The QT-Prolonging Effects of a Histamine H1 Receptor Antagonist Terfenadine Assessed in the In Vivo Canine Heart Model: Comparison of the Rate-Correcting Methods for the Ventricular Repolarization Periods

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Abstract: The QT-prolonging effects of the histamine H1 receptor antagonist terfenadine were assessed using the halothane-anesthetized canine model to evaluate four mathematical methods for rate-correction of the QT interval. Administration of a therapeutic dose of terfenadine (0.3 mg/kg, i.v., n = 4) prolonged intraventricular conduction without affecting the other cardiovascular parameters. Additional administration of supra-therapeutic dose of terfenadine (3 mg/kg, i.v.) decreased the blood pressure as well as preload and afterload of the left ventricle, increased the heart rate and cardiac output, and prolonged the monophasic action potential duration (MAP90), effective refractory period and intraventricular conduction. The QTc obtained using Fridericia’s, Van de Water’s and Matsunaga’s formulas was prolonged at 3 mg/kg while that obtained using Bazett’s formula increased from 0.3 mg/kg. Correlation between the MAP90 during the sinus rhythm corrected by the formula of Van de Water or Matsunaga (cMAP90) versus MAP90(CL400) gave higher correlation coefficients than that corrected by Bazett’s or Fridericia’s formulas. Therefore, Matsunaga’s and Van de Water’s formulas would be better mathematical methods in comparison with Bazett’s and Fridericia’s for the assessment of QT-prolonging drugs in dogs for preclinical studies.

Key words: terfenadine, monophasic action potential, QTc, Bazett, Van de Water

INTRODUCTION

Prolongation of the QT interval by a drug given to patients for a long period is currently a topic of concern for pharmaceutical companies as well as clinicians1-3. When the QT interval is prolonged by a drug that inhibits the delayed rectifying K+ currents (IKr), there is an increased risk of ventricular tachyarrhythmias, including torsades de pointes, particularly when combined with other risk factors, including hypokalemia, structural heart disease and bradyarrhythmias1-3. Since the repolarization period of the ventricular muscle is affected by the ventricular rate4, normalization of the QT interval for the heart rate has been attempted using mathematical procedures to predict a net effect of drugs. For humans, Bazett’s formula5 is frequently used to normalize the QT interval. However, since this formula has been shown to overcorrect the QT interval especially at fast
heart rate\(^6,7\), it may be inappropriately applied to QT data from experimental animals including dogs, which show considerable variations in the heart rate from 80 to 220 beats/min. Thus, other rate-correction formulas have been proposed for animals to assess it for preclinical experiments\(^8–10\). However, it is controversial regarding which correction formula should be selected for experimental animals to detect potential risks of the acquired long QT syndrome.

In this study, we analyzed four rate-correction formulas; Bazett’s, Fridericia’s, Van de Water’s and Matsunaga’s formulas, using the well-established in vivo canine model, in which we could detect the end of the repolarization phase of ventricular muscle with a 1-ms scale by measuring duration of monophasic action potentials (MAPs) together with a real-time full automatic data analysis system\(^11–15\). We administered a histamine H\(_1\) receptor antagonist, terfenadine, as a typical QT prolonging drug\(^15–17\) to the model, and analyzed the correlation between the MAP duration at a fixed pacing rate and corrected MAP duration by these formulas\(^5,8–10\) during the sinus rhythm, which will provide useful information to better estimate preclinical examinations of repolarization in dogs.

Materials and Methods

The experiments were carried out using four beagle dogs of either sex weighing approximately 10 kg. All experiments were performed in accordance with the rules and regulations of the Committee for Research at the University of Yamanashi.

Cardiohemodynamic and electrophysiological parameters

The dogs were initially anesthetized with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1.0 % halothane vaporized with 100 % oxygen was inhaled with a volume-limited ventilator (SN-480-3, Shinano, Tokyo, Japan). The tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 IU/kg) was intravenously administered. A heparinized catheter was inserted through the right femoral artery for continuous monitoring of the systemic blood pressure. A thermodilution catheter (TC-704, Nihon Kohden, Tokyo, Japan) was positioned at the right side of the heart via the right femoral vein, and the cardiac output was measured by a standard thermodilution method using a cardiac output computer (MFC-1100, Nihon Kohden). Total peripheral resistance was calculated using the basic equation: mean blood pressure/cardiac output. A pig-tail catheter was positioned at the left ventricle through the right femoral artery to measure the left ventricular pressure. The maximum upstroke velocity of the left ventricular pressure (LVdP/dt\(_{max}\)) and the left ventricular end-diastolic pressure (LVEDP) were obtained during sinus rhythm to estimate the contractility and preload of the left ventricle, respectively.

Electrophysiological parameters

The surface lead II electrocardiogram (ECG) was obtained from the limb electrodes. Corrected QT intervals were calculated using the formulas of Bazett (QTc (B)), Fridericia (QTc (F)), Van de Water (QTc (V)) and Matsunaga (QTc (M))\(^5,8–10\), whose equations are as follows; Bazett’s formula: \(\text{QTc} = \frac{\text{QT}}{\sqrt{\text{RR}/1000}}\)

Fridericia’s formula: \(\text{QTc} = \frac{\text{QT}}{\sqrt{\text{RR}/1000}}\)

Van de Water’s formula: \(\text{QTc} = \frac{\text{QT}}{\sqrt{\text{RR}/1000}}\)

Matsunaga’s formula: \(\text{QTc} = \log_{600} \)
QT/logRR, where a unit of the RR interval is given in ms. A quad-polar electrodes catheter was positioned at the non-coronary cusp of the aortic valve through the left femoral artery to obtain the His bundle electrogram. A bi-directional steerable monophasic action potential (MAP) recording/pacing combination catheter (1675P, EP Technologies Inc., Sunnyvale, CA, U.S.A.) was positioned at the endocardium of the interventricular septum in the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (300, EP Technologies Inc.). The duration of the MAP signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level, and the interval (ms) at 90 % repolarization was defined as MAP90.

The heart was electrically driven using a cardiac stimulator (SEC-3102, Nihon Kohden) with the MAP recording/pacing combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 1-2 V (about twice the threshold voltage) and of 1 ms duration. The MAP90 was measured during sinus rhythm (MAP90(sinum)) and at a pacing cycle length of 400 ms (MAP90(CL400)) and 300 ms (MAP90(CL300)). Rate-corrected MAP90(sinum) was calculated with the formulas of Bazett, Fridericia, Van de Water and Matsunaga5,8-10, where each value of the QT interval was replaced with that of MAP90(sinum). The effective refractory period (ERP) was assessed by the programmed electrical stimulation to the right ventricle. The pacing protocol consisted of five beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting in the late diastole, the coupling interval was shortened in 5- to 10-ms decrements until refractoriness occurred.

Experimental protocol

The cardiohemodynamic and electrophysiological parameters were continuously monitored using a polygraph system (RM-6000, Nihon Kohden), and analyzed using a real time full automatic data analysis system (MP/VAS 3 for Macintosh ver 1.0, Physio-Tech, Tokyo, Japan). Each measurement of the ECG, MAP, atrio-His (AH) and His-ventricular (HV) intervals was the mean of three consecutive recordings. The cardiovascular variables were assessed in the following order. The cardiac output was measured twice, and then the ECG, His bundle electrogram, systemic and left ventricular pressure and the MAP signals were recorded under the sinus rhythm. Then, the MAP signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Finally, the ERP was assessed with a programmed electrical stimulator as described above.

After the basal assessment, terfenadine in a low dose of 0.03 mg/kg was administered over 10 min, and each parameter was assessed 5, 10, 15, 20 and 30 min after the start of the infusion. Next, terfenadine in a middle dose of 0.3 mg/kg was additionally administered over 10 min and each parameter was assessed in the same manner. Finally, terfenadine in a high dose of 3 mg/kg was administered over 10 min and each parameter was assessed 5, 10, 15, 20, 30, 45 and 60 min after the start of the infusion.

Drugs

Terfenadine was obtained from Sigma (St. Louis, MO, U.S.A.) and dissolved in 1 % of lactate. The following drugs were also purchased: thiopental sodium (Tanabe Seiyaku, Osaka, Japan), halothane (Takeda Chemical Industries, Tokyo, Japan) and heparin calcium (Mitsui Pharmaceuticals, Tokyo, Japan).
Data analysis

The data are expressed as the mean ± S.E.M. Statistical significances within a parameter were evaluated by one-way, repeated-measures analysis of variance (ANOVA) followed by Contrast for mean values comparison. A $P$ value < 0.05 was considered to be statistically significant.

RESULTS

Effects on the blood pressure and heart rate

The time courses of changes in the heart rate and mean blood pressure are summarized in Fig. 1 ($n = 4$), of which pre-drug control values were 116 ± 5 beats/min and 116 ± 6 mmHg, respectively. After the low dose of 0.03 mg/kg as
well as middle dose of 0.3 mg/kg of terfenadine infusion, no significant change was detected in the heart rate or mean blood pressure. After the high dose of 3 mg/kg of terfenadine infusion, the heart rate transiently increased and mean blood pressure transiently decreased. The significant changes were detected in the heart rate for 5–10 min and mean blood pressure for 5–30 min.

**Effects on the cardiac output and TPR**

The time courses of changes in the cardiac output and TPR are summarized in Fig. 1 (n = 4), of which pre-drug control values were 2.00 ± 0.22 L/min and 59 ± 6 mmHg/L/min, respectively. After the low dose as well as the middle dose of terfenadine infusion, no significant change was detected in the cardiac output and TPR. After a high dose of terfenadine infusion, the cardiac output increased and TPR decreased. The significant changes were detected in the cardiac output for 5–10 min and TPR for 5–15 min.

**Effects on the LVdP/dtmax and LVEDP**

The time courses of changes in the LVdP/dtmax and LVEDP are summarized in Fig. 1 (n = 4), of which pre-drug control values were 2211 ± 157 mmHg/s and 13 ± 1 mmHg, respectively. After the low dose as well as the middle dose of terfenadine infusion, no significant change was detected in the LVdP/dtmax or LVEDP. After the high dose of terfenadine infusion, the LVEDP decreased transiently. The significant changes were detected in the LVEDP at 5 min. Meanwhile, no significant change was detected in the LVdP/dtmax.

**Effects on the ECG during the sinus rhythm**

Typical tracings of the effects of terfenadine on the ECG are depicted in Fig. 2, and the time courses of changes in the ECG parameters are summarized in Fig. 3 (n = 4). The pre-drug control values of the PR interval, QRS width, QT interval, QTc (B), QTc (F), QTc (V), and QTc (M) were 119 ± 9, 64 ± 5, 279 ± 5, 389 ± 9, 347 ± 7, 322 ± 5 and 286 ± 5 ms, respectively. After the low dose of terfenadine infusion, no significant change was detected in the ECG parameters. After the middle dose of terfenadine infusion, the QTc (B) increased at 10 and 30 min. After the high dose of terfenadine infusion, the QRS width, QT interval QTc (B), QTc (F), QTc (V) and QTc (M) prolonged and the significant changes were detected in the QRS width and QTc (B) for 5-60 min, QTc (F) for 15–20 min, and QT interval, QTc (M) and QTc (V) for 20–60 min. No ventricular premature beat was observed during the whole experimental period.

**Effects on the His bundle electrogram and MAP signals during the sinus rhythm**

Typical tracings of the effects of terfenadine on the His bundle electrogram and MAP signals are depicted in Fig. 2, and the time courses of changes in the AH and HV intervals and MAP90(sinus) during the sinus rhythm are summarized in Fig. 3 (n = 4). The pre-drug control values of the AH and HV intervals and MAP90(sinus) were 78 ± 6, 23 ± 1 and 243 ± 12 ms, respectively. After the low dose terfenadine infusion, no significant change was detected in these parameters. After the middle dose of terfenadine infusion, the HV interval was prolonged at 10, 20 and 30 min. After a high dose of terfenadine infusion, the HV interval and MAP90(sinus) were prolonged and the significant changes were detected in the HV intervals at 5–15 and 30–60 min and MAP90(sinus) for 20–60 min. Meanwhile, no significant change was detected in the AH interval.
Effects on the monophasic action potential and effective refractory period during the ventricular pacing

The time courses of changes in the MAP_{90(CL300)}, MAP_{90(CL400)} and ERP are summarized in Fig. 4 (n = 4), of which pre-drug control values of the MAP_{90(CL300)}, MAP_{90(CL400)} and ERP were 218 ± 7, 237 ± 10 and 201 ± 10 ms, respectively. After the low dose as well as middle dose of terfenadine infusion, no significant change was detected in these parameters. After the high dose of terfenadine infusion, the MAP_{90(CL300)}, MAP_{90(CL400)} and ERP were prolonged, and the significant changes were detected in the MAP_{90(CL300)} for 10-60 min, MAP_{90(CL400)} for 20-60 min and ERP for 5-60 min.

Correlation between the MAP_{90(CL400)} and corrected MAP_{90(sinus)} calculated with the Bazett, Fridericia, Van de Water and Matsunaga formulas

The relationships within each dog between
the MAP$_{90\text{(CL400)}}$ and corrected MAP$_{90\text{(sinus)}}$ are summarized in Fig. 5. The average of the correlation coefficients obtained from 4 dogs of the MAP$_{90\text{(CL400)}}$ and MAP$_{90\text{(sinus)}}$ corrected by the Bazett, Fridericia, Van de Water and Matsunaga formulas were 0.688 ± 0.14, 0.822 ± 0.091, 0.898 ± 0.046 and 0.900 ± 0.044, respectively.

DISCUSSION

To better apply mathematical methods for rate-correction of repolarization period for preclinical studies, we analyzed the correlation between the MAP$_{90}$ at a fixed pacing rate (MAP$_{90\text{(CL400)}}$) and corrected MAP duration by these formulas$^{5,8-10}$ during the sinus rhythm (cMAP$_{90\text{(sinus)}}$) with current data. The morphology of the T wave may change after drug administration. To solve the difficulty in assessing the drug effect on the repolarization period, we used the MAP$_{90}$, instead of ECG, which correlates well with the QT interval$^{18}$. The cardiac effects of terfenadine have been extensively examined$^{15-17}$, and the present results were essentially in accordance with these previous reports. Since the high dose of terfenadine in this study provided supra-therapeutic level, leading to prolongation of the repolarization period$^{15}$, the results would give us important information regarding a guide for selection of a rate-correcting method of the QT interval using dogs for preclinical studies.

Fig. 3. Time courses of the effects of terfenadine on the PR interval (circles), QRS width (squares) and QT interval (triangles), QTc (B) (triangles: corrected by Bazett’s formula), QTc (F) (diamonds: corrected by Fridericia’s formula), QTc (V) (circle: corrected by Van de Water’s formula), QTc (M) (triangles: corrected by Matsunaga’s formula), atrio-His interval (AH, circles), His-ventricular interval (HV, squares), and duration of the monophasic action potential at 90 % repolarization (MAP$_{90\text{(sinus)}}$) during the sinus rhythm (MAP$_{90\text{(sinus)}}$, triangles). Data are presented as the mean ± S.E.M. (n = 4). The closed symbols represent the significant differences from each pre-drug control (C) value by $P < 0.05$. 

QT Prolongation by Terfenadine
The high dose of terfenadine decreased the mean blood pressure, total peripheral vascular resistance and LVEDP with increasing cardiac output, which may reveal vasodilator actions at both arterioles and venulae. Since the in situ heart, as used in this study, is physiologically regulated by neuronal and humoral control, the positive chronotropic effects observed after the high dose would be induced by the sympathetic reflex as a result of its hypotensive action. Also, a lack of the positive inotropic effects was indicative of its direct cardio-depressing effects.

The high dose of terfenadine prolonged the ventricular repolarization phase, as previously reported. It should be noted that the extent of changes in the MAP90 by terfenadine was greater at a slower heart rate, indicating reverse use-dependent prolongation of the ventricular repolarization process, which is usually observed by I_{Ks} blockade. Terfenadine after administration of the middle and high doses also delayed intraventricular conduction, which can be affected by Na⁺ channel blockers like class I antiarrhythmic drugs. Furthermore, the ERP was also prolonged after the high dose administration, which may be associated with its inhibitory actions on the Na⁺ and/or K⁺ channels. Since terfenadine inhibits the Na⁺ and K⁺ currents in addition to Ca²⁺ channels, the in vivo hemodynamic and electrophysiological properties of this drug can be explained by its multiple ion channel-blocking properties.

The action potential duration generally decreases as the pacing cycle length decreases. Thus, to estimate the net effect of a QT prolonging drug like terfenadine, experiments should be performed at a fixed ventricular pacing rate, as we previously did for various drugs. As shown in Fig. 5, corrected values for the MAP90(CL400) (cMAP90) using Matsunaga’s and Van de Water’s formulas, in comparison with those with Bazett’s and Fridericia’s ones, are better correlated with MAP90(CL400), suggesting that Bazett’s formula, a classically used rate-correction method, may overestimate the repolarization period in the case of dogs. Recently, similar results have been reported for conscious dogs, in which the QT-RR relationship was analyzed. In those studies, however, each formula was analyzed with the assumption that the QT-RR is constant at different heart rates. This hypothesis does not correspond with

![Fig. 4. Time courses of the effects of terfenadine on the duration of the monophasic action potential at 90% repolarization (MAP90) during the electrical pacing at a cycle length of 400 ms (MAP90(CL400)) and 300 ms (MAP90(CL300)), and effective refractory period (ERP). Data are presented as mean ± S.E.M. (n = 4). The closed symbols represent the significant differences from each pre-drug control (C) value by P < 0.05.](image-url)
the observation that the action potential duration decreases as the pacing cycle length decreases\cite{27}. In contrast, since changes in the MAP_{90(\text{CL}400)} by QT-prolonging drugs may reflect a net effect on the repolarization phase, the current analysis method involving the MAP_{90(\text{CL}400)} and cMAP_{90} may be accurate for the verification of these formulas. It should be noted that significant prolongation of the QTc (M) and QTc (V) was detected for 20–60 min after high dose administration, which was equal to that assessed using the value of the MAP_{90(\text{CL}400)}, as shown in Fig. 3. Therefore, these results suggest that rate-correction of the QT interval by a mathematical method such as Matsunaga’s or Van de Water’s formulas can be used as an alternative procedure for preclinical studies when use of a MAP catheter is limited.

In conclusion, in the halothane-anesthetized dogs, terfenadine prolonged the ventricular repolarization processes together with hemodynamic changes associated with its multiple ion channel–blocking properties\cite{25,26}. Matsunaga’s and Van de Water’s formulas for rate-correction of the QT interval would be better mathematical methods than Bazett’s and Fridericia’s for the assessment of the QT-prolonging drugs in dogs.

REFERENCES


