

Original article

# A method for QT correction based on beat-to-beat analysis of the QT/RR interval relationship in conscious telemetred beagle dogs

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## Abstract

**Introduction:** Drug-induced QT prolongation is a major clinical risk factor for arrhythmia induction, particularly torsades de pointes. QT interval is rate dependent, and many formulae exist that attempt to correct QT for changes in heart rate. Most correction factors are acknowledged to overcorrect at high heart rates, undercorrect at low heart rates, and tend to be species specific. Data collected from computerised data acquisition systems are normally reported as means over a given logging period, and so extremes of heart rate are averaged out. Therefore, the aim of this study was to develop a technique for assessing drug-induced changes in the QT/RR relationship, which is simple, suitable for small group sizes, and better able to determine rate-dependent effects of drugs. **Methods:** Telemetred beagle dogs ( $n=4$ ) instrumented for the measurement of electrocardiogram (ECG) were monitored for four separate 20-h periods to define the control QT/RR relationship. Data were binned by RR interval, in 10 ms bins, to produce a control curve. Each dog was treated with vehicle and sotalol (4, 8, 32 mg/kg) in a crossover design to determine whether drug-induced changes in the QT/RR relationship could be detected using the data binning technique. **Results:** The control QT/RR relationship was curvilinear with a steep section for RR intervals below 580 ms, and was much less steep after this point. Sotalol produced QT prolongation and bradycardia—Fridericia's correction (QT<sub>f</sub>) reduced the magnitude of this prolongation. The data analysed by the binning technique showed a larger prolongation in QT than was suggested by QT<sub>f</sub>, and an inverse frequency-dependent response. **Discussion:** Beat-to-beat analysis and binning allows accurate determination of the QT/RR relationship and assessment of QT prolongation without recourse to mathematical modelling. It also highlights the importance of assessing QT effects in well-trained animals over a range of heart rates.

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## 1. Introduction

The QT interval of the electrocardiogram (ECG) represents the duration of ventricular depolarisation and repolarisation. Prolongation of the QT interval, an electrocardiographic manifestation of prolonged ventricular repolarisation, is a known clinical risk factor for the development of severe, life-threatening, ventricular arrhythmias, including torsades de pointes (Ackerman, 1998). Although torsades is not normally seen without concomitant QT prolongation, not all QT-prolonging drugs are known to induce torsades de pointes. However, increasing regulatory attention is being paid to in vivo and in vitro indicators of drug-induced changes in repolarisation and

QT concerns can seriously hamper development of an otherwise promising drug (Haverkamp et al., 2000). Interpretation of in vivo data can often be made more difficult by changes in heart rate because the QT interval is rate dependent. Furthermore, many drugs that prolong repolarisation exhibit inverse rate-dependent block of cardiac ion channels responsible for repolarisation, exhibiting greater channel block at lower stimulation frequencies (Yang & Roden, 1996). Therefore, it is important to be able to accurately determine the magnitude of any change in QT interval, particularly in the presence of concomitant changes in the heart rate.

Historically, the relationship between QT and RR intervals was noted by Bazett in the early 20th century. He reported a regression formula of  $QT = a\sqrt{RR}$ . This was later used to produce the more common Bazett's correction, where  $QT_b = QT/RR^{1/2}$  (for a discussion, see Molnar, Weiss, & Rosenthal, 1995). This is mainly used clinically, as it tends to

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severely overcorrect at high heart rates, making it less suitable for most laboratory animals. For canine data, the cube root correction factor, as described by [Fridericia \(1920\)](#), may produce slightly more accurate results. This is defined as  $QT_f = QT/RR^{1/3}$ . Although easy to use, the results are still difficult to interpret if more than mild heart rate effects are seen, as both formulae overcorrect at high heart rates and undercorrect at low heart rates. To try to overcome this, various groups have used more complex regression formulae to define their own data populations, as typified by [Carlsson, Abrahamsson, Andersson, Duker, and Schiller-Linhardt \(1993\)](#). However, the relationship between QT and RR interval is far from linear and any attempt at mathematical correction inevitably fails at certain heart rates.

Cardiac rhythm generally, and canine rhythm in particular, can show marked sinus arrhythmia, whereby the RR and QT intervals can vary greatly on a beat-by-beat basis, within the respiratory cycle. The types of computerised data acquisition used today are capable of performing beat-to-beat analysis; however, it is common practice for data to be reported by the computer as mean values over a given logging period, say 30 s, 1 min, or longer. This inevitably reduces the range of heart rates that are reported and, by averaging, masks the exact relationship between QT and RR. Beat-to-beat analysis of spontaneous changes in QT and RR intervals may allow a more adequate description of the QT/RR relationship than would be possible using standard correction formulae; optimising the detection of drug-induced QT changes from a small number of animals.

Beat-to-beat analysis of canine data has been previously reported by [Raunig, DePasquale, Huang, Winslow, and Fossa \(2001\)](#). Data were fitted using a multicoefficient formula (the Sarma equation; [Sarma, Sarma, Bilitch, Katz, & Song, 1984](#)), which has been shown by [Matsunaga et al. \(1998\)](#), to provide a good fit for canine data. Raunig et al. used statistical analysis on pre- and postdose data curve fit parameters and the incidence of QT interval outliers to determine the effects of E-4031 and cisapride on repolarisation.

Therefore, the purpose of this study was to develop a method for analysing beat-to-beat QT and RR intervals from a small group of dogs instrumented for telemetric recording of ECG and to understand further the relationship between RR and QT intervals. As part of this assessment, the intra- and intersubject variability was determined. The sensitivity of this method to detect drug-induced changes in QT was then assessed using sotalol. Racemic sotalol is a Class III antiarrhythmic drug with  $\beta$ -adrenoceptor blocking actions, known to prolong QT interval and reduce heart rate in vivo ([Patterson, Lynch, & Lucchesi, 1984](#)).

## 2. Methods

All animal work described in this paper was carried out under the authority of a UK Home Office licence, and was also approved by internal ethical review processes.

### 2.1. Telemetry

Male beagle dogs ( $n=4$ ), aged 1.6–2.4 years, of weights 14.0–17.5 kg were premedicated with acetylpromazine (0.03–0.125 mg/kg sc) and pethidine (3.3 mg/kg) and anaesthesia was induced with propofol (4–8.0 mg/kg) and maintained with isoflurane in oxygen, for surgical instrumentation with ITS, totally implantable radio telemetry transmitters (TD27E, RMISS, Wilmington; DE, USA). A small incision was made in the abdominal wall for implantation of the transmitter, battery housing, and induction switch coil and a thermistor probe was inserted into the peritoneal cavity for the measurement of body temperature. Leads attached to ECG electrodes, aortic pressure (BP) and the left ventricular pressure (LVP) transducers were tunnelled through the diaphragm into the thorax. A left thoracotomy was performed between the fifth and sixth intercostal space to expose the left ventricle apex for insertion of the left ventricular Konigsberg transducer for the measurement of LVP. The aortic transducer, which also served as one electrode of the ECG, was inserted into the thoracic aorta just below the aortic arch, and a second ECG electrode was attached to the chest wall, approximating to a Lead II ECG configuration across the heart. The abdominal and chest incisions were closed, analgesics (buprenorphine) and antibiotics (Ceporex) were administered, and the pneumothorax evacuated. Dogs were allowed to recover for at least 4 weeks prior to use on study. During study days, dogs were housed singly in home pens, which had been modified slightly for telemetry recording. Husbandry routines were standardised, and disturbances kept to a minimum on study days. Data were received and converted to analogue by an ITS base station, prior to being cabled to a remote office area for data capture, analysis, and storage. Data capture and analysis was carried out using CA Recorder software (CAR version 1.4, DISS LLC, Michigan, USA). Raw data were saved to a compact disk at the end of each study day for subsequent beat-to-beat analysis (Titan, DISS).

### 2.2. Experimental design

Four treatments were administered: vehicle (1% aqueous methylcellulose w/v) and sotalol at 4, 8, and 32 mg/kg (Beta-cardone, Evans Medical) in a  $4 \times 4$  Latin square design. Dosing occurred weekly, with each animal randomised to receive each treatment once. One animal had to be removed from the study, and so the 8 and 32 mg/kg dose groups represent  $n=3$ . On each day prior to treatment, data were captured for a 20-h control period from every animal. On dosing days, animals were dosed by oral gavage 1 h prior to feeding. Animals were then left undisturbed for the remainder of the 20-h period, but monitored by CCTV for welfare reasons. ECG signals were regularly monitored on screen to verify correct triggering and placement of interval markers.

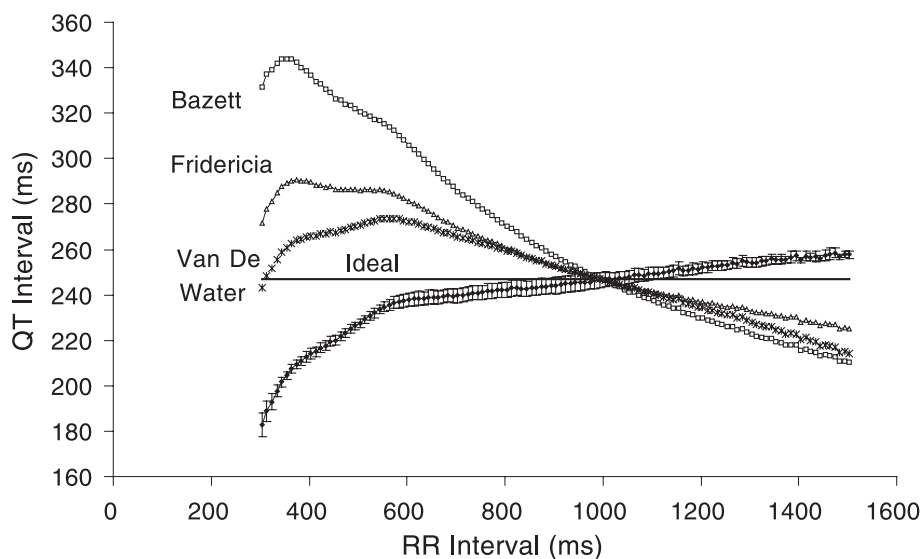


Fig. 1. Mean data from four animals, showing curvilinear nature of the QT/RR relationship ( $\pm$  S.E.M.). For comparison, the same data is shown transformed by Bazett's, Fridericia's, and Van de Water's correction factors. Correction is by definition to an RR interval of 1 s, so the line of ideal correction is shown to highlight the deviation of both the real and the transformed data.

### 2.3. Measurement of QT and RR

Accurate and reliable computerised detection of the QT and RR interval is a prerequisite to using computerised measurement of the ECG, and extensive validation of these systems against classical hand measurements is a requirement for regulatory authorities. The RR interval was calculated as the time difference between the peak of the QRS complex and the peak of the preceding QRS complex. The QT interval was determined as the time difference from the start of the Q wave to the point where the ECG returns to the isoelectric point following the T wave. Computerised measurements of these intervals were compared to hand measurements from strip chart by two trained readers using electronic calipers (S225, Sylvac, Switzerland).

### 2.4. Data analysis

Derived data were reported on line as 30-s means. To enable beat-to-beat analysis, the raw files were replayed to report instantaneous heart rate and QT interval. These data were then imported into an Excel worksheet (Excel 98, Microsoft, USA). The worksheet was designed to sort the data, by RR interval, into 10-ms bins. There were 121 bins from 300 to 1500 ms (equivalent to instantaneous heart rates of 200 to 40 bpm). This produced a table containing RR bins, with average RR and QT intervals for that bin, and the number of beats within that bin. To produce the control data, each dogs' four control days were averaged together, and then all four dogs' mean data were used to produce a group mean  $\pm$  S.E.M. Data from treatment days were averaged together by dose.

Initial analysis of data used 30-min averages of heart rate and both uncorrected QT and Fridericia's corrected QT

interval ( $QT_f = QT/RR^{1/3}$ ). This provided an overview of the time-course of the experiment. The beat-to-beat data were compared by plotting the QT/RR curves for each dose. Finally, the 30-min averaged uncorrected data were compared to control by estimating the expected QT interval from the actual RR interval at that time.

## 3. Results

### 3.1. Validation

The maximum difference between the two readers' hand measurements of the RR interval was 1%, and 11% for the QT interval. The maximum difference between computer-

Table 1  
Day-to-day variation in QT/RR interval relationship in telemetred beagle dogs

Animal	RR interval (ms)	QT interval (ms)				Mean
		Day 1	Day 2	Day 3	Day 4	
1	500	229	235	231	–	231 $\pm$ 2
	1000	242	245	243	–	243 $\pm$ 1
	1500	262	268	254	–	261 $\pm$ 4
2	500	219	227	224	220	223 $\pm$ 2
	1000	235	245	242	236	240 $\pm$ 2
	1500	251	256	253	256	254 $\pm$ 1
3	500	227	233	223	227	227 $\pm$ 2
	1000	248	258	252	256	254 $\pm$ 2
	1500	256	262	261	260	260 $\pm$ 1
4	500	227	229	227	232	229 $\pm$ 1
	1000	250	254	246	253	251 $\pm$ 2
	1500	255	259	253	258	256 $\pm$ 1

Data show average QT interval for four animals over four separate days, at three different RR interval values.

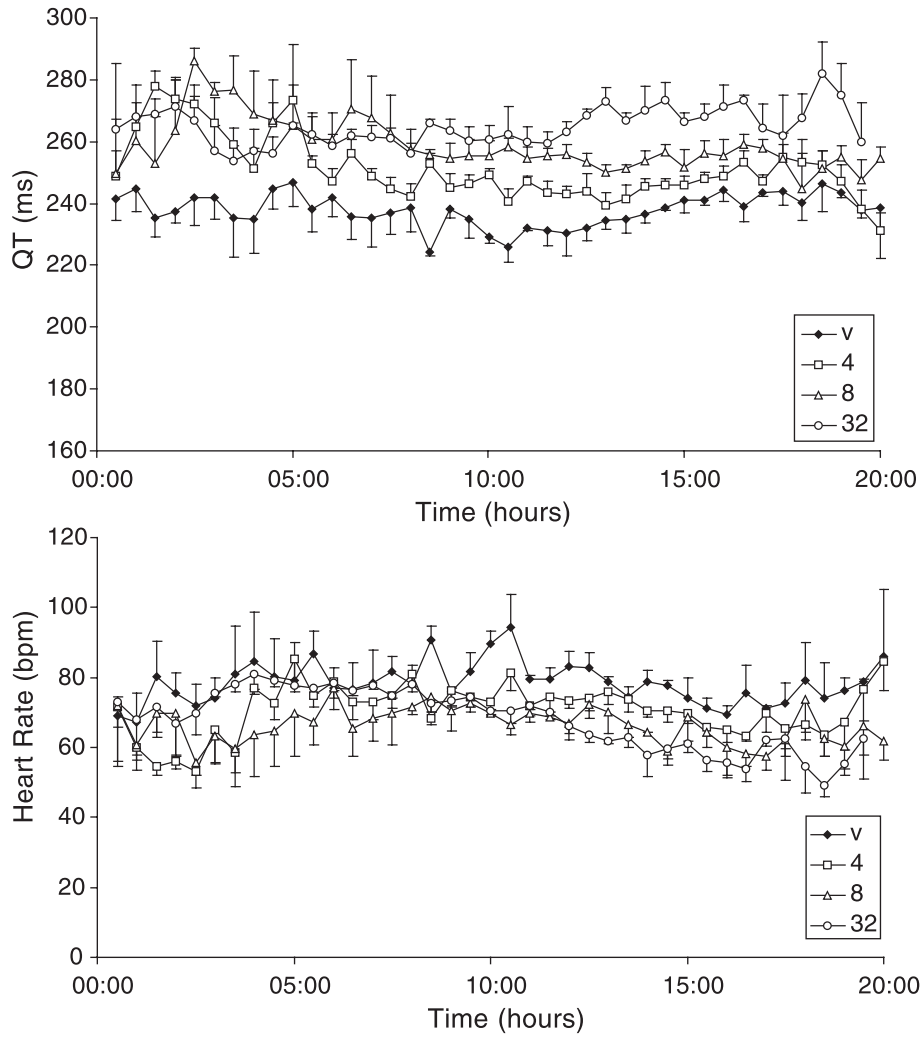


Fig. 2. Effect of vehicle and sotalol 4, 8, and 32 mg/kg on QT interval and heart rate. QT is apparently lengthened in the treatment groups, but it is not known how much is due to the decrease in heart rate. Data shown are 30-min means  $\pm$  S.E.M,  $n = 3$  (8 and 32 mg/kg) or 4.

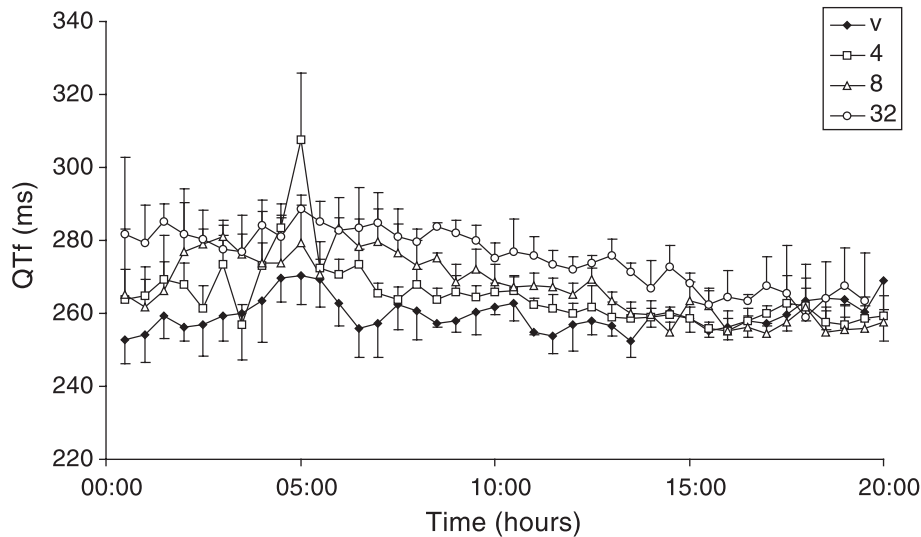


Fig. 3. Transformation of the QT data by Fridericia's cube root correction removes some of the heart rate dependency, but also decreases the apparent QT prolongation. Data shown are 30-min means  $\pm$  S.E.M,  $n = 3$  (8 and 32 mg/kg) or 4.

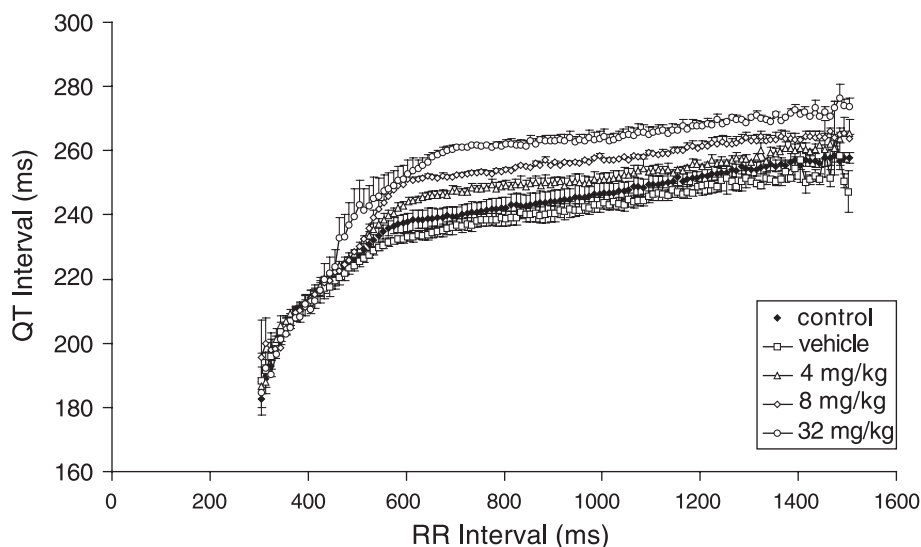


Fig. 4. QT/RR plot of sotalol data shows a dose-dependent trend towards increasing QT at RR intervals greater than 500 ms, but a convergence at RR intervals below this value. ANOVA of these data with Dunnett's correction shows that all sotalol groups (4, 8, and 32 mg/kg) are significantly different to control ( $P < .001$ ). For vehicle compared to control,  $P > .05$ ,  $n = 3$  (8 and 32 mg/kg) or 4.

derived measurement and the average of the hand measurement was 11% for the QT interval and 0.8% for the RR interval.

### 3.2. Control data

The control beat-to-beat QT/RR relationship is plotted in Fig. 1. This indicates that spontaneous changes in RR interval in the telemetered dogs occur over a wide range, and that this relationship is not linear, especially at the higher heart rates. To illustrate the correction errors, the same data are shown transformed by Bazett's and Fridericia's formulae. These corrections indicate a strong deviation

from the line of ideal correction, positively at rates greater than 60 bpm, and negatively at rates below 60 bpm. The uncorrected QT/RR plot exhibits two distinct regions separated by a marked inflexion at an RR interval of approximately 500 ms. The most linear region runs from approximately 580 to 1500 ms, where QT only rises by 20 ms (237–257 ms). Between 310 and 580 ms is an area of much steeper rise, where QT rises by 48 ms (189–237 ms).

To assess the day-to-day variability within a particular dog, QT was assessed at RR intervals of 500, 1000, and 1500 ms, chosen to reflect the point of inflexion, the standard RR interval to which QTf and QTb correct and a very long QT interval (Table 1). These data indicate close

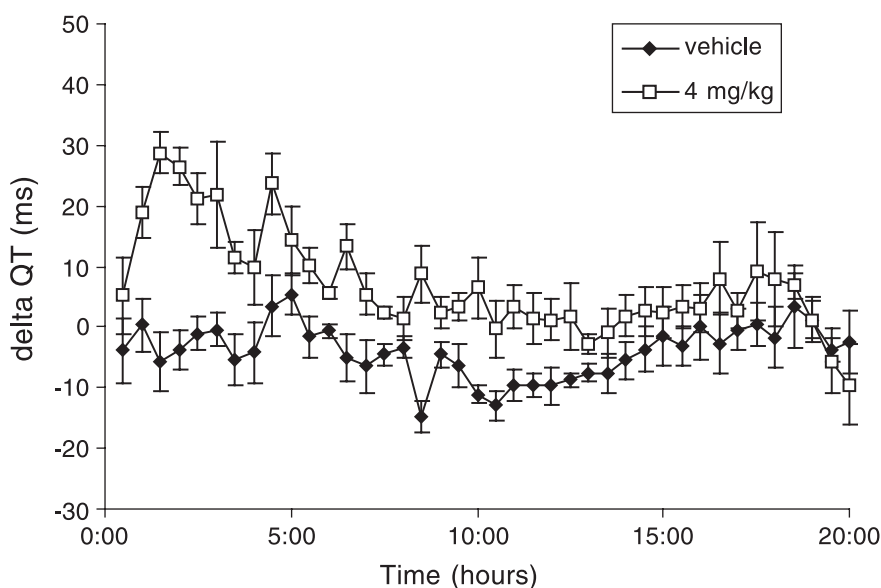


Fig. 5. Sotalol-induced changes in QT interval (mean  $\pm$  S.E.M.), with actual QT (4 mg/kg sotalol) compared to estimated QT using RR interval. ANOVA with Dunnett's correction shows a significant difference between vehicle and 4 mg/kg sotalol ( $P < .001$ ),  $n = 4$ .

agreement with a maximum day-to-day QT difference of 14 ms for a particular dog; the maximum dog-to-dog difference was also 14 ms.

### 3.3. Sotalol data

Following administration of sotalol, the time-averaged data indicate a prolongation of the uncorrected QT interval, which was also associated with a decrease in heart rate (Fig. 2). However, it is difficult to say with certainty how much of the apparent increase in QT interval could be ascribed to the decrease in heart rate. For comparison, Fig. 3 shows the same data corrected using Fridericia's formula. Here it is apparent that heart rate correction has reduced the magnitude of the sotalol-induced prolongation compared to the uncorrected QT.

If the treatment data are plotted in the binned form (Fig. 4), a dose-dependent effect of sotalol can be seen. In addition, transforming the data using this technique reveals a rate-dependent effect of sotalol on the QT interval, with apparently no effect of drug at RR intervals below 500 ms and divergence of the control and vehicle curves at longer RR intervals.

To unravel the time-course of drug effect, the control QT/RR relationship was used as a "calibration curve." Heart rate data from the treatment groups were converted to RR intervals and the expected QT read off from the control QT/RR relationship. This value was then subtracted from the post-treatment QT value. These data were plotted for the low dose of sotalol (Fig. 5). This dose produced the least sustained effect on QT using conventional analysis methods (Figs. 2 and 3). However, Fig. 5 indicates a greater maximal change in QT and more separation between groups than Fridericia's data (Fig. 3) might have suggested.

## 4. Discussion

QT correction methods have long been a topic of much debate. Realisation that classical methods such as Fridericia's and Bazett's fail to adequately correct the QT interval for changes in heart rate have led to the application of a plethora of linear or nonlinear fitting equations, which force a mathematical function to the QT/RR relationship with varying degrees of success. However, many of these techniques exhibit species dependence and the fitting parameters cannot easily be interpreted in terms of the electrophysiological events associated with ventricular repolarisation.

The data binning technique described here, using beat-to-beat changes in RR and QT intervals, unveiled a curvilinear relationship that is often hidden within the time-averaged data normally used. Furthermore, the QT/RR relationship exhibits "reverse rate dependence," a characteristic which has been well described in multicellular *in vitro* preparations such as the Purkinje fibre assay (Puisieux, Adamantidis, Dumotier, & Dupuis, 1996) or papillary muscles (Baskin &

Lynch, 1994), but is often averaged out or ignored in preclinical ECG evaluation. Application of this technique enables a relatively simple physiological description of the relationship between RR interval and ventricular repolarisation at the whole organ level, but perhaps more importantly it adequately describes the rate dependence of drug-induced changes in repolarisation without recourse to mathematical modelling. Therefore, we have demonstrated, using only a small number of animals, the ability to accurately detect drug-induced changes in ventricular repolarisation following sotalol, in the presence of concomitant changes in heart rate.

The application of telemetry systems to the field of safety pharmacology not only provides the ability to determine the long-term effects of drugs, but the free-ranging animal provides a more realistic environment, similar to man, in which autonomic control of the heart can vary enormously in a short space of time. This is evident from the large range of heart rates observed in this study. Although the number of data points obtained from a 20-h recording in this study is large, with modern computerised analysis systems, it is still possible to perform beat-to-beat analysis of the ECG to determine the effects of the preceding time interval between cardiac cycles (RR interval) on the QT interval. One difficulty often encountered with computerised analysis of the ECG is the accurate determination of ECG intervals. We have performed a formalised validation of this system for use in the GLP environment to support regulatory studies, and the results indicate that the differences between computer-derived and hand measurements were small, being of similar magnitude to the differences observed between hand measurements made by two individuals. Therefore, this system was considered reliable for the beat-to-beat measurement of the QT and RR intervals.

The control heart rate data indicate a wide range of heart rates and QT intervals during a 20-h recording period. However, binning data according to RR interval provides a method for assessing QT over this large range of RR intervals and more adequately describes the rate dependence of QT in the dog than would be possible using standard rate corrections. Each animal showed a marked curvilinearity of QT/RR relationship. The curve exhibited three distinct phases, a very steep section at high heart rates, a much flatter area at low heart rates, and an inflection zone linking the two, with a moderate slope. This triphasic relationship has been noted in humans (Franz, 1994; Karjalainen et al., 1994). A QT/RR plot was obtained from healthy young men, at heart rates of 40–120 bpm. It was demonstrated that the data could be described well by the Framingham equation ( $QT_c = QT + 0.154[1 - RR]$ ) at normal heart rates but, unsurprisingly, failed at extremes of heart rate. It was shown that a linear approach, using three different segments, yielded the best fit. Regressions fitted to data from heart rates of < 60, 60–100, and > 100 bpm yielded slopes of 0.116, 0.156 (cf. Framingham, 0.154), and 0.384, respectively. This suggests

that canine and human QT/RR relationships may share a similar trend, albeit with different heart rate ranges for each of the segments. Karjalainen et al. (1994) concluded that a nomogram approach, similar to the approach used in Table 1 of this paper, may provide more accurate results than mathematical correction. Toivonen (2002) concurs with this view, but concedes that there is not yet enough background data to create accurate nomograms.

The concept of a threshold point for RR interval, above which the QT/RR relationship is linear, was explored by Schoenwald and Isaacs (1974). The threshold was investigated for mice, guinea pigs, rabbits, dogs, and humans yielding values of 108, 190, 265, 677, and 669 ms, respectively. Although the value reported in this paper is somewhat shorter, it does demonstrate that there may be a common electrophysiological theme between species, which is tailored to the normal physiological heart rate for that species.

A study by Malik, Färbon, Batchvarov, Hnatkova, and Camm (2002), using Holter data from humans, modelled individual QT/RR relationships with six different formulae. Regardless of the regression used, there was wide interindividual variation. Taking the simplest formula ( $QT_c = QT/RR^\alpha$ ), optimised for each person,  $\alpha$  varied between 0.234 and 0.486. At the one extreme,  $\alpha$  is smaller than Fridericia's (0.33), and at the other is approaching Bazett's (0.5). From this, they conclude that general formulae should only be used for approximate measurements over narrow heart rate ranges. Detailed studies should address individual QT/RR relationships. In a similar study, Molnar, Weiss, Zhang, and Rosenthal (1996) concluded that any personalised regression-based formula will provide good correction, but that a group-based correction provides no advantage over Bazett's. Contrary to this high interindividual variation in humans, the QT/RR relationship presented here is remarkably similar from day to day within a single animal, and despite the curvilinearity of the relationship, there is close agreement between animals. Therefore, the good reproducibility of this technique suggests that this is a suitable method for use with small numbers of animals. We have demonstrated that correction methods such as Fridericia's or Bazett's exhibit the worst mix of errors: overcorrection at high heart rates and undercorrection at low heart rates. Typical Class III actions show reverse rate dependency, so arguably it is possible that undercorrection at low heart rates may mask a drug-induced prolongation of ventricular repolarisation. Indeed, bradycardia associated with a long QT is considered an additional risk factor in the incidence of torsade. At higher heart rates, above about 100 bpm, we have shown that QT prolongation was much less marked; precisely the area where the overcorrection observed with Bazett's and Fridericia's correction would suggest there is an effect. Application of the data binning technique does not assume a mathematical relationship between QT and RR, and therefore the likelihood of detecting false negative or false positive effects of drugs on the QT interval due to mathematical assumption is reduced.

Administration of vehicle was devoid of an effect on the QT/RR relationship. However, sotalol, as expected, was associated with a mild decrease in average heart rate and a prolongation of the QT interval. Despite the bradycardia associated with drug treatment, beat-to-beat variability in heart rate was sufficient to observe a similar range of RR intervals to vehicle or untreated control, enabling an adequate comparison of the QT relationship obtained in the absence of treatment and following administration of vehicle or sotalol.

A striking feature of the sotalol QT/RR relationship was the apparent absence of a QT response at heart rates above 120 bpm, corresponding to RR intervals less than 500 ms. It could be argued that QT intervals at the shorter RR intervals reflect the effects prior to the onset of drug action, since  $\beta$ -adrenoceptor blockade associated with racemic sotalol tends to reduce heart rate. However, using beat-to-beat analysis, it is clear that although average heart rate is decreased following sotalol, there is sufficient variability in heart rate that a large range of RR intervals also occurs in the postdose period. It seems more likely that the apparent absence of an effect of sotalol at high heart rates may reflect the phenomenon of inverse rate dependence, frequently observed with sotalol and other Class III agents in vitro. Indeed, an interesting study by Funck-Brentano et al. (1991) studied the effects of sotalol in humans, both at rest and during exercise. They discovered a rate-related threshold for QT prolongation with sotalol. Doses of 160, 320, and 640 mg were used, all of which caused QT prolongation at RR intervals greater than 800 ms, but only the highest dose produced significant prolongation at an RR interval of 600 ms. This has important consequences for the interpretation of ECG data in conscious animals that exhibit a high heart rate either due to a drug effect or to poor acclimatisation to the laboratory; data from these animals could erroneously suggest the absence of a QT effect.

A criticism of this type of analysis is that sorting the QT data by RR interval removes the time-course of a drug's effect. However, we have demonstrated that the QT/RR relationship in the absence of drug can be used as a calibration curve to determine the expected QT value for an observed heart rate. Subtraction of the expected QT from the observed QT enabled us to remove the effect of rate on the QT interval and unravel the time-course of the rate-independent QT effect of sotalol (Fig. 5). It is interesting to note that the maintained effect of the low dose of sotalol up to 18 h postdose was attenuated when Fridericia's correction was applied (Fig. 2).

Other correction methods can yield less extensive overcorrection, but again are only effective within a relatively narrow heart rate window. Van de Water et al. (1989) describe a popular QT-correcting formula, where  $QT_c = QT - 0.087(RR - 1)$ . Use of this formula with our data yielded virtually identical  $QT_c$  values to Fridericia's when applied to RR intervals between 700 and 1300 ms. However, outside this range, Van de Water's correction still

showed a tendency to overcorrection at high heart rates and undercorrection at low heart rates, although the errors at these extremes were smaller than those associated with Fridericia's. Below RR intervals of approximately 400 ms, Van de Water's correction does rapidly curve back down to the line of ideal correction.

More complicated exponential equations have been described by various groups. Sarma et al. (1984) collected data from both exercising and paced human subjects, at rates between 50 and 180 bpm. The data showed clear curvilinearity, the best fit being described by the formula  $QT_c = A1 - B1 \times \text{Exp}(-k1 \times RR)$ . Similar findings were reported by Matsunaga et al. (1998), using beagle dogs, where exponential formulae provided a better fit than linear formulae. As mentioned previously, Raunig et al. (2001) expanded and improved on this by applying the Sarma equation to beat-to-beat canine data, both predose and after exposure to QT-prolonging compounds. In common with the data reported here, beat-to-beat analysis provided a wide range of RR intervals, with all-important overlap between pre- and postdose data. The application of curve fitting allowed for statistical comparison of the entire QT/RR relationship pre- and postdose. It was also shown that outlier analysis could, in this model, provide a more sensitive detection of QT prolongation than mere comparison of curve fit parameters.

Spence, Soper, Hoe, and Coleman (1998) used analysis of covariance in a large (99 males/99 females) beagle population, reporting that predose QT was best estimated by  $\log(QT) = \alpha + \beta \log(HR)$ . They concluded that, although accurate with a large predose cohort, Van de Water's correction would be preferable for small samples because bias associated with a fixed formula is likely to be less than the variance obtained by estimating  $\beta$  from a small sample.

A final cautionary note on potential pitfalls with QT correction is illustrated by Aytemir et al. (1999) and Benetar and Decraene (2001). The former study, involving human subjects on exercise bikes, demonstrated an exercise-induced lengthening of QT interval when corrected by Bazett's, Fridericia's, and a nomogram. Correction by the Framingham equation suggested that exercise-induced shortening of the QT interval was occurring. Similarly, in the latter study, peak effort in children's exercise testing was associated with QT prolongation when corrected by Bazett's, but with a shortening when corrected with either Fridericia's or the Framingham equation.

In conclusion, although the small number of animals used in this study limits the statistical treatment of these data, the study was designed to be realistic and typical of those performed in many safety pharmacology laboratories. These studies have to balance the need to assess the potential liability for a drug to cause adverse QT effects at high dose, with the ethical considerations towards limiting animal usage. Despite these limitations, we have clearly demonstrated that beat-to-beat analysis provides a simple but potent tool for unravelling the rate dependency of drug-

induced QT prolongation without recourse to mathematical modelling. Furthermore, there is no reason why similar techniques could not be used in other preclinical species or for the analysis of Holter ECGs recorded from man.

## References

- Ackerman, M. J. (1998). The long QT syndrome. *Pediatrics in Review*, *19*, 232–238.
- Aytemir, K., Maarouf, N., Gallagher, M. M., Yap, Y. G., Waktare, Y. G., & Malik, M. (1999). Comparison of formulae for heart rate correction of QT interval in exercise electrocardiograms. *PACE*, *22*, 1397–1401.
- Baskin, E. P., & Lynch, J. J. (1994). Comparative effects of increased extracellular potassium and pacing frequency on the Class III activities of methanesulfonanilide  $I_{Kr}$  blockers dofetilide, D-sotalol, E-4031 and MK-499. *Journal of Cardiovascular Pharmacology*, *24*, 199–208.
- Bazett, J. (1920). An analysis of time relation of electrocardiograms. *Heart*, *7*, 353–367.
- Benatar, A., & Decraene, T. (2001). Comparison of formulae for heart rate correction of QT interval in exercise ECGs from healthy children. *Heart*, *86*, 199–202.
- Carlsson, L., Abrahamsson, C., Andersson, B., Duker, G., & Schiller-Linhardt, G. (1993). Proarrhythmic effects of the Class III agent almokalant: importance of infusion rate, QT dispersion, and early afterdepolarisations. *Cardiovascular Research*, *27*, 2186–2193.
- Franz, M. R. (1994). Time for yet another QT correction algorithm? Bazett and beyond. *JACC*, *23*, 1554–1556.
- Fridericia, L. (1920). Dir Systolendæur in Elektrokardiogram bei normalen Menschen und bei Herzkranken. *Acta Medica Scandinavica*, *53*, 469–486.
- Funck-Brentano, C., Kibleur, Y., Le Coz, F., Poirier, J., Mallet, A., & Jaillon, P. (1991). Rate dependence of sotalol-induced prolongation of ventricular repolarization during exercise in humans. *Circulation*, *83*, 536–545.
- Haverkamp, W., Breithardt, G., Camm, A. J., Janse, M. J., Rosen, M. R., Antzelevitch, C., Escande, D., Franz, M., Malik, M., Moss, A., & Shah, R. (2000). The potential for QT prolongation and pro-arrhythmia by non-anti-arrhythmic drugs: clinical and regulatory implications. Report on a Policy Conference of the European Society of Cardiology. *Cardiovascular Research*, *47*, 219–233.
- Karjalainen, J., Viitasalo, M., Manttari, M., & Manninen, V. (1994). Relation between QT intervals and heart rates from 40 to 120 beats/min in rest electrocardiograms of men and a simple method to adjust QT interval values. *Journal of the American College of Cardiology*, *23*, 1547–1553.
- Malik, M., Färholm, P., Batchvarov, V., Hnatkova, K., & Camm, A. J. (2002). Relation between QT and RR intervals is highly individual among healthy subjects: implications for heart rate correction of the QT interval. *Heart*, *87*, 220–228.
- Matsunaga, T., Mitsui, T., Harada, T., Inokuma, M., Murano, H., & Shibutani, Y. (1998). QT corrected for heart rate and relation between QT and RR intervals in beagle dogs. *Journal of Pharmacological and Toxicological Methods*, *38*, 201–209.
- Molnar, J., Weiss, J. S., & Rosenthal, J. E. (1995). The missing second: what is the correct unit for the Bazett corrected QT interval? *American Journal of Cardiology*, *75*, 537–538.
- Molnar, J., Weiss, J. S., Zhang, F., & Rosenthal, J. E. (1996). Evaluation of five QT correction formulas using a software-assisted method of continuous QT measurement from 24-hour Holter recordings. *American Journal of Cardiology*, *78*, 920–926.
- Patterson, E., Lynch, J. J., & Lucchesi, B. R. (1984). Antiarrhythmic and antifibrillatory actions of the beta-adrenergic receptor antagonist, DL-sotalol. *Journal of Pharmacology and Experimental Therapeutics*, *230*, 519–526.
- Puisieux, F. L., Adamantidis, M. M., Dumotier, B. M., & Dupuis, B. A. (1996). Cisapride induced prolongation of cardiac action potential and

- early after depolarisations in rabbit Purkinje fibres. *British Journal of Pharmacology*, 117, 1377–1379.
- Raunig, D., DePasquale, M. J., Huang, C., Winslow, R., & Fossa, A. A. (2001). Statistical analysis of QT interval as a function of changes in RR interval in the conscious dog. *Journal of Pharmacology and Toxicological Methods*, 46, 1–11.
- Sarma, J. S. M., Sarma, R. J., Bilitch, M., Katz, D., & Song, S. L. (1984). An exponential formula for heart rate dependence of QT interval during exercise and cardiac pacing in humans: reevaluation of Bazett's formula. *American Journal of Cardiology*, 54, 103–108.
- Schoenwald, R. D., & Isaacs, V. E. (1974). QT corrected for heart rate: a new approach and its application. *Archives Internationales de Pharmacodynamie*, 211, 34–48.
- Spence, S., Soper, K., Hoe, C., & Coleman, J. (1998). The heart rate-corrected QT interval of conscious beagle dogs: a formula based on analysis of covariance. *Toxicological Sciences*, 45, 247–258.
- Toivonen, L. (2002). More light on QT interval measurement. *Heart*, 87, 193–194.
- Van de Water, A., Verheyen, J., Xhonneux, R., & Reneman, R. S. (1989). An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. *Journal of Pharmacological Methods*, 22, 207–217.
- Yang, T., & Roden, D. M. (1996). Extracellular potassium modulation of drug block of IKr. Implications for torsades de pointes and reverse use-dependence. *Circulation*, 93, 407–411.