QT PRODACT: In Vivo QT Assay in the Conscious Dog for Assessing the Potential for QT Interval Prolongation by Human Pharmaceuticals

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Abstract. The goal of the present study was to examine the utility of the conscious dog model by assessing the QT-interval-prolonging potential of ten positive compounds that have been reported to induce QT interval prolongation in clinical use and seven negative compounds considered not to have such an effect. Three doses of test compounds or vehicle were administered orally to male beagle dogs (n = 4), and telemetry signals were recorded for 24 h after administration. All positive compounds (astemizole, bepridil, cisapride, E-4031, haloperidol, MK-499, pimozide, quinidine, terfenadine, and thioridazine) caused a significant increase in the corrected QT (QTc) interval, with a greater than 10% increase achieved at high doses. In contrast, administration of negative compounds (amoxicillin, captopril, ciprofloxacin, diphenhydramine, nifedipine, propranolol, and verapamil) did not produce any significant change in the QTc interval, with the exception of nifedipine that may have produced an overcorrection of the QTc interval due to increased heart rate. The estimated plasma concentrations of the positive compounds that caused a 10% increase in the QTc interval were in good agreement with the plasma/serum concentrations achieved in humans who developed prolonged QT interval or torsade de pointes (TdP). Although careful consideration should be given to the interpretation of QT data with marked heart rate change, these data suggest that an in vivo QT assay using the conscious dog is a useful model for the assessment of QT interval prolongation by human pharmaceuticals.

Keywords: conscious dog, QT interval, telemetry, ECG, safety pharmacology
Introduction

A variety of drugs can prolong the QT interval of the electrocardiogram (ECG) and lead to torsade de pointes (TdP), a polymorphic ventricular tachycardia that can progress to ventricular fibrillation and sudden death (1). Therefore, much emphasis has been placed on the assessment of the potential risk for QT interval prolongation caused by pharmaceuticals both in clinical and non-clinical tests. Indeed, the International Conference on Harmonisation (ICH) S7B guideline describes a non-clinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization (2).

According to the ICH S7B guideline, an in vivo QT assay is included as one of the most important components of the general testing strategy. Intact animal models are believed to be very informative for extrapolations to the clinical situation because they enable evaluation of metabolites and estimation of safety margins. The use of conscious unrestrained animals with a telemetry device has great advantages for eliminating the influences of anesthetic and restraint-induced stress since these factors may alter the sensitivity of the models to detect an effect on QT interval. Because of these advantages, the use of conscious unrestrained animals has been recommended in safety pharmacology studies (3). The beagle dog is a popular species for evaluating the effects on ECG; and the conscious dog model, which allows monitoring of blood pressure, heart rate (HR), and ECG, has been widely used as a primary test system for the in vivo QT assay (4). However, the utility of the in vivo QT assay in the conscious dog has not been systematically verified. In order to construct a database and support the finalization of the ICH S7B guideline, a project named “QT Interval Prolongation: Project for Database Construction (QT PRODACT)” consisting of pharmaceutical companies belonging to Japan Pharmaceutical Manufacturers Association (JPMA) and contract laboratories belonging to Japan Association of Contract Laboratories for Safety Evaluation was organized. The goal of the present study was to examine the utility of the conscious dog model by assessing the QT interval-prolonging potential of ten positive compounds that have been reported to induce QT interval prolongation with or without TdP in clinical use (1, 5, 6) and seven negative compounds considered not to have such effects.

Because this study was intended to evaluate the utility of the conscious dog model, the safety of each compound in clinical use should be assessed by integrating the non-clinical, clinical, and other relevant information.

Materials and Methods

Experiments were conducted at nine separate facilities in accordance with the following standard protocol, which was created based on our preliminary investigations and can be applicable to routine safety pharmacology studies.

Test compounds and vehicle

The study used ten positive and seven negative compounds. These compounds cover a broad range of therapeutic classes including antiarrhythmics (E-4031, MK-499, propranolol, and quinidine); antihistaminics (astemizole, diphenhydramine, and terfenadine); antimicrobial agents (amoxicillin and ciprofloxacin); antipsychotics (haloperidol, pimozide, and thioridazine); antihypertensive/antianginal agents (bepridil, captopril, nifedipine, and verapamil); and a gastroprokinetic agent (cisapride) (also see Appendices 3-1 to 3-9 in the Supplemental Materials available with this article online). A 0.5% (w/v) methylcellulose solution was used as the vehicle.

Preparation of test compound solutions

Methylcellulose (Metolose SM-400; Shin-Etsu Chemical Co., Ltd., Tokyo) was weighed and suspended in a suitable quantity of moderately warm water. Then water was further added to completely dissolve the methylcellulose to make a 0.5% (w/v) solution. The solution was stored at 4°C and used within 15 days after preparation.

An appropriate amount of each test compound was suspended in the vehicle to obtain the target concentration for the high-dose treatment. This suspension was then diluted with the vehicle to prepare the target concentrations for the middle- and low-dose treatments. Each suspension was prepared at the time of use.

Animals

In each facility, four male beagle dogs (8–21-month-old, 7–14 kg) were housed in individual stainless steel cages in an air conditioned room with a 12-h light/dark cycle. Approximately 300 g of solid diet was given daily, and drinking water was provided ad libitum. Animals were kept in the fasting state for approximately 16 h before administration of the test compound. On the day of drug administration, diet was supplied after completion of the electrocardiogram (ECG) recording and the blood collection at the time point of 4 h after drug dosing. The experimental protocol and design were approved by the Animal Care and Use Committee of each facility, and it was performed in accordance with the Guidelines for Animal Experi-
mentation of the respective institutions.

Telemetry systems

The telemetry system consisted of a transmitter (TL11M2-D70-PCT; Data Science International, Arden Hills, MN, USA), a receiver (RMC-1, Data Science International), a data acquisition system (A.R.T. Analog, Data Science International), and data analysis software (HEM ver 3.4 or 3.5; NOTOCORD Systems SAS, Croissy sur Seine, France).

Surgical implantation of the telemetry transmitter

By basic veterinary surgical procedures, a telemetry transmitter was implanted in the left lateroabdominal subcutaneous area of dogs under anesthesia with pento-barbital, ketamine/xylazine, thiopental, or isoflurane/nitrous oxide. A catheter for measuring blood pressure was passed subcutaneously to the area of the left femoral artery and was inserted into the abdominal aorta via the femoral artery. The ECG electrodes were implanted subcutaneously at the area of the right thorax and the left abdomen (lead II configuration) through a subcutaneous tunnel. Dogs were treated with antibiotics appropriately and were allowed to recover for at least 2 weeks before experimentation.

Group composition and administration

Two test compounds were assigned to each facility. Each dog received the vehicle or three doses of the test compound randomly according to a Latin square design at weekly intervals. The intervals were set based on pharmacokinetic/pharmacodynamic properties of the test compounds. Astemizole and thioridazine were administered in a dose-escalation design to avoid the confounding factors of long half-life and induction of vomiting, respectively (see Appendices 3-1 and 3-2). Dosage was selected based on the results of previous reports or our preliminary dose-finding study. The dosing suspension (5 mL/kg) was administered orally to each dog at approximately 10:00 AM. After completion of the series of experiments, the vehicle and dl-sotalol hydrochloride (sotalol), a common positive compound, were administered to the same animals to investigate the interfacility variability. The results are reported by Sasaki et al. (7) in this issue.

Recording of the telemetry signals

Telemetry signals were recorded continuously from 24 h before to 24 h after administration. During the recording, access to the animal room was restricted except for personnel to complete room cleaning between 8:30 and 9:00 AM, administration of the test compound, feeding at 4 h after administration, and blood collec-

tions.

Analysis procedures

Mean arterial blood pressure (MBP), heart rate (HR), and ECG were analyzed before and at 1, 2, 4, 8, and 24 h after dosing using data analysis software; other time points were added if necessary. The telemetry signals at the time points between before and 8 h and those at 24 h after administration were recorded in the light period. The signals were averaged over 30-s periods for measurement of MBP. ECG waveforms over 10 serial beats were used to analyze RR and PR intervals, QRS duration, and QT interval. If the ECG waveform at the time point was judged to be unanalyzable due to contamination of the electromyogram, another 10 serial beats nearest to the point were analyzed. The analysis results were reviewed and corrected manually on a computer display using commercial software (XLECG ver 2.0.4, NOTOCORD Systems SAS). The average values for 10 beats were defined as the values at each time point. The RR intervals were used to analyze HR. The QT interval was corrected for HR using Fridericia’s method (QTcF) (8), one of the most common correction formulae for the conscious dog.

Evaluation of the effects of test compounds

The effects of the test compounds were evaluated as the percentage change from the vehicle average baseline (VAB) value. The VAB value was calculated individually by averaging values obtained at all time points between before and 24 h after the vehicle treatment. Percentage changes from the VAB values were calculated individually for all time points in all treatments. The mean percentage changes from the VAB values for four animals were compared statistically between the vehicle and each dose of the test compound. In addition, differences in percentage changes between the vehicle and each dose of the test compound were calculated individually, and their group-mean-differences were also calculated. The maximum group-mean-difference toward prolongation of the QTcF interval at each dose level was subsequently used for the evaluation of the magnitude of the effect.

Determination of plasma concentrations of positive compounds

In animals treated with the positive compounds, blood was collected from the cephalic vein with a heparinized syringe for subsequent determination of the plasma concentrations of the test compound. Blood collection was conducted within 5 min after the scheduled time points after recording the ECG. Plasma was obtained and then stored at −80°C until the assay was performed.
When the negative compounds were administered, blood was collected but discarded without assaying for drug concentrations.

Plasma concentrations of the positive compounds were determined as follows. Astemizole, desmethylastemizole, and E-4031 concentrations were measured by high-performance liquid chromatography (HPLC). Bepridil was measured by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Cisapride was measured by modified methods of Xu et al. (12) and Adams et al. (13) using a LC/MS/MS. Thioridazine was measured by HPLC according to modified methods of Daniel et al. (14), Eap et al. (15), and Carrillo et al. (16). The assay of plasma pimozide concentration was not performed. For further details of the measuring methods and equipment used at each facility, see Appendices 3-1 to 3-9. Minor deviations from the standard protocol occurred. However, these deviations were not considered to have impacted the outcome of the studies or altered the interpretation of the results because significant changes in QTcF interval in response to sotalol were observed in all facilities (7).

Results

Effects of blood collection procedure on cardiovascular and ECG parameters

To evaluate the relationship between exposures to the test compounds and pharmacodynamic responses, plasma drug concentration assays were performed. This also allows for estimation of the margin between the concentration required to induce QT interval prolongation and its effective therapeutic plasma concentration. Since the blood collection procedure may affect cardiovascular and ECG parameters, preliminary studies were conducted to determine the acceptable interval for recovery from the procedure.

The MBP, HR, and QT interval were measured after blood collection from four conscious dogs from two facilities. Increases in MBP and HR and shortening of the QT interval were observed after the blood collection procedure, but these parameters returned to the baseline levels within 45 min after the procedure in all the animals, even when the collection was repeated multiple times (data not shown). Furthermore, significant prolongation of the corrected QT (QTc) interval in response to oral administration of sotalol with repeated blood collections was observed. These results suggest that the blood collection procedure does not affect the outcome of the in vivo QT assay using the conscious dogs if a collection interval of longer than 45 min is allowed.

Summary of changes in QTcF interval

Typical ECG waveforms before and 8 h after oral administration of 30 mg/kg terfenadine are shown in Fig. 1, and changes in QTcF interval in response to the positive compounds and pharmacokinetic parameters are summarized in Table 1. All positive compounds prolonged the QTcF interval by greater than 10% of the maximum group-mean-difference, mostly in a dose-
dependent manner. These compounds did not induce ventricular arrhythmia at any time point, although ECG waveforms between each time point were not examined. Some of the positive compounds displayed a good plasma concentration-response relationship. As typified by MK-499 in Fig. 2A, QTcF interval prolongation rates correlated with plasma concentrations of E-4031 \( (r = 0.553) \), MK-499 \( (r = 0.655) \), and quinidine \( (r = 0.526) \). In contrast, as typified by thioridazine in Fig. 2B, the prolongation caused by astemizole \( (r = 0.098) \), bepridil \( (r = 0.285) \), cisapride \( (r = 0.328) \), haloperidol \( (r = 0.321) \), terfenadine \( (r = 0.117) \), and thioridazine \( (r = 0.194) \) had only weak or no correlations with plasma concentrations of the drug.

Changes in QTcF interval caused by the negative compounds are summarized in Table 2. The negative compounds, with the exception of nifedipine and verapamil, had no significant effects on QTcF interval. The maximum group-mean-differences induced by negative compounds were less than 10%, with the exception of nifedipine, which produced an 11% increase. None of the negative compounds induced ventricular arrhythmia at any time point.

Effects of positive compounds on HR, MBP, and ECG, and changes in compound plasma concentrations

**Astemizole (3, 10, and 30 mg/kg; Table 1, Appendices 1-1, 2-1, and 3-1):** Statistically significant transitory differences were occasionally observed in MBP and PR interval. Although significant changes in HR including marked increases in it were occasionally observed, they were not considered to be related to the treatment since the changes were transient and no significant effect on HR was observed in anesthetized dogs (17, 18). There was no significant change in QRS duration. Significant prolongation of QT interval was occasionally observed at all dosage levels, with the exception of significant shortening at 12 h at 30 mg/kg. Administration of astemizole at doses of 3, 10, and 30 mg/kg resulted in a significant and dose-dependent prolongation of QTcF interval. These changes were observed between 1 and 8 h after administration, except for the 4-h time point, and the effects induced by 30 mg/kg lasted until 24 h after administration. The maximum group-mean-difference in QTcF interval from the time-matched vehicle values at 3, 10, and 30 mg/kg was 9%, 12%, and 15%, respectively. The \( C_{max} \) for astemizole and its metabolite, desmethylastemizole, were 13 and 15 ng/mL at 3 mg /kg, 93 and 87 ng/mL at 10 mg/kg, and 178 and 153 ng/mL at 30 mg/kg, respectively. The \( T_{max} \) was 2.7 – 3.0 h for astemizole and 2.3 – 8.0 h for desmethylastemizole, for all concentrations of the respective drug.

**Bepridil (10, 30, and 100 mg/kg; Table 1, Appendices 1-2, 2-2, and 3-2):** Bepridil had no effect on MBP, HR, or QRS duration at any dose, but did significantly prolong the PR interval at 1 and 2 h at 100 mg/kg. The QT interval was prolonged significantly between 2 and 20 h at 30 mg/kg and between 1 and 24 h at 100 mg/kg, with the exception of the 8- and 12-h time points at 30 mg/kg. Significant prolongation of QTcF interval was observed at 16 h at 30 mg/kg and at 1, 16, and 24 h at 100 mg/kg. The maximum group-mean-difference in QTcF interval at 10, 30, and 100 mg/kg was 7%, 9%, and 13%, respectively. The \( C_{max} \) was 561 ng/mL at 10 mg/kg, 919 ng/mL at 30 mg/kg, and 1475 ng/mL at 100 mg/kg; the \( T_{max} \) was 1.0 – 2.0 h at all doses.

**Cisapride (0.6, 2, and 6 mg/kg; Table 1, Appendices 1-3, 2-3, and 3-3):** HR was significantly increased by administration of cisapride at 6 mg/kg but not at the dose of 0.6 or 2 mg/kg. Cisapride had no significant effect on MBP, PR interval, QRS duration, or QT interval, with the exception of MBP at 8 h at 6 mg/kg. Significant changes in QTcF interval were observed between 2 and 8 h at 0.6 mg/kg and 1 and 24 h at 6 mg /kg. The maximum group-mean-difference in QTcF interval at 0.6 and 6 mg/kg was 11% and 18%, respectively. There was no significant change in QTcF interval at 2 mg/kg, but 10% of the group-mean-difference was observed. The \( C_{max} \) following the 0.6, 2, and 6 mg/kg doses was 143, 625, and 2348 ng/mL, respectively. The \( T_{max} \) was 1.8 – 2.8 h for all concentrations.

**E-4031 (0.3, 1, and 3 mg/kg; Table 1, Appendices 1-4, 2-4, and 3-4):** E-4031 had no effect on MBP, HR, or QRS duration at any dose, but did significantly prolong the PR and QT intervals at all dosage levels. Significant prolongation of QTcF interval was observed between 0.5 and 4 h at 0.3 mg/kg and between 0.5 and 8 h at 1 and 3 mg/kg. The maximum group-mean-difference in QTcF interval at 0.3, 1, and 3 mg/kg was 9%, 20%, and 21%, respectively. The \( C_{max} \) was 8 ng/mL at 0.3 mg/kg,
Table 1. Statistical significance, maximum group-mean-difference in QTcF interval, and pharmacokinetic parameters after oral administration of positive compounds in conscious dogs

<table>
<thead>
<tr>
<th>Dosage (mg/kg)</th>
<th>Statistical significance</th>
<th>Maximum group-mean-difference in QTcF interval toward prolongation and time points (%)</th>
<th>Pharmacokinetic parameters</th>
<th>Published plasma/serum compound concentration in humans with prolonged QT interval or TdP (ng/mL)</th>
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<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
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<tr>
<td><strong>Astemizole (Desmethylastemizole)</strong></td>
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<tr>
<td>3</td>
<td>S</td>
<td>9 ± 3</td>
<td>8</td>
<td>3.0 (2.3)</td>
</tr>
<tr>
<td>10</td>
<td>S</td>
<td>12 ± 3</td>
<td>8</td>
<td>2.7 (6.7)</td>
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<tr>
<td>30</td>
<td>S</td>
<td>15 ± 5</td>
<td>2</td>
<td>3.0 (8.0)</td>
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<td><strong>Bepridil</strong></td>
<td>NS</td>
<td>7 ± 9</td>
<td>20</td>
<td>1.0</td>
</tr>
<tr>
<td>30</td>
<td>S</td>
<td>9 ± 4</td>
<td>16</td>
<td>1.5</td>
</tr>
<tr>
<td>100</td>
<td>S</td>
<td>13 ± 6</td>
<td>1</td>
<td>2.0</td>
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<tr>
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<td></td>
<td>11 ± 7</td>
<td>8</td>
<td>1.8</td>
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<tr>
<td>0.6</td>
<td>S</td>
<td>9 ± 4</td>
<td>1</td>
<td>0.9</td>
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<tr>
<td>2</td>
<td>NS</td>
<td>10 ± 11</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>S</td>
<td>18 ± 14</td>
<td>2</td>
<td>2.8</td>
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<tr>
<td><strong>E-4031</strong></td>
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<td>9 ± 4</td>
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<td>0.9</td>
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<tr>
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<td>1</td>
<td>S</td>
<td>21 ± 7</td>
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<td>0.5</td>
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<tr>
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<td>8</td>
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<tr>
<td>1</td>
<td>S</td>
<td>20 ± 6</td>
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<tr>
<td><strong>MK-499</strong></td>
<td></td>
<td>6 ± 4</td>
<td>8</td>
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<tr>
<td>0.03</td>
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<td>14 ± 5</td>
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<tr>
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<td>S</td>
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<td>6.0</td>
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<td>S</td>
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<td>4</td>
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<td>S</td>
<td>20 ± 3</td>
<td>4</td>
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<tr>
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<tr>
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<td>1.3</td>
</tr>
<tr>
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<td>NS</td>
<td>14 ± 11</td>
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<tr>
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<td>NS</td>
<td>11 ± 8</td>
<td>6</td>
<td>13.5</td>
</tr>
<tr>
<td><strong>Thioridazine</strong></td>
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<tr>
<td>5</td>
<td>S</td>
<td>16 ± 6</td>
<td>4</td>
<td>2.3</td>
</tr>
<tr>
<td>10</td>
<td>S</td>
<td>18 ± 7</td>
<td>4</td>
<td>2.5</td>
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</table>

Differences in percentage change in QTcF interval between the vehicle and each dose of the test compounds were calculated individually, and the difference values from four dogs were averaged at each time point to obtain the group-mean-difference values. The maximum group-mean-differences are shown as means ± S.D. The C<sub>max</sub> and T<sub>max</sub> represent means of values from four dogs. S: significant difference from the vehicle value, NS: no significant difference from the vehicle value, NT: not tested, Ref: reference number.
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37 ng/mL at 1 mg/kg, and 147 ng/mL at 3 mg/kg. The T\text{max} was 0.5 – 0.9 h at all concentrations.

Although the blood collection interval of longer than 45 min had been recommended as described above, the procedure was performed every 30 min until the 1-h time point in consideration of the metabolic rate of E-4031. However, significant changes in QTcF interval were observed at the subsequent time points. Therefore, the blood collection procedure was not considered to have impacted the outcome of the study.

Haloperidol (0.3, 1, and 3 mg/kg; Table 1, Appendices 1-5, 2-5, and 3-5): Haloperidol had no effect on MBP, HR, PR interval, or QRS duration at any dose. Bell-shaped dose/concentration-effects curves were observed in QT and QTcF intervals. Haloperidol at the dose of 1 mg/kg resulted in significant prolongation of the QTcF interval between 2 and 8 h after administration. The maximum group-mean-difference in QTcF interval at this dose was 19%. There were no significant changes in QTcF interval at the doses of 0.3 and 3 mg/kg, but a 11% group-mean-difference was observed at 3 mg/kg. The reason for the bell shaped relationship in QTc interval is unclear. The C\text{max} with the 0.3, 1, and 3 mg/kg doses was 15, 44, and 166 ng/mL, respectively. The T\text{max} was 2.5 – 3.5 h at all tested doses.

MK-499 (0.03, 0.1, and 0.3 mg/kg; Table 1, Appendices 1-6, 2-6, and 3-6): No treatment-related changes were observed in MBP, HR, PR interval, or QRS duration. Significant prolongation of QT interval was observed between 2 and 8 h after administration. The maximum group-mean-difference in QTcF interval at this dose was 19%. There were no significant changes in QTcF interval at the doses of 0.3 and 3 mg/kg, but a 11% group-mean-difference was observed at 3 mg/kg. The reason for the bell shaped relationship in QTc interval is unclear. The C\text{max} with the 0.03, 0.1, and 0.3 mg/kg doses was 1.3, 5.6, and 19.7 ng/mL, respectively.

Quinidine (2, 10, and 50 mg/kg; Table 1, Appendices 1-8, 2-8, and 3-8): Quinidine did not affect MBP, HR, QRS duration, or QT interval at any dose. Administration of 50 mg/kg quinidine shortened PR interval significantly between 0.5 and 24 h, with the exception of the 8-h time point. Quinidine at the dose of 50 mg/kg caused significant prolongation of QTcF interval between 0.5 and 8 h after administration, with the exception of the 4-h time point. The maximum group-mean-difference in QTcF interval at 2, 10, and 50 mg/kg was 6%, 12%, and 14%, respectively. The C\text{max} was 1325 ng/mL at 2 mg/kg, 3175 ng/mL at 10 mg/kg, and 6375 ng/mL at 50 mg/kg. The T\text{max} was 1.0 – 2.8 h for all doses.

Terfenadine (10, 30, and 100 mg/kg; Table 1, Appendices 1-9, 2-9, and 3-9): Administration of 100 mg/kg of terfenadine resulted in significant prolongation of the PR interval between 2 and 8 h, with the exception of the 8-h time point. There were no significant changes in MBP, HR, QRS duration, or QT interval except for the QRS duration at 24 h after administration at 10 mg/kg.
and the QT interval at 24 h at 100 mg/kg. The QTcF interval was prolonged significantly between 2 and 24 h after administration of 30 mg/kg, with the exception of the 4-h time point. The maximum group-mean-difference in QTcF interval at doses of 10, 30, and 100 mg/kg was 8%, 13%, and 11%, respectively. The Cmax with 10, 30, and 100 mg/kg was 22, 71, and 181 ng/mL, respectively. The Tmax, which increased with increasing compound doses, ranged from 1.7 – 13.5 h. The plasma terfenadine levels were maintained at high levels until 24 h after administrations at 30 and 100 mg/kg.

The change in QTcF interval at 100 mg/kg was not statistically significant, and the maximum difference at this dose was lower than that produced by 30 mg/kg. This was probably due to induction of vomiting that occurred in one of the four dogs within 1 h after administration of 100 mg/kg, with little transitory changes in HR and QRS duration were also observed at 24 h. The QT interval was occasionally prolonged significantly at all dosage levels. Doses ≥ 5 mg/kg caused significant and dose-dependent prolongation of the QTcF interval that was observed continuously from 1 – 24 h after administration, with the exceptions of the 20-h time point at all doses and the 24-h time point at 5 mg/kg. The maximum group-mean-difference in QTcF interval at 5, 10, and 20 mg/kg was 12%, 16%, and 18%, respectively. The Cmax of thioridazine was 163 ng/mL at 5 mg/kg, 402 ng/mL at 10 mg/kg, and 614 ng/mL at 20 mg/kg. For all doses tested, the Tmax was 1.8 – 2.5 h.

Effects of negative compounds on HR, MBP, and ECG

**Amoxicillin** (70, 200, and 500 mg/kg; Table 2, Appendices 1-11, 2-11, and 3-2): Amoxicillin had no significant effect on any parameters measured at any dose. The maximum group-mean-difference in QTcF interval from the time-matched vehicle values at 70, 200, and 500 mg/kg was 0%, 2%, and 4%, respectively.

**Captopril** (3, 10, and 100 mg/kg; Table 2, Appendices 1-12, 2-12, and 3-5): Captopril had no significant effect on any parameters measured at any dose, with the exception of a significant increase in HR at 100 mg/kg. The maximum group-mean-difference in QTcF interval at 3, 10, and 100 mg/kg was 2%, 2%, and 7%, respectively.

**Ciprofloxacin** (5, 30, and 200 mg/kg; Table 2, Appendices 1-13, 2-13, and 3-1): Ciprofloxacin had no signifi-

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**Table 2.** Statistical significance and maximum group-mean-difference in QTcF interval after oral administration of negative compounds in conscious dogs

<table>
<thead>
<tr>
<th>Dosage (mg/kg)</th>
<th>Statistical significance</th>
<th>Maximum group-mean-difference in QTcF interval toward prolongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>NS</td>
<td>0 ± 5</td>
</tr>
<tr>
<td>200</td>
<td>NS</td>
<td>2 ± 5</td>
</tr>
<tr>
<td>500</td>
<td>NS</td>
<td>4 ± 6</td>
</tr>
<tr>
<td>Captopril</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td>2 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>NS</td>
<td>2 ± 7</td>
</tr>
<tr>
<td>100</td>
<td>NS</td>
<td>7 ± 12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>NS</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>30</td>
<td>NS</td>
<td>4 ± 14</td>
</tr>
<tr>
<td>200</td>
<td>NS</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NS</td>
<td>2 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td>2 ± 3</td>
</tr>
<tr>
<td>10</td>
<td>NS</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>S</td>
<td>5 ± 6</td>
</tr>
<tr>
<td>1</td>
<td>S</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>3</td>
<td>S</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Propranolol</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td>3 ± 6</td>
</tr>
<tr>
<td>10</td>
<td>NS</td>
<td>4 ± 7</td>
</tr>
<tr>
<td>30</td>
<td>NS</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>Verapamil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NS</td>
<td>3 ± 5</td>
</tr>
<tr>
<td>5</td>
<td>S</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>15</td>
<td>S</td>
<td>4 ± 3</td>
</tr>
</tbody>
</table>

Differences in the percentage change in the QTcF interval between the vehicle and each dose of the test compounds were calculated individually, and the difference values from four dogs were averaged at each time point to obtain the group-mean-difference values. The maximum group-mean-differences are shown as means ± S.D. S: significant difference from the vehicle value, NS: no significant difference from the vehicle value. †: significant difference toward shortening.
cant effect on any parameters measured at any dose. The maximum group-mean-difference in QTcF interval at 5, 30, and 200 mg/kg was 5%, 4%, and 10%, respectively. Since small volume vomiting was observed in three of the four dogs between 2 and 4 h after administration at 200 mg/kg and the timing coincided with that of the maximum QTcF change, the 10% increase in QTcF interval may have resulted from autonomic changes associated with vomiting.

Diphenhydramine (1, 3, and 10 mg/kg; Table 2, Appendices 1-14, 2-14, and 3-6): Diphenhydramine had no significant effect on any parameters measured at any dose. The maximum group-mean-difference in QTcF interval at 1, 3, and 10 mg/kg was 2%, 2%, and 4%, respectively.

Nifedipine (0.3, 1, and 3 mg/kg; Table 2, Appendices 1-15, 2-15, and 3-7): MBP was decreased significantly at 0.5 h at 1 mg/kg of nifedipine and between 0.5 and 2 h at 3 mg/kg of nifedipine. Significant increases in HR were observed at 0.5 h at 0.3 mg/kg and between 0.5 and 2 h at 1 and 3 mg/kg. The maximum rates of increase in HR at 1 and 3 mg/kg were greater than 130% and 170%, respectively. Although QRS duration was not affected at any dose, PR and QT intervals decreased in response to the 1 and 3 mg/kg doses. Furthermore, prolongation of QTcF interval was observed. The maximum group-mean-difference in QTcF interval at 0.3, 1, and 3 mg/kg was 5%, 6%, and 11%, respectively.

Propranolol (3, 10, and 30 mg/kg; Table 2, Appendices 1-16, 2-16, and 3-9): The MBP showed a tendency to decline with increasing propranolol dose, but this difference did not reach statistical significance. Administration of propranolol did not induce significant changes in PR, QT, or QTcF intervals. Occasional significant transitory differences in HR and QRS duration were observed. The maximum group-mean-difference in QTcF interval following the 3, 10, and 30 mg/kg doses was 3%, 4%, and 5%, respectively.

Verapamil (1, 5, and 15 mg/kg; Table 2, Appendices 1-17, 2-17, and 3-4): Verapamil had no effect on MBP or QRS duration. Significant increases in HR were observed at 0.5 and 1 h at 5 mg/kg and between 0.5 and 4 h at 15 mg/kg. The PR interval decreased in response to the 15 mg/kg dose. Significant shortening of QT interval was observed between 0.5 and 2 h at 5 and 15 mg/kg. The QTcF interval was significantly prolonged between 2 and 4 h at 15 mg/kg and significantly shortened at 2 h at 5 mg/kg. The maximum group-mean-difference in QTcF interval at 1, 5, and 15 mg/kg was 3%, 3%, and 4%, respectively.

Discussion

Compound dosage levels

Plasma/serum concentrations of the positive compounds have been reported in patients who developed prolonged QTcF interval or TdP. The Cmax, that resulted in increased QTcF intervals after administration of the positive compounds in our present studies were comparable to those previously reported for astemizole (19–22), bepridil (23), cisapride (24, 25), E-4031 (6), haloperidol (26, 27), MK-499 (5), pimozide (28), quinidine (29), terfenadine (30, 31), and thioridazine (32, 33) (Table 1). Although the plasma concentration of pimozide was not determined in this study, pimozide has been reported to prolong QTc interval in patients who were receiving 0.093 mg/kg (34), which is less than the doses given in the present study. Thus, the plasma pimozide concentration achieved in the present study was likely greater than the concentration required to induce QTc interval prolongation or TdP in humans.

All negative compounds, with the exception of nifedipine, had no effect on the QTcF interval. Although plasma concentrations of the negative compounds were not determined in this study, the plasma compound concentrations achieved in dogs by oral administration of 20 mg/kg amoxicillin (35), 2.5 mg/kg captopril (36), 3 mg/kg nifedipine (37), 40 mg/kg propranolol (38), or 5 mg/kg verapamil (39) were comparable to the Cmax achieved in humans given the therapeutic doses. The plasma concentration following administration of 200 mg/kg ciprofloxacin (40) was more than 3 times higher in dogs than the Cmax in humans given the therapeutic dose. The pharmacokinetic parameters after oral administration of diphenhydramine have not been reported in dogs, but the highest dose of the compound used in this study was approximately 4 times higher than the therapeutic dose in humans. Based on these data, the dosages of the negative compounds used in this study were sufficient to assess the potential risk of QT interval prolongation.

Effect of a change in heart rate on the QTc interval

In the present study, hypotension, tachycardia, and QTcF interval prolongation were observed in response to nifedipine treatment. There are two previous reports in which nifedipine induced TdP in patients with cardiovascular diseases (41, 42). Zhabyeyev et al. (43) reported that a high concentration of nifedipine inhibited the rapidly activating component of the delayed rectifier K+ current (Ikr) in guinea-pig ventricular myocytes with the IC50 value of 275 µmol/L. The Cmax has been reported to be only 190 ng/mL (0.55 µmol/L) when 3 mg/kg of nifedipine was orally administered to male
beagle dogs (37). These data suggest that these two cases were not attributed to the effect on $I_{k1}$, and nifedipine is unlikely to have the potential to prolong the QTc interval. Since a change in HR affects QT interval, coincident changes in HR should be taken into account when interpreting in vivo data. Although the most common approach to this problem is to use conventional correction formulae, such as Fridericia or other single-coefficient models, these models may not be sufficient to describe the QT-RR relationship in conscious dogs and may produce overestimation at short RR intervals (44, 45). Therefore, it is likely that nifedipine resulted in a false-positive indication of QTcF prolongation, since the change was accompanied by a marked increase in HR.

An increase in HR is associated with QT interval shortening. Indeed, significant decreases in QT interval accompanied with marked increases in HR were observed in response to nifedipine and verapamil treatments in the present study. On the other hand, pimozide had no significant effect on QT interval despite a marked increase in HR. These data suggest that QT interval as well as QTc interval may provide important information for understanding the effect of a test compound on ventricular repolarization even when the change in HR is large.

Criteria to determine the potential for QTc interval prolongation by a test compound

The method of analysis using the VAB value is not common in safety pharmacology studies. In this study, we aimed to assess the QT interval-prolonging potential of the test compounds on the basis of the statistical significance and percentage change in QTc interval. The percentage change data were also considered to be very useful for comparing the responses to each test compound among different animal models in the QT PRODACT. We, therefore, aimed to perform a statistical analysis of the percent change data. However, if the changes are calculated based on the actual measurement values obtained for the vehicle treatment, differences in values between the vehicle and drug treatments cannot be analyzed statistically. Because of the specific circumstances of our project, we decided to use the VAB as a reference value. Meanwhile, it was considered that the maximum group-mean-differences can be treated as the prolongation rates that were calculated based on the actual measurement values obtained for the vehicle treatment since we confirmed that the differences of values calculated by both methods were small.

As summarized in Table 1, all positive compounds produced statistically significant prolongation of QTcF interval. The maximum group-mean-differences in QTcF interval between the vehicle and the positive compounds were greater than 10%. In contrast, the changes in QTcF interval induced by the negative compounds were not significant and were less than 10%, with the exception of that induced by nifedipine as discussed above. Thus, this model was able to properly classify test compounds into “positive” and “negative” based on the statistical significance and 10% prolongation of QTcF interval in the present study. Although some investigators consider a 10% change in QT interval to be important (4), there is no international consensus on the magnitude of QT change considered to be biologically significant. In general, a 10% change is inappropriate as a criterion, since the magnitude of the change can vary with dosage levels. Plasma concentrations of the positive compounds that cause a 10% increase in the QTcF interval ($EC_{10}$) in conscious dogs were calculated based on the results of the present study and are reported by Omata et al. (46) in this issue. The estimated $EC_{10}$ values of the positive compounds in conscious dogs were in good agreement with the previously published plasma/serum concentrations in humans with prolonged QT interval or TdP (Table 1). These results show the usefulness of using the VAB as a reference value, and they also suggest that a 10% increase in QTc interval can be a useful standard to determine the potential for QT interval prolongation in the conscious dog model.

Plasma concentration-response relationship

Time points for evaluation can be based on the $T_{max}$ of a test compound. In the present study, there were positive correlations between plasma concentrations of E-4031, MK-499, and quinidine and the prolongation rates of the QTcF interval (Fig. 2). However, this relationship was not present for astemizole, bepridil, cisapride, haloperidol, terfenadine, and thioridazine. A previous report suggests that drug concentrations in ventricles are responsible for changes in QTcF interval (47). Therefore, one of the reasons for any weak correlations may be secondary to delayed distribution of the compounds from the blood to the myocardial cells. Actually, some compounds showed delayed QT interval-prolonging effects with reference to their $T_{max}$ values (Table 1). Other explanations may include accumulation of the compounds in the myocardial cells, mixed ion channel blocking properties of the compounds, interactions of their active metabolites, or individual variability. Thus, changes in QTcF interval do not always parallel the plasma concentrations, and time points for evaluation should be chosen with consideration of the factors that may affect this relationship.
Utility and limitations of an in vivo QT assay in a conscious dog model

This study was conducted at nine facilities in accordance with the standard protocol that is applicable to routinely conducted safety pharmacology studies. All positive compounds showed significant QTcF interval prolongation. Thus, the in vivo QT assay in the conscious dog was considered to be a sensitive and useful model for the assessment of QT interval prolongation by human pharmaceuticals.

This study possesses several limitations. The changes in QTc interval in response to negative compounds suggest that the maximum group-mean-difference of up to 10%, which is considered an experimental variation, will be detected in this test system. In addition, the present study did not assess the influence of gender difference, which is particularly important, as there is a higher incidence of QT interval prolongation in women compared with men (48), and there are also gender-based differences in action potential parameters in canine Purkinje fibers (49). These issues may be addressed by setting appropriate dosage levels that can provide a sufficiently high plasma concentration of a test compound compared with the C\text{max} in humans given the therapeutic doses, because all the positive compounds had positive results in our present study using conscious male dogs. However, doses should be chosen carefully to avoid induction of vomiting that may lead to a false-positive result as seen in the case of ciprofloxacin in this study. Furthermore, interpretation of data from this model may often be made more difficult by changes in HR as described above. Since the effects of the slow component of delayed rectifier K+ current (I_{Ks}) blockers were not assessed in this study, the utility of this test system in evaluating the risk of QT interval prolongation in response to I_{Ks} blockers remains unknown.

All positive compounds induced a greater than 10% increase in QTcF interval, and the estimated plasma concentrations of positive compounds that caused a 10% increase were in good agreement with the plasma/serum concentrations in humans who developed prolonged QT interval or TdP. In contrast, administration of negative compounds did not produce any significant change in the QTc interval, with the exception of nifedipine, which may have produced an overcorrection of the QTc interval due to increased heart rate. Although careful consideration should be given to the interpretation of QT data with marked HR change, these data suggest that an in vivo QT assay using the conscious dog is a useful model for the assessment of QT interval prolongation by human pharmaceuticals.

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In Vivo QT Assay in Conscious Dog


