QT-RR RELATIONSHIPS AND SUITABLE QT CORRECTION FORMULAS FOR HALOTHANE-ANESTHETIZED DOGS

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(Received June 20, 2006; Accepted August 4, 2006)

ABSTRACT — Several QT correction (QTc) formulas have been used for assessing the QT liability of drugs. However, they are known to under- and over-correct the QT interval and tend to be specific to species and experimental conditions. The purpose of this study was to determine a suitable formula for halothane-anesthetized dogs highly sensitive to drug-induced QT interval prolongation. Twenty dogs were anesthetized with 1.5% halothane and the relationship between the QT and RR intervals were obtained by changing the heart rate under atrial pacing conditions. The QT interval was corrected for the RR interval by applying 4 published formulas (Bazett, Fridericia, Van de Water, and Matsunaga); Fridericia’s formula (QTcF = QT/RR^{0.33}) showed the least slope and lowest R^2 value for the linear regression of QTc intervals against RR intervals, indicating that it dissociated changes in heart rate most effectively. An optimized formula (QTcX = QT/RR^{0.3879}) is defined by analysis of covariance and represents a correction algorithm superior to Fridericia’s formula. For both Fridericia’s and the optimized formula, QT-prolonging drugs (d,l-sotalol, astemizole) showed QTc interval prolongation. A non-QT-prolonging drug (d,l-propranolol) failed to prolong the QTc interval. In addition, drug-induced changes in QTcF and QTcX intervals were highly correlated with those of the QT interval paced at a cycle length of 500 msec. These findings suggest that Fridericia’s and the optimized formula, although the optimized is a little bit better, are suitable for correcting the QT interval in halothane-anesthetized dogs and help to evaluate the potential QT prolongation of drugs with high accuracy.

KEY WORDS: QT interval prolongation, QT correction formula, Halothane-anesthetized dogs, Fridericia’s formula, Analysis of covariance

INTRODUCTION

Over the past decades several classes of non-cardiac drugs have caused a rare but life-threatening arrhythmia termed Torsades de Pointes (TdP) (De Ponti et al., 2001). Although the entire mechanism of drug-induced TdP remains unclear, all drugs that induce TdP in man prolong the QT interval (Redfern et al., 2003) and the International Conference on Harmonization (ICH) S7B guideline requires an in vivo QT study for the risk assessment of drug-induced lethal arrhythmia (ICH S7B). The ICH S7A guideline also requires an in vivo study for cardiovascular safety assessment of new drugs (ICH S7A), and recommends the study be performed using conscious animals in consideration of the various effects of anesthetics on the cardiovascular system (Hammond et al., 2001). However, in conscious animals, spontaneous physiological changes in hemodynamics dependent on posture or activity make the measurement of the QT interval generally more variable than in anesthetized animals. In addition, the Japan Pharmaceutical Manufacturers Association (JPMA) QT PRODICT suggests that a QT study using isoflurane-anesthetized dogs is more accurate to drug-induced QT interval prolongation than one using conscious animals (Ando et al., 2005; Tashibu et al., 2005;...
Halothane, an anesthetic used for anesthetized studies as well as isoflurane, would help detect the potential risk of drugs to prolong the QT interval with high sensitivity because it inhibits the human ether-a-go-go-related gene (hERG) channel (Li and Correa, 2002), the alpha subunit of rapidly activating delayed rectifier K+ currents (I_Kr) mainly responsible for ventricular repolarization, and might potently reduce the repolarization reserve (Biliczki et al., 2002). Takahara et al. (2005) actually demonstrated that halothane strengthened the extent of QT interval prolongation caused by sematilide-induced I_Kr blockade in anesthetized dogs.

Since the QT interval adapts to changes in heart rate (or RR interval), several formulas have been proposed to correct the QT interval for the RR interval. Correction formulas tend to be specific to species and experimental conditions such as conscious or anesthetized. Correction formulas proposed by Bazett (Bazett, 1920), Fridericia (Fridericia, 1920), Van de Water (Van de Water et al., 1989), and Matsunaga (Matsunaga et al., 1997) have been used in canine experiments in order to evaluate the QT liability of drugs. However, there is inadequate information about which formula corrects most appropriately for RR interval in anesthetized dogs.

The present study was designed to clarify the suitability of a correction formula in halothane-anesthetized dogs highly sensitive to drug-induced QT interval prolongation. For this purpose, we established the relationship between QT and RR intervals under atrial pacing conditions and clarified the correction formula, of the four published ones and an optimized formula calculated by analysis of covariance (Spence et al., 1998). Furthermore, the accuracy of the corrections of the selected formulas was then assessed using two clinically QT-prolonging drugs, d,l-sotalol and astemizole, and one clinically non-QT-prolonging drug, d,l-propranolol. To our knowledge, this is the first report to identify a QT correction formula suitable for use with halothane-anesthetized dogs.

MATERIALS AND METHODS

Animals

Twenty male beagle dogs (10-15 months old, 10.0-12.5 kg) were used in this study. The animals were obtained from Chugai Research Institute for Medical Science, Inc. (Nagano, Japan) and handled according to the protocol approved by the Ethical Committee for Animal Welfare at Chugai Pharmaceutical Co., Ltd.

Anesthesia and animal preparations

Beagle dogs were initially anesthetized by an intravenous injection of thiopental sodium (30 mg/kg). After intubation with a cuffed endotracheal tube, each animal received 1.5% halothane vaporized with 100% oxygen from a volume-controlled ventilator (Model 613, Harvard Apparatus, MA, USA). Tidal volume and respiratory rate were set at 20 ml/kg and 15 breaths/min, respectively. The left cephalic and right femoral veins were then cannulated with polyethylene catheters for infusion of the test substances and blood sampling for measurement of plasma drug concentrations, respectively. A pressure catheter was positioned at the aorta through the right femoral artery to measure systemic blood pressure. Electrocardiogram (ECG) limb electrodes for recording were positioned in standard lead II configuration. During the surgical procedure and following experimentation, the core body temperature was controlled to maintain it at 37°C with a homeothermic system (TP-401, Gaymar Industries, Inc., NY, USA). A left thoracotomy was performed, the myocardium exposed, and a bipolar electrode was attached onto the right atrial appendage for pacing (1 msec duration, 2 times the threshold voltage) by an electronic stimulator (SEN-3201, Nihon Kohden, Tokyo, Japan) through an isolation unit (SS-102J, Nihon Kohden).

Drugs

d,l-Sotalol hydrochloride (d,l-sotalol), astemizole and d,l-propranolol hydrochloride (d,l-propranolol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Test substances were dissolved in distilled water containing 1% lactic acid (Sigma Chemical Co.).

Experimental protocols

After stabilizing cardiovascular parameters for at least 1 hr, two studies commenced. In the first study, in 20 dogs several right atrial pacings were conducted for 20 sec each to fix the heart rate to establish a relationship between QT and RR intervals. The first pacing rate was set to about 10 beats/min above each resting heart rate (70-130 beats/min) and the rate was increased every 10 beats/min up to a maximum of 160 beats/min. Data samples were accepted only when no irregular events occurred during sampling and the end of the T wave returned to baseline without overlapping the subsequent paced P wave.
In the second study, 18 dogs were assigned to four groups for administration of drugs: 1% lactate (vehicle control, n = 4), d,l-sotalol (n = 4), astemizole (n = 5), and d,l-propranolol (n = 5). Two of 20 dogs were disallowed because each had a resting heart rate of over 120 beats/min, the constant pacing rate used in the study. Each test compound was intravenously infused over 10 min (infusion volume: 2 ml/kg) using an infusion pump (STC-521, Terumo, Tokyo, Japan) with 20-min intervals between each dose infusion. Five consecutive doses were given cumulatively during each experiment. Vehicle control was administered 5 times into control animals. The cumulative intravenous doses used were 0, 0.15, 0.5, 1.5, and 5 mg/kg for d,l-sotalol; and 0, 0.1, 0.3, 1, and 3 mg/kg for astemizole and d,l-propranolol. Effects of each dose on the cardiovascular system were recorded at 0, 5, 10, 20, and 30 min after the start of infusion. After sampling the QT and RR interval data at sinus rhythm, atrial pacing was conducted at the rate of 120 beats/min (cycle length: 500 msec) for 20 sec to collect the QT interval data on the constant heart rate (QT_{CL500}). Venous blood was drawn at the same sampling points in all animals, and the samples obtained from dogs treated with d,l-sotalol or astemizole were analyzed to measure the plasma drug concentrations. The blood samples were centrifuged for 10 min at 4°C. The supernatant plasma was removed and stored at −80°C until analysis of the drug concentrations. Sensitive and specific determinations of the concentrations of d,l-sotalol and astemizole were performed using high-performance liquid chromatographic methods (Laer et al., 2001; Yamamoto et al., 2001).

Data acquisition and analysis

Blood pressure and ECG were amplified using an amplifier system (RMP-6000, Nihon Kohden), and the heart rate was deduced from the blood pressure. Blood pressure and heart rate were continuously recorded on a rectilinear recorder (WR3101, GRAPHTEC, Tokyo, Japan) at a paper speed of 10 mm/min. ECG signals were collected using computer software (SP97, Softron, Tokyo, Japan) at 500 Hz and averaged the beats during 8 sec just before the start of pacing (sinus rhythm data) and the last 8 sec of each pacing sequence (pacing data). QT and RR intervals were analyzed using the same software.

The QT interval measured at sinus rhythm was corrected for the RR interval by applying Bazett’s formula (QT_{CB} = QT/RR^{0.5}), Fridericia’s formula (QT_{CF} = QT/RR^{0.33}), Van de Water’s formula (QTcV = QT/RR^{0.33}), and Yan de Water’s formula (QTcV = QT/RR^{0.5}), Fridericia’s formula (QTcF = QT/RR^{0.55}), and Matsunaga’s formula (QTcM = Log600*QT/LogRR), and an optimized formula. The optimized formula was determined by analysis of covariance in accordance with Spence et al. (1998) in order to find a more suitable formula for this experimental condition. First, the slope of the linear regression obtained by the logarithmic scale of QT and RR intervals was determined as the correction coefficient (β) (LogQT = α+β*LogRR). Then the optimized formula (QTcX = QT/RR^{β}) was defined using the determined correction coefficient (β).

Statistical analysis

In the first study, linear regression equations for the corrected QT (QTc) intervals against RR intervals were obtained by the method of least squares, and the slope and R square (R^2) of equations were calculated. When both the slope and R^2 were close to zero, the correction formula was judged to eliminate the effect of heart rate changes on QT interval.

In the second study, all values in the data represent the mean ± S.E. of 4 or 5 animals. For a few groups, data could not be obtained because of a difficulty in determining the end of the T wave due to excessive QT interval prolongation in some animals. When the number of animals with data available in a group was less than three, the data was not included. For all other data, values were statistically compared with the time-matched vehicle control values using unpaired Student’s t-test (JIS, 1965). When no homogeneity of variance was obtained by F test (JIS, 1965), the data was analyzed by Welch’s t-test (Welch, 1938). All data of the QT intervals corrected by the suitable correction formulas and the QT_{CL500} interval treated with test substances and vehicle control were calculated as percentage of change from each individual baseline. The changes in the QTc intervals were then plotted against those in the QT_{CL500} interval, and the slope and R^2 of the linear regression lines were calculated as mentioned above.

These statistics were calculated using the SAS system (Version 8.2, SAS Institute, Cary, NC, USA) and values of p<0.05 were considered significant.

RESULTS

QT-RR relationships in halothane-anesthetized dogs

The heart rates and QT intervals of 20 dogs at sinus rhythm were 95 ± 4 beats/min (656 ± 33 msec for RR interval) and 317 ± 5 msec, respectively. The rela-
relationship between QT and RR intervals under an atrial pacing condition was obtained from 20 dogs (Fig. 1). The lowest and highest individual paced heart rates were 70-130 beats/min (461-857 msec for RR interval) and 130-160 beats/min (375-461 msec for RR interval), respectively. The QT-RR relationship indicates that when the RR interval is prolonged, prolongation of the QT interval follows.

Comparative evaluation of QT correction formulas

The QT-RR data were transformed into QTc-RR plots using four published formulas and the optimized formula (Fig. 2). Concerning the published formulas, both the slope and R² of linear regression obtained by Fridericia’s formula (slope = 0.0273, R² = 0.0466) (Fig. 2B) were closer to zero than those obtained by Bazett’s (slope = -0.0913, R² = 0.3174) (Fig. 2A), Van de Water’s (slope = 0.1142, R² = 0.5438) (Fig. 2C), and Matsunaga’s formulas (slope = 0.1174, R² = 0.5619) (Fig. 2D). Fridericia’s formula showed high independence from the RR interval, but Bazett’s, Van de Water’s, and Matsunaga’s formulas showed relatively strong deviations from the flat line of the ideal correction; Bazett’s formula overestimated at shorter RR intervals, whereas both Van de Water’s and Matsunaga’s formulas underestimated at shorter RR intervals. The optimized formula (QTcX = QT/RR^0.3879) was established by a correction coefficient β (0.3879), which was determined from the linear regression analysis of a logarithmical scaled equation (LogQT = 1.4193+0.3879*LogRR) and is more similar to that of Fridericia’s formula (0.33) than that of Bazett’s formula (0.5). Regarding the correcting accuracy, the optimized formula (slope = -0.0092, R² = 0.0053) (Fig. 2E) showed values closer to zero for both slope and R² than did Fridericia’s formula and showed near independence of the RR interval.

Effects of QT-prolonging and non-QT-prolonging drugs on QTcF, QTcX, and QTCL500 intervals

Time courses of QTcF, QTcX, QTCL500 intervals of test drugs, and drug concentrations of d,l-sotalol and astemizole are shown in Fig. 3. The baseline values of the QTcF, QTcX, QTCL500 intervals were 370 ± 5, 379 ± 7, 302 ± 3 msec in the vehicle control group; 376 ± 6, 381 ± 6, 302 ± 3 msec in the d,l-sotalol group; 379 ± 7, 387 ± 7, 301 ± 5 msec in the astemizole group and 370 ± 14, 377 ± 15, 304 ± 3 msec in the d,l-propranolol group. Vehicle control showed no effects on any parameters tested. d,l-Sotalol showed no effects on QTcF, QTcX, or QTCL500 intervals at 0.15 mg/kg, but at 0.5 mg/kg significantly prolonged the QT CL500 interval by 24 msec from the baseline at maximum and showed no significant effects on QTcF and QTcX intervals (Fig. 3A). d,l-Sotalol significantly prolonged QTcF, QTcX, and QTCL500 intervals by 88, 81, and 42 msec at 1.5 mg/kg, and by 146, 140, and 58 msec at 5 mg/kg, respectively. The peak plasma concentrations administered at 0.15, 0.5, 1.5, and 5 mg/kg were 0.53 ± 0.05, 1.95 ± 0.19, 6.33 ± 0.69 and 24.0 ± 1.64 μg/ml, respectively. Astemizole showed no effects on QTcF, QTcX, or QTCL500 intervals at 0.1 mg/kg, but showed dose-dependent prolongations of these QT intervals at 0.3 and 1 mg/kg (Fig. 3B). A dosage of 3 mg/kg also prolonged these QT parameters, but the degree of prolongation in QTcF and QTcX intervals was less than at 1 mg/kg. The form of the T wave considerably changed after administration of 3 mg/kg, possibly leading to the shortening of QTc intervals at 3 mg/kg compared with at 1 mg/kg. The maximum prolongations of QTcF, QTcX, and QTCL500 intervals were 99, 95, and 31 msec at 0.3 mg/kg; and 224, 217, and 52 msec at 1 mg/kg; and 181, 174 msec, and no available data at 3 mg/kg, respectively. The peak plasma concentrations administered at 0.1, 0.3, 1, and 3 mg/kg were 0.044 ± 0.020, 0.140 ± 0.035, 0.500 ± 0.188 and 1.346 ± 0.529 μg/ml, respectively. d,l-Propranolol failed to prolong QTcF, QTcX or QTCL500 intervals at all doses tested.
Fig. 2. Corrected QT (QTc) intervals against RR intervals. The QT interval was corrected using Bazett’s (A), Fridericia’s (B), Van de Water’s (C), Matsunaga’s (D), and the optimized formula (E). Solid lines represent linear regression. The slopes and $R^2$ values are shown on the figures.
(Fig. 3C). None of the test drugs or vehicle control developed arrhythmias. In the groups given 5 mg/kg of d,l-sotalol and 1 and 3 mg/kg of astemizole, there were some animals in which it was impossible to determine the end of the T wave when paced (QT_{CL500} interval) because of excess QT interval prolongations.

Changes in the QTcF and QTcX intervals were plotted against those in the QT_{CL500} interval from the data treated with vehicle control and test drugs (Fig. 4). There were apparent correlations between the QTcF interval (R² = 0.5986) (Fig. 4A) or QTcX interval (R² = 0.5792) (Fig. 4B) and QT_{CL500} interval. These relationships appear to shift to greater prolongations in the QTcF (Slope = 1.5069) (Fig. 4A) and QTcX intervals (Slope = 1.4455) (Fig. 4B) compared with QT_{CL500} interval prolongation.

**DISCUSSION**

This study aimed to find a suitable QT correction formula for halothane-anesthetized dogs that are highly sensitive to drug-induced QT interval prolongation (Takahara et al., 2005). We clarified the relationship between QT and RR intervals under conditions of atrial pacing and applied four well-known correction formulas—Bazett’s (1920), Fridericia’s (1920), Van de Water’s (1989), and Matsunaga’s (1997)—and one optimized in accordance with the analysis of covariance (Spence et al., 1998) to determine the most suitable formula. Based on a linear regression analysis of the QTc interval against the RR interval, it was demonstrated that, among the formulas, Fridericia’s formula best corrected the effect of RR interval on QT interval. Furthermore, the optimized formula showed superior dissociation of the QTc interval from the RR interval than Fridericia’s because of the least slope and lowest R² value for the linear regression.

Fridericia’s formula—division of the QT interval by the cube root of the RR interval—was effectively used in a large-scale QT assessment trial performed by JPMA QT PRODACT using isoflurane-anesthetized dogs (Tashibu et al., 2005). Although the group did not demonstrate the appropriateness of the formula scientifically, the study did detect the effects of clinically QT-prolonging and non-QT-prolonging drugs on QTcF interval with high accuracy. The other three formulas showed some correcting errors in the present study. Bazett’s formula—division of the QT interval by the square root of the RR interval—over-corrected at higher heart rates. This phenomenon is a common problem when applying the formula not only to dogs but also to humans (Cavero et al., 2000). The formula proposed by Van de Water under-corrected at higher heart rates contrary to Bazett’s. This formula was established under pacing conditions in anesthetized dogs as in the current study, but the anesthesia was maintained by scopolamine/lofentani and the QT interval was defined as the interval between the start of the Q wave and the peak of the T wave instead of the end of the T wave (Van de Water et al., 1989). These differences might have led to less of an ability to correct in the present study. Matsunaga’s formula is a logarithmic analysis and was developed using conscious dogs (Matsunaga et al., 1997), in which bradycardia at the RR interval of more than 1000 msec is commonly observed. At such longer RR intervals, a plot of QT versus RR is almost flat, whereas the QT interval lengthened with an increase in RR interval at shorter RR intervals, suggesting that a logarithmic correction formula might be more suitable for a wide range of RR intervals. In anesthetized dogs, however, such severe bradycardia is not usually observed even when administering bradycardiac drugs (Tashibu et al., 2005). Therefore, Matsunaga’s formula might show a poor fit in the present study because of the relative short range of RR intervals.

When corrected by Fridericia’s and the optimized formula, we detected QTcF interval prolongation from d,l-sotalol and astemizole, which are known to lengthen QT intervals in man, and no QTcF interval prolongations from the vehicle or d,l-propranolol known to not lengthen QT intervals. In addition, peak plasma concentrations treated with the minimum QT-prolonging dose of d,l-sotalol and astemizole in this study, 1.95 and 0.140 μg/ml, respectively, were within the plasma concentration associated with QT interval prolongation caused by these drugs in man, approximately 1-2.5 μg/ml (Funck-Brentano, 1993) and 0.0799-0.25 μg/ml (Hoppu et al., 1991; Simons et al., 1988), respectively. From these results, it is suggested that applying Fridericia’s and the optimized formula is suitable for identifying the liability of drugs for QT interval with high accuracy and appropriate sensitivity.

To confirm the correcting accuracies of Fridericia’s and the optimized formula when applying QT-prolonging or non-QT-prolonging drugs, we calculated the correlations between the QT interval at a fixed pacing rate (QT_{CL500}), which is a robust index of QT interval not influenced by change in heart rate, and QT intervals corrected by these formulas (QTcF and QTcX). As shown in Fig. 4, good correlations were...
QT correction formula in halothane-anesthetized dogs.

Fig. 3. Time courses of QTcF (circles), QTcX (triangles), and QTCL500 (squares) intervals in the groups administered by d,l-sotalol (n = 4) (A), astemizole (n = 5) (B), and d,l-propranolol (n = 5) (C). Plasma drug concentrations of d,l-sotalol and astemizole are shown with circle symbols at each bottom figure. The results of the vehicle control group (n = 4) and the drug-administered groups are shown with open and closed symbols, respectively. Data points represent the mean ± S.E. of 4 (vehicle control, d,l-sotalol) or 5 animals (astemizole, d,l-propranolol), except the QTCL500 data at the time point of 100 min in the astemizole group (n = 4). In addition, the QTCL500 data at the time points of 140 and 150 min in the d,l-sotalol group and 110 min and later in the astemizole group are not shown because the number of animals for which data was available was less than three due to the impossibility of determining the end of the T wave. *: p<0.05, **: p<0.01; statistically significant difference from the time-matched vehicle control group.
observed in these two algorithms. These results suggest that the correcting abilities of Fridericia’s and the optimized formula are excellent even when drug-induced changes in QT intervals occur.

It is known that drug-induced QT interval prolongation becomes more obvious at lower cardiac stimulation rates (Hondeghem and Snyders, 1990) called ‘reverse frequency dependency’, because the contribution of the I_k channel, which is the main target of most QT-prolonging drugs, to the ventricular repolarization at lower rates is greater than that at higher rates. This phenomenon was also observed in the current study, since the slopes of prolongations in QTcF and QTcX intervals measured at sinus rhythm (lower rates) against QT_{CL500} interval measured at pacing condition (higher rates) were more than 1 (Fig. 4). Therefore, the current in vivo study could precisely analyze the QT-prolonging effects of drugs on the ventricular repolarization process.

There is a limitation in the present study. Halothane is known to inhibit several cardiac channels including the hERG channel (Davies et al., 2000; Stadnicka et al., 2000; Li and Correa, 2002; Shibata et al., 2004) and might potently reduce the repolarization reserve (Biliczki et al., 2002), suggesting that the QT-RR relationship under anesthesia with halothane might be different from other anesthetics. Although we do not suggest that the formulas proposed in this study be used to correct the QT interval data in dogs under other anesthetics, we do strongly consider the formulas to be suitable for QT studies using halothane-anesthetized dogs.

In conclusion, it was demonstrated that of the published formulas tested, Fridericia’s formula (QTcF = QT/RR^{0.33}) effectively dissociated the effects of heart rate change on QT interval. The optimized formula (QTcX = QT/RR^{0.3879}) furthermore, showed a correction algorithm superior to Fridericia’s, and both did detect drug-induced changes in the QT interval with high accuracy and the appropriate sensitivity. Therefore, these two formulas, although optimized is a little bit better, are suitable for correcting the QT interval in halothane-anesthetized dogs highly sensitive to drug-induced QT interval prolongation. The current study of halothane-anesthetized dogs could accurately evaluate the potential QT prolongation of drugs.

ACKNOWLEDGMENT

The authors would like to thank H. Igarashi and

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**Fig. 4.** The relationship between the values of QT_{CL500} interval changes and QTcF (A) or QTcX interval changes (B). Values show the percentage of the baseline value. Solid lines represent lines of linear regression, and the slope and R^2 values are shown on the figure.
QT correction formula in halothane-anesthetized dogs.

S. Takata in the pre-clinical research department at Chugai Pharmaceutical Co., Ltd. for measuring plasma drug concentrations.

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