correction factors applied to the same beats interpreted the cloud of data as a 30–40% decrease in the QTc response from the original prolongation (even though this was visually not evident from each plot), whereas the beat-to-beat technique indicated only a 2 ms reduction. Conversely, nitroprusside produced hysteresis above the heart rate correction line (interpreted as QTc prolongation of between 16 and 55 ms) but not beyond the 95% confidence bounds of the normal resting QT–RR interval relationship. Reflex bradycardia with phenylephrine produced a clear increase in the QT interval when visualized dynamically and is reflected by a subtle increase in the beat-to-beat QT interval of 3–6 ms. However, corrections applied to the same data clouds interpret this response as a 4–20 ms decrease in QTc. Thus the magnitude of a safe drug, such as nitroprusside, can be interpreted as a greater risk of arrhythmia than a dangerous drug such as E-4031. This demonstrates that with conditions of autonomic change, the incremental changes with correction factors can allow qualitative errors as well as quantitative errors (Fossa *et al.*, 2005). Beat-to-beat analyses may provide a useful means to assess the dynamics of normal and abnormal states to compare the boundaries related to where arrhythmia liability exists.

Are we applying our knowledge of cardiac physiology to drug evaluation?

Moving beyond the way we currently examine the QT–RR interval relationship, we must ask ourselves whether the right biomarkers are being applied to ECG data to assess arrhythmia liability. One approach has been to examine spatial heterogeneity of the QT interval measurement through either lead dispersion (longest–shortest interval) across all leads (Day *et al.*, 1990) or transmural dispersion within the T-wave morphology (peak of T to Tend) of the same beat (Lubinski *et al.*, 1998; Antzelevitch *et al.*, 1999). Both measures are based on evidence that demonstrates that spatial discordance, which arises from apical to basal (Choi and Salama, 2000) or from epicardial/endocardial to mid-myocardial regions (Yan and Antzelevitch, 1998) of the heart, is related to predisposition to arrhythmic states. Considerable effort to translate these findings to predictive biomarkers is in progress (Taggart *et al.*, 2001; Topilski *et al.*, 2007). These spatial measures are primarily examined at steady state during a single beat cycle, and consequently the impact of autonomic tone is less apparent but nonetheless cannot be discounted (Umetani *et al.*, 1999;

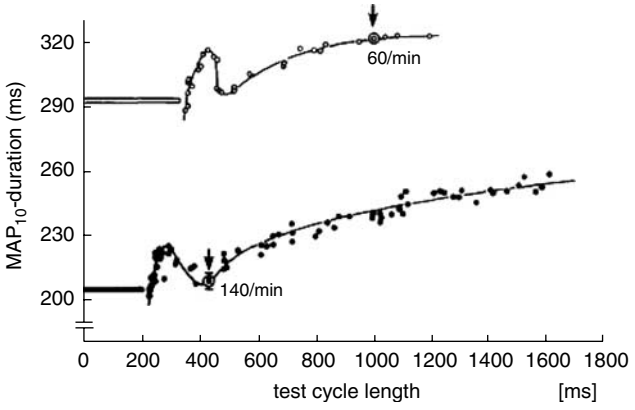

Figure 3 Human restitution curves at two different right ventricular pacing rates. Data points (marked arrows) show steady-state responses (mean ± s.d.) at indicated rate. The horizontal bar before each curve indicates the duration of the membrane depolarization above 70% as part of the test cycle length. From Franz *et al.* (1983).

Couderc *et al.*, 2007). However, for the purpose of this discussion, an alternative approach to assess autonomic influences is to examine the impact of temporal changes across a series of beats leading towards arrhythmia generation.

Dynamically, action potentials throughout the heart are influenced by autonomic state. The duration of the cardiac action potential is largely dependent on the duration of the preceding diastolic interval (DI) (Bass, 1975). This relationship is referred to as action potential duration (APD) restitution and is an important determinant of the beat-to-beat cardiac dynamics at all heart rates or RR intervals. The APD restitution measured in normal human endocardium is a triphasic curve characterized by a modest reduction in APD in relation to shortening DIs near resting heart rates, followed by a transient incline and a steep decrease when approaching fast heart rates (Figure 3). The increasing heart rate reduces the effective refractory period thus accommodating for shorter DIs.

The restitution function itself is highly dynamic and varies with normal and abnormal physiological conditions including autonomic state. The relationship of APD/DI is influenced by both α- and β-adrenergic stimulation (Ng *et al.*, 2001; Taggart *et al.*, 2003), species (Boyett and Jewell, 1980), disease state (Taggart *et al.*, 1986; Kurz *et al.*, 1994) or sudden changes in heart rate (Franz *et al.*, 1988).

Increasing the delay of repolarization can also affect APD restitution. When the DI is progressively shortened within each cardiac cycle, steady-state ion kinetics must be maintained for consistent beat-to-beat APD. However, if steady-state kinetics cannot be maintained, an oscillation of consecutive short–long–short DI intervals is followed by a subsequent short–long–short APD to maintain stability at a given cycle length or heart rate. This oscillatory pattern of APD is referred to as alternans. The initial causes for this were thought to be mediated by reductions in ion channels currents, such as IK_1, *I*Kr (rapid potassium-delayed rectifier current) or IKs (slow potassium-delayed rectifier current), or increases in L-type calcium at higher heart rates (Fox *et al.*, 2002). More recently, the latency of calcium handling at fast heart rates (Chudin *et al.*, 1999) has gained attention, as the causative factor resulting in greater action potential alternans (Diaz *et al.*, 2004; Pruvot *et al.*, 2004). The initiation of alternans may be partially reflected in the triphasic restitution curve, as the inflection point at which the relationship becomes steep (large changes in APD for small changes in DI). As the magnitude of these oscillations is increased to the point where the DI is extinguished (that is, due to refractoriness), the synchronous pattern is terminated leading to triggered unstable re-entry and arrhythmia (Karma, 1994).

The application of restitution principles to the analogous measurements on the ECG may now be possible with greater advances in computer waveform algorithms (Fossa *et al.*, 2006, 2007). QT interval changes generally reflect changes made to the APD, although spatial differences do matter (Shimizu and Antzelevitch, 1998; Choi *et al.*, 2001) and investigators are working to understand that impact on the ECG (Badilini *et al.*, 1999; Smetana *et al.*, 2004; Extramiana *et al.*, 2005; Moss, 2005, Couderc *et al.*, 2007). Few studies have examined TQ interval as a measure of the preceding DI (Gross, 1952, Gilmour *et al.*, 1997), and to date, no other laboratories have reported ECG restitution findings. Thus, if the same examination of the beat-to-beat QT–RR interval relationship previously described (as in Figure 1) is applied to the QT–TQ interval relationship, as TQ diminishes towards zero, a theoretical region of arrhythmia vulnerability could possibly be defined (Figure 4). Under normal circumstances, TQ only approaches zero when QT equals RR or as a premature ventricular beat (R on T). This scenario may be most likely to occur when the QT interval is prolonged in combination with heart rate acceleration or abnormal hysteresis due to altered calcium handling (Walker *et al.*, 2003). Consequently, drugs or conditions that affect the QT–TQ interval relationship may modulate this substrate for re-entry by several mechanisms (1) by prolonging QT directly to the point where the TQ interval is diminished between beats, (2) by reducing the heart rate at which the restitution steepness occurs making progression of instability more likely and (3) by increasing the sensitivity of autonomic states, such as acceleration of heart rate, to induce increased heterogeneity (probably both spatial and temporal). This latter point may be the reason why sudden cardiac death in congenital long QT syndromes (LQT)1 and LQT2 is generally associated with exercise or startle (Schwartz *et al.*, 2001), and with rapid acceleration and oscillations of heart rate before TdP in acquired QT prolongation (Locati *et al.*, 1995). To examine these concepts in a situation similar to LQT1 and LQT2, non-invasive restitution measurements under normal sinus rhythm were conducted using conscious dogs at rest and during autonomic challenge with isoproterenol (Fossa *et al.*, 2006). Repolarization was severely impaired with either the IKs blocker L-768,673 or the *I*Kr blocker E-4031 or a combination of both, and the QT–TQ dynamics were compared to both the vehicle baseline response and baseline before treatment in the same dogs. The combination produced greater QT prolongation than the additive responses to the single agents attributed most likely to a depletion of the repolarization reserve (Roden, 1998). The combination increased temporal heterogeneity at rest 55–91% and increased hysteresis 40–60% with isoproterenol

challenge. At rest with the combination, the QT variability increased with a diminution of the TQ, and an apparent bump in the restitution was visible at high heart rates in three of the five dogs (Figure 5). During the isoproterenol challenge, the minimum TQ boundary observed after the combination was dramatically diminished 72% to only 28 ms. The increase in temporal heterogeneity of hysteresis was predominately a result of the effect on the acceleration component and no change was observed in deceleration (Figure 6). Isoproterenol increases the cardiac intracellular calcium release during β-adrenergic stimulation (Steinberg *et al.*, 2002) and this may amplify heterogeneities (O'Rourke *et al.*, 1999; Katra *et al.*, 2004). These findings are consistent with studies in humans examining direct cardiac restitution measurement of APD/DI with isoproterenol (Taggart *et al.*, 2003), QT hysteresis (Krahn *et al.*, 2002) and increased QT variability in patients with LQT1 and LQT2 syndromes (Satomi *et al.*, 2005).

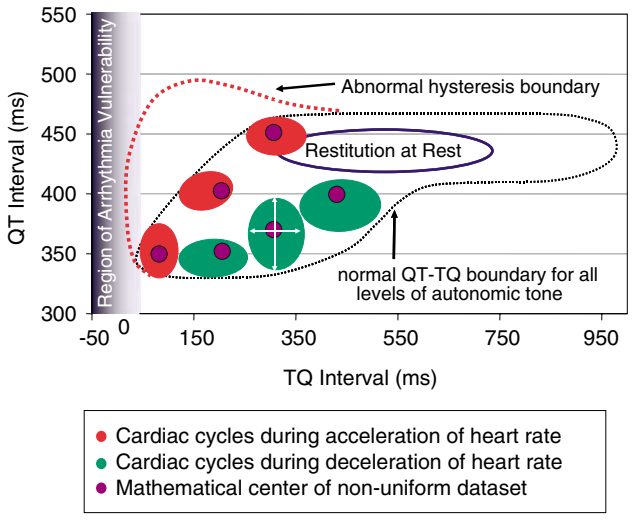

Figure 4 Human restitution with conceptualized view of boundaries during different autonomic states. Black-dotted boundary conveys normal unstressed autonomic boundary as described in Figure 2. As TQ interval diminishes towards zero, a theoretical region for arrhythmia vulnerability can be hypothesized, whereas beats nearing this QT–TQ relationship in higher frequency may be associated with an increase in arrhythmias liability. Impaired hysteresis may allow a higher percentage of beats to approach this region of arrhythmia vulnerability. Autonomic challenges can be induced from the resting restitution state to assess dynamic clouds of data during both acceleration and deceleration phases. Circles within clouds reflect the non-uniform centre and boundaries of these data. Temporal heterogeneity of these responses can help determine the magnitude of the hysteresis by examining the confidence bounds of the clouds during particular phases or throughout the total response. The minimum TQ boundary may also be assessed for arrhythmia liability.

Where do we go from here?

As dynamic beat-to-beat restitution measurements can also be obtained non-invasively in humans using high-quality digital Holter systems, validation of this new approach (biomarker) for assessment of QT prolongation and quantification of arrhythmogenic risk could be accomplished (Fossa *et al.*, 2007). The validation would require well-designed prospective studies and examination of selective retrospective data sets. Characterization of the continuum of risk for arrhythmia from normal physiology to disease with TdP arrhythmia could be obtained from discrete populations with known arrhythmia liability outcomes. A similar approach could be conducted in parallel with drugs to determine the relationship of these biomarkers with known torsadogens using autonomic challenges to expose effects on hysteresis, temporal and spatial heterogeneities. This will allow us to not only begin to define boundaries for our

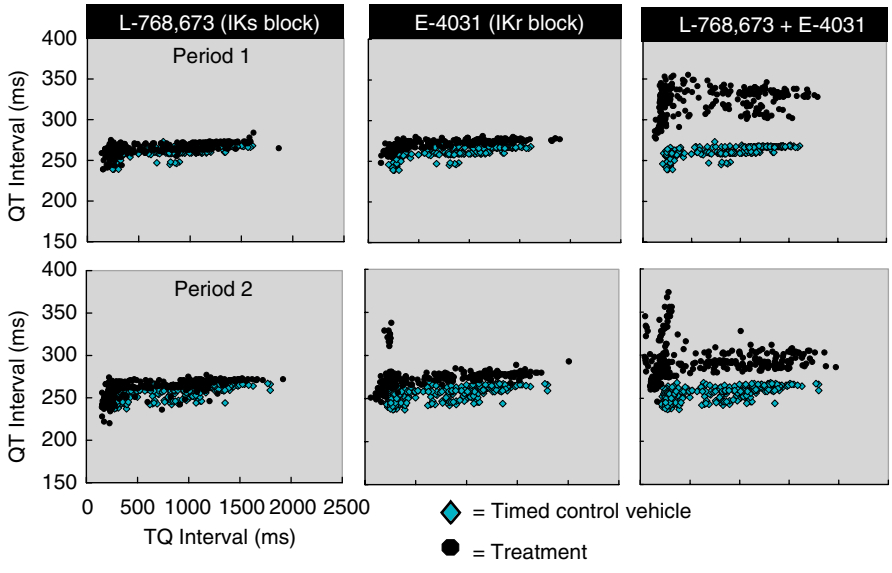

Figure 5 Effect of impaired repolarization on restitution in the same resting conscious dog. Comparison of the resting QT–TQ interval relationship when given either L-768,673 or E-4031 or the combination of both to the time-matched vehicle responses in the same individual dog during Period 1 (timed-matched vehicle or L-768,673 plus 1.7 or 0.5 nM × therapeutic concentration of E-4031; Ceff = 3.5 nM) or Period 2 (timed-matched vehicle or L-768,673 plus 8.8 nM or 2.5-fold therapeutic concentration of E-4031). Modified from Fossa *et al.* (2006).

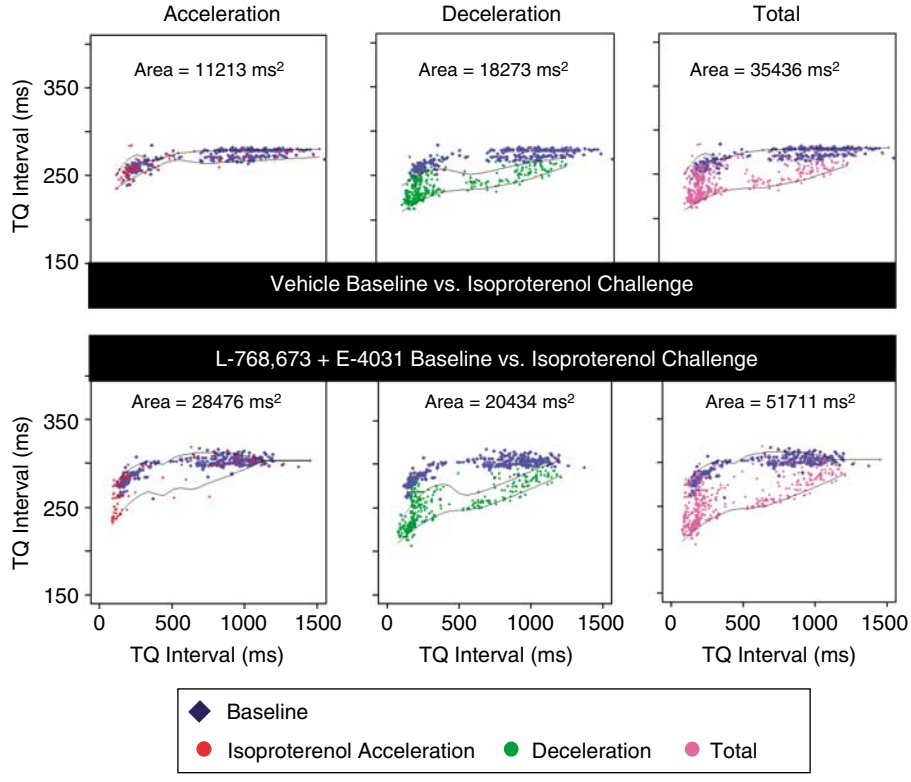

Figure 6 Effect of time-matched isoproterenol challenge on the heterogeneity of restitution hysteresis after either vehicle or the combination of L-768,673 plus E-4031 treatment in the same dog during Period 4B when compared to resting state before isoproterenol. Areas of 98% confidence bounds for cardiac cycles after isoproterenol are separated by TQ intervals during acceleration, deceleration and total response. Only dots within specified TQ interval were used in the analyses. From Fossa *et al.* (2006).

biomarkers associated with known outcomes but also to assess the transition areas where we can quantify the risk/benefit ratios to put future drug studies in perspective for better development decisions.

Conflict of interest

The author states no conflict of interest.

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