

Original article

Assessment of drug-induced QT interval prolongation in conscious rabbits

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Abstract

Introduction: Most preclinical trials are designed to identify potential torsadogenicity test only for surrogates of torsade de pointes, most commonly prolongation of the heart rate corrected QT interval (QTc). This study was conducted to determine which correction method best accounts for the effects of changes in the RR interval on the QT interval of conscious rabbits. This study was also conducted to validate the use of conscious, sling-trained rabbits to assess the QTc interval, and to evaluate the reliability and accuracy of this preparation in predicting drug-induced QTc prolongation in humans. **Methods:** ECGs were recorded via bipolar transthoracic ECG leads in 7 conscious rabbits previously trained to rest quietly in slings. The heart rate was slowed with 2.0 mg/kg zatebradine to assess the effects of heart rate on the QT interval. The same ECG and sling preparation was used to evaluate the effects in of three drugs known to be torsadogenic in humans (cisapride, dofetilide and haloperidol), two drugs known to be non-torsadogenic in humans (propranolol and enalaprilat) and a control article (vehicle). All of the test articles were administered intravenously to 4 rabbits, and both RR and QT intervals were measured and the corrected QT values were calculated by an investigator blinded to the test article, utilizing our own algorithm ($QTc = QT / (RR)^{0.72}$) which permitted the least dependency of QTc on RR interval. **Results:** The following regression equations were obtained relating QT to RR: $QT = 2.4RR^{0.72}$, $r^2 = 0.79$, with RR intervals varying between 210 and 350 ms. QTc lengthened significantly in all conscious rabbits given intravenous cisapride, dofetilide and haloperidol ($p < 0.05$), and QTc did not change with DMSO (vehicle control), propranolol or enalaprilat. **Discussion:** Results indicate that a bipolar transthoracic ECG recorded in conscious, sling-trained rabbits may provide an easy and economical methodology useful in predicting QTc lengthening of novel pharmacological entities.

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1. Introduction

No potential pharmacological agent is studied in clinical trials without first evaluating its potential to lengthen QTc, a known sentinel for predicting torsadogenicity in humans. Anesthetics used in preclinical testing on intact animals either alter QTc, or alter the relationship between QTc and the test article to be evaluated (Hamlin, Kijawornrat, Keene, & Hamlin, 2003). Furthermore, meaningful preclinical tests of potential therapeutic articles that effect

ventricular repolarization by altering specific ion channels must be conducted on species that possess the same spectrum of ion channels known to be present in humans. The rabbit has been shown to be one such species (Kaab & Nabauer, 2001). Finally, in order to determine whether a test article lengthens ventricular repolarization directly or merely because it lengthens RR interval (i.e., slows heart rate), the method of correcting the QT interval for RR interval must produce QTc intervals that are as independent of RR interval as possible (Bednar, Harrigan, Anziano, Camm, & Ruskin, 2001). Previous authors have proposed an equation, $QTcL = QT - 0.704(RR - 250)$, to correct QT for RR interval in rabbits anesthetized with sodium pentobarbital (Batey & Coker, 2002), and these authors

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claim that an existing equation, $QT_c = QT - 0.175(RR - 300)$, developed from rabbits anesthetized with methohexitone sodium and alpha chloralose, did not produce a QT_c independent of RR interval (Carlsson, Abrahamsson, Andersson, Duker, & Schiller-Linhardt, 1993). We have demonstrated previously that drugs that prolong QTc intervals in humans also prolong QTc intervals in conscious guinea pigs (Hamlin, Kijawornrat et al., 2003), but conscious rabbit models for assessment of QT liability have not been reported.

The purposes of this study were (1) to validate a method of correcting QT for RR interval that produces, in rabbits, a QT_c nearly independent of RR interval, and (2) to use this validated method to determine whether the conscious rabbit may be a useful sentinel to predict QT_c lengthening in humans.

2. Methods

2.1. Approvals

This study was approved by the *Institutional Laboratory Animal Care and Use Committee* (ILACUC) of the Ohio State University.

2.2. Animal preparation and ECG recording

A total of 31 rabbits were used. All weighed between 2.2 and 3.2 kg. Four males and 3 females were used to assess the relationship between RR interval and QT interval and for calculation of the rate-corrected QT interval (QT_c). Twenty-four additional rabbits, distributed equally by sex, received test articles. Each of 6 test articles (cisapride, DMSO (vehicle control), dofetilide, enalaprilat, haloperidol, propranolol) was given to 4 rabbits each.

After clipping the hair from the ventral region of the thorax, rabbits were placed without chemical restraint in a ventrally recumbent position in a comfortable, padded sling. The sling is fitted with copper plates, which “sandwich” the ventro-cranial aspect of the thorax, such that a bipolar transthoracic electrocardiogram between points rV2 (right, 4th intercostal space at the costochondral juncture) and V2 (left, 5th intercostal space at the costochondral juncture) can be obtained. The electrodes are made with a central hole so that electrode paste can be applied from outside the sling directly onto the rabbit–electrode interface to minimize impedance between the electrodes and the skin without disturbing the rabbit’s position in the sling. To obtain the ECG recordings, the right and left arm electrodes of the electrocardiograph were attached to the right and left sided sling electrodes, the electrocardiograph was switched to limb lead I, and a bipolar transthoracic ECG was obtained on a Biopac MP100 Data Acquisition Unit (Biopac Systems, Inc., Santa Barbara, CA). The high pass filter was set at 0.01 Hz and the low pass filter at 1 kHz, and

signals were sampled at 2 kHz. Tracings were obtained for 15 to 60 s while the rabbits were conscious and quiet.

To examine the effect of RR interval on QT interval, rabbits were given 2.0 mg/kg of zatebradine (Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT), a funny channel (I_f) blocker that reduces heart rate without direct effects on other electrophysiological parameters (Goethals, Raes, & Van Bogaret, 1993; Hamlin, Nakayama, Nakayama & Carnes, 2003). ECG tracings were obtained at numerous RR intervals generated in response to zatebradine, and these measurements were used to generate curves of QT versus RR interval.

2.3. Data analysis

ECG intervals from beats originating from the sinoatrial node (sinus rhythms) and displayed at 100 mm/s were measured manually using on-screen cursors, taking the mean of at least 12 consecutive cardiac cycles at each time point. The QT interval was measured from the onset of Q wave to the end of T wave, including U wave if present (Batey & Coker, 2002). Because the conscious, quiet rabbit has a relatively slow heart rate, the T wave was always over prior to the onset of the next P wave, and the end of the T wave was always easily identifiable (Fig. 1). Furthermore, because all rabbits had regular sinus rhythms, no interval measurements were excluded because of arrhythmias.

The RR interval and the QT interval of the following QRS-T complex were measured at all recorded heart rates, and plots of QT versus RR intervals were constructed. Using Sigma Plot (SPSS Inc., Chicago, IL), the equation relating QT to RR interval and its regression coefficient (r^2) were calculated using a simple regression model of the generic form $QT = \beta \times RR^\alpha$. QT was corrected for the preceding RR interval using the formula, $QT_c = QT / RR^\alpha$. In addition, the QT intervals were corrected for the preceding RR intervals using common exponential (Bazett, Fridericia) (Bazett, 1920; Fridericia, 1920) and linear (Carlsson, Liverpool) equations (Batey & Coker, 2002; Carlsson et al., 1993). Plots were made of QT_c versus RR interval, and regression lines and r^2 were calculated. These plots were made to determine which method(s) for the conscious rabbits produced the least dependence of QT_c on RR.

2.4. Validation of the QT_c formula

In a separate study, groups of four conscious rabbits resting quietly in slings were each given one of the following compounds intravenously over 10 min in a marginal ear vein: 2 mg/kg cisapride, 20 μ g/kg dofetilide, 0.5 mg/kg haloperidol, 0.5 mg/kg propranolol, 0.5 mg/kg enalaprilat, or 0.1 ml/kg DMSO. All doses were selected from the literature (Bohmer, Loffelholz, Schmid, Raach, & Gouzoulis, 1986; Carlsson, Almgren, & Duker, 1990; Madwed & Winquist, 1994; Poisson, Christen, & Sannajust,

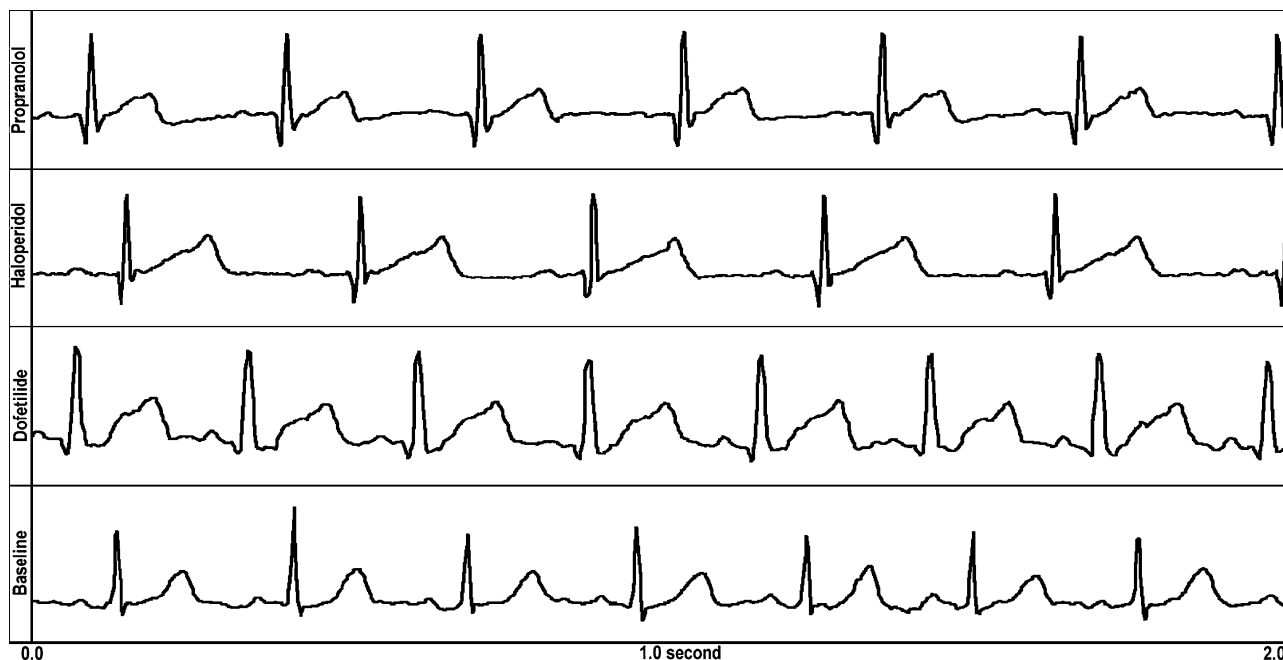


Fig. 1. Examples of bipolar, transthoracic ECG in conscious rabbits which were receiving (from top panel to bottom panel) propranolol, haloperidol, dofetilide and baseline ECG. Notice the absence of baseline artifacts, the ease of identification of the onset of QRS and the end of T.

2000; Wu, Su, & Sun, 2003). Bipolar transthoracic ECGs were obtained before dosing and every 5 min during dosing and for 20 min after each compound had been administered. QT and RR interval measurements were made on at least 12 consecutive cardiac cycles at measurement time, and the average of 12 cycles was recorded for each measurement. Significant differences between test articles and vehicle were sought by a one-way ANOVA with repeated measures design on time. When indicated by a significant F -statistic, specific means were compared by the Tukey post-hoc test. Plots were made of the RR, QT and QTc interval differences from baseline versus time.

3. Results

High quality electrocardiograms were obtained from all of the rabbits used all phases of the study (Fig. 1). A plot of QT versus RR interval for the seven conscious rabbits that received zatebradine is shown (Fig. 2). The relationships between QT and RR interval for both sexes, as well as for males and females separately, respectively are $QT = 2.4 (RR)^{0.72}$, having an r^2 of 0.79; $QT = 2.4 (RR)^{0.72}$, having an r^2 of 0.81; $QT = 2.5 (RR)^{0.72}$, having an r^2 of 0.79, respectively. This demonstrates that 79% of the variability in QT can be explained by the RR interval, and the relationship between the two is highly significant ($p < 0.001$). Graphs made for each sex independently (Fig. 3) demonstrate the absence of a significant effect of gender on the relationship between RR and QT or QTc intervals. Plots of QTc versus RR interval using each of the 5 methods

of correction for RR interval are shown, with their slopes and r^2 (Fig. 4). With our equation, plotting rate-corrected QT interval (QTc-QTest) against RR interval produces a regression line with a slope of zero, indicating that this correction removes the influence of heart rate. For this reason, all QT intervals in the rests of the study were corrected according to this QTest formula. All other potential correction equations demonstrated at least some dependence of QTc on RR interval, with positive or negative slopes and an r^2 , although relatively small, different from zero.

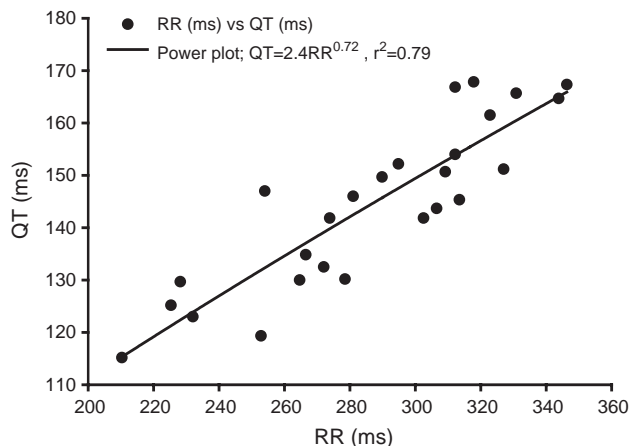


Fig. 2. Plot of QT (ms) duration versus the preceding RR (ms) interval for conscious rabbits (4 males and 3 females) receiving zatebradine. The line of regression and its equation and r^2 are shown. Notice that the RR intervals varied from approximately 210 ms (a heart rate of 286 beat per minute) to 346 ms (a heart rate of 173 beat per minute). Each data point is an average of 12 consecutive cardiac cycles from a different rabbit.

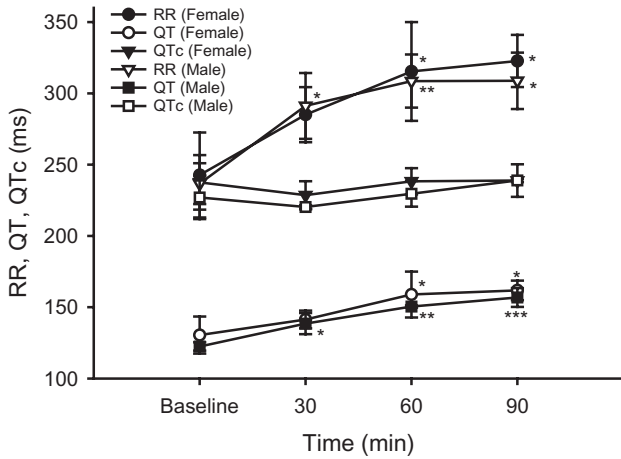


Fig. 3. Plots of RR, QT and QTc duration (ms) versus time (min) for rabbits that received zatebradine. Notice that zatebradine significantly lengthened RR and QT interval compared to baseline but there is no difference between male and female. Each data point is the average of 12 consecutive cardiac cycles. An asterisk indicates a difference between baseline and a post-dosing measurement. One asterisk (*) indicates $p < 0.05$, two asterisks (**) indicate $p < 0.01$, and three asterisks (***) indicate $p < 0.001$.

Correction factors varied in independence as follows: $QTest < Bazett < Carlsson \approx Fridericia < Liverpool$, having r^2 of, respectively, 0.00, 0.26, 0.50, 0.52, 0.75.

Plots of RR (Fig. 5), QT (Fig. 6) and QTc (Fig. 7) versus time (min) after injection are shown for groups of 4 rabbits each exposed to the test articles known to, and known not to lengthen QTc. All test articles except enalaprilat either lengthened (cisapride, dofetilide, haloperidol, and propranolol) or tended to lengthen (DMSO) RR interval when compared to RR intervals obtained at

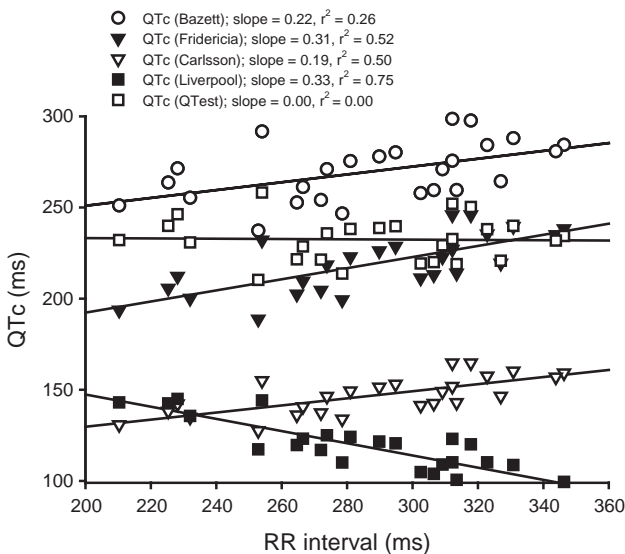


Fig. 4. Plots of QTc, their slopes, and r^2 , using many equations for removing influence of heart rate, versus RR (ms) interval for all conscious rabbits. Notice that the slope for the QTest equation is zero with an r^2 of 0.00. Each data point is an average of 12 consecutive cardiac cycles from a different conscious rabbit.

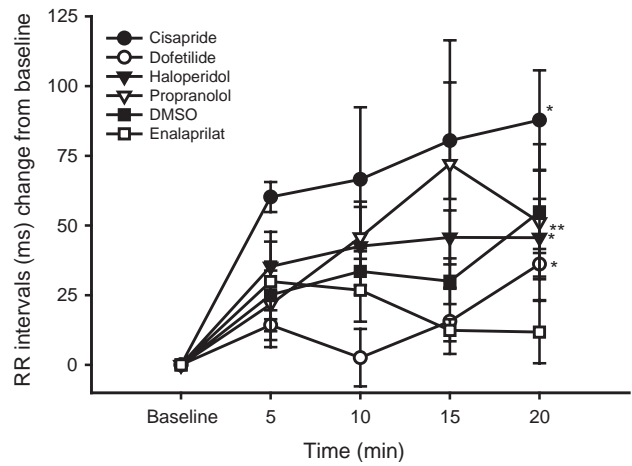


Fig. 5. Plots of mean and SEM of values of changes between baseline and values at each time after dosing of 3 test articles known to lengthen QTc and 2 negative controls known not to lengthen QTc, and for the DMSO vehicle. Each data point is the average of 12 consecutive cardiac cycles. Notice that all test articles other than enalaprilat and DMSO lengthened RR interval. Each mean is the mean of four rabbits receiving each test article. Doses of test articles were in mg/kg except indicated: cisapride (2), dofetilide (20 $\mu\text{g}/\text{kg}$), haloperidol (0.5), propranolol (0.5), Enalaprilat (0.5) and DMSO (0.1 ml/kg). An asterisk (*) indicates when a difference changed with statistical significance from baseline. One asterisk (*) indicates $p < 0.05$ and two asterisks (**) indicate $p < 0.01$.

baseline. Enalaprilat, propranolol, and DMSO caused no QTc prolongation. QTc prolonged significantly in response to injections of cisapride, haloperidol and dofetilide. Most of the QTc prolongation had already occurred within 5 min

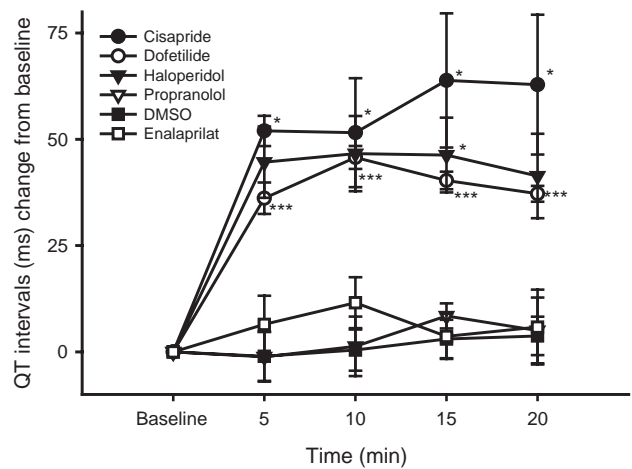


Fig. 6. Plots of Change in QT (ms) between baseline and times post-dosing for 3 test articles known to lengthen QT and 2 negative controls known not to lengthen QT, and for the DMSO vehicle. Each data point is the average of 12 consecutive cardiac cycles. Notice the lengthening of QT for the three test article but not for the negative controls and vehicle. Each mean is the mean of four rabbits receiving each test article. Doses of test articles were in mg/kg except indicated: cisapride (2), dofetilide (20 $\mu\text{g}/\text{kg}$), haloperidol (0.5), propranolol (0.5), Enalaprilat (0.5) and DMSO (0.1 ml/kg). An asterisk (*) indicates when a difference changed with statistical significance from baseline. One asterisk (*) indicates $p < 0.05$, and three asterisks (***) indicate $p < 0.001$.

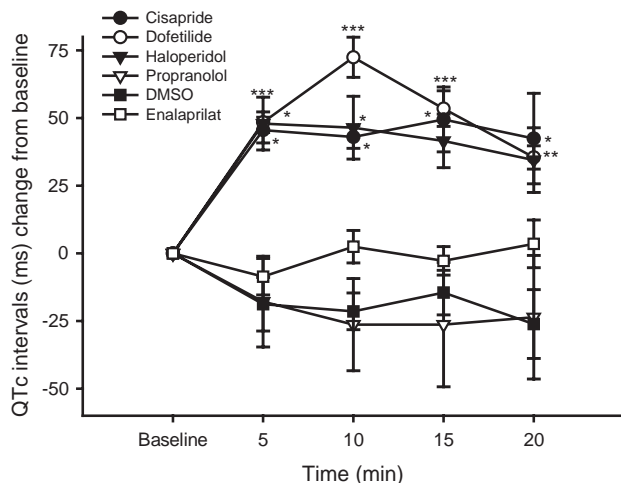


Fig. 7. Plots of difference between baseline and values at each time post-dosing for 3 test articles known to lengthen QTc and 2 negative controls known not to lengthen QTc, and for the DMSO vehicle. Each data point is the average of 12 consecutive cardiac cycles. Notice the lengthening of QTc for the three test article but not for the negative controls and vehicle. Each mean is the mean of four rabbits receiving each test article. Doses of test articles were in mg/kg except indicated: cisapride (2), dofetilide (20 μ g/kg), haloperidol (0.5), propranolol (0.5), Enalaprilat (0.5) and DMSO (0.1 ml/kg). An asterisk (*) indicates when a difference changed with statistical significance from baseline. One asterisk (*) indicates $p < 0.05$, two asterisks (**) indicate $p < 0.01$, and three asterisks (***) indicate $p < 0.001$.

after these test articles were injected, however QTc prolongation due to cisapride peaked at the 15-min recording.

4. Discussion

The purposes of this study, (1) to validate a method of correcting QT for RR interval that produces, in rabbits, a QTc nearly independent of RR interval, and (2) to use this validated method to determine whether the conscious rabbit may be a useful sentinel to predict QTc lengthening in humans, were achieved. It was demonstrated that QTc prolongation occurred in this model in response to all three test articles known to lengthen QTc in humans, and QTc failed to prolong in conscious rabbits in response to all three test articles known to not lengthen QTc in humans. This study demonstrates that a single bipolar, transthoracic ECG, from which RR and QT may be measured easily, can be obtained from a conscious rabbit placed in a comfortable sling, and that a relationship between QT and RR can be successfully modeled by a power plot relationship.

Heart rates in the conscious rabbits used during this study varied between 170 and almost 300 beats per minute, a wide range that we utilized to confirm the dependence of QT on the preceding RR interval ($QT = 2.4RR^{0.72}$, $r^2 = 0.79$), and to demonstrate that QTc could be independent of RR using the equation $QTc = QT / (RR)^{0.72}$. In the present study, Bazett, Carlsson, Fridericia, and Liverpool QT correction factors

failed to correct adequately for the influence of heart rate in conscious rabbits. However, failure in this experiment should not imply that the QTc formulas would be equally applicable to the data of other studies, since there may be significant individual differences in QT/RR patterns (Malik, 2002). This is in agreement with the finding of Batey and Coker (2002) who reported that the Carlsson's correction formula (Carlsson et al., 1993) for anesthetized rabbits did not correct data of rabbits in their study.

This study also demonstrated the potential utility of the conscious rabbit for detecting the liability of test articles to lengthen QTc in humans. Three test articles of different pharmacological classes (pure antiarrhythmic class III, GI motility agents, and antipsychotics) known to lengthen human QT intervals also lengthened this parameter in the rabbits; and 2 test articles, also of different pharmacological classes (antiarrhythmic class II and ACE inhibitor) as well as DMSO, known not to lengthen QT interval in humans, did not lengthen QT interval in this study. Based solely upon these 6 test articles, sensitivity and specificity would appear to be 1.0 for the detection of QT liability in humans—this does not imply that sensitivity and specificity would remain 1.0 for a greater number of test articles, nor does it imply that sensitivity and specificity achieved from conscious rabbits would necessarily differ from those obtained from rabbits anesthetized with any number of pre-anesthetic/anesthetic combinations, or in other models. Equally high sensitivities and specificities have been obtained using 50 test articles in the isolated, perfused guinea pig heart (Hamlin et al., 2004).

There are, however, several advantages of using the conscious rabbit to test for drug-induced prolongation of QTc. (1) Results are free of any possible interference from an anesthetic regimen. (2) Animals can be studied repeatedly without risk of death by anesthesia. (3) Recordings using the bipolar transthoracic electrocardiogram permit easy and accurate measurements of the beginning of the QRS complex and the end of the T wave. (4) Uniform electrode placement can be accomplished without discomfort to the rabbit. (5) The rabbit heart shares with humans all of the transmembrane ion channels specific for controlling ventricular repolarization (Kaab & Nabauer, 2001). (6) A QTc formula now exists for conscious rabbits that appear to completely correct the QT for the effects of heart rate, even at extremely short and long RR intervals.

An interesting finding in this study was that correcting the QT interval for the preceding RR interval was actually unnecessary to achieve high sensitivity and specificity. Examination of Figs. 5 and 6 shows that the additional information gained from QT correction does not change the conclusions regarding QT liability. This result is clearly limited to these 6 test articles, however, and it is clearly possible that test articles exist for which correction of the QT interval for the RR interval may be necessary.

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