Rate-Correction Technique for QT Interval in Long-Term Telemetry ECG Recording in Beagle Dogs

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Abstract: The establishment of a new rate-correction method for the QT interval is presented for long-term telemetry ECG recording in free-moving beagle dogs. First, in order to define the QT-RR relation to derive the correction formula, the diurnal variations of the QT and RR intervals and the influencing factors were analyzed, and the QT-RR regression coefficient \(\beta\) was estimated under various conditions: steady and non-steady states of animal, light and dark periods, and over 24 h. In the results, the diurnal rhythm of the QT interval was synchronized with the RR interval reflecting the physical and emotional states of the animal. The coefficient \(\beta\) had considerable variation during the day: \(\beta\) = 0.276 ± 0.052 (maximum to minimum: 0.495 to 0.153). Thus, it was considered that the ideal rate-correction technique for telemetry ECG requires the selection of a flexible coefficient \(\beta\) adjusted to the condition of the measurement. Therefore, rate-correction utilizing analysis of covariance estimating the coefficient \(\beta\) for each dog, was compared with previously proposed formulas which fix the rate-correction coefficient, based on the capacity to dissociate the effects of heart rate on the QT interval. This was then tested by the levels of discrimination apparent in the QT prolongation caused by a class III antiarrhythmic drug, which ranked the formulas on the levels of correction achieved as follows: covariance adjustment> Matsunaga> Van de Water> Bazett. Thus, the rate-correction method utilizing analysis of covariance is proven adequate for data from telemetry ECG recordings.

Key words: Analysis of Covariance, Dog, ECG, QTc, Telemetry

Introduction

Delayed cardiac repolarization may result in ventricular arrhythmias, including torsade de pointes, a potentially life-threatening polymorphic ventricular tachycardia. Recently, it has been observed clinically that there are a number of cardiovascular and non-cardiovascular drugs with the potential to cause delayed cardiac repolarization. In new drug development, in vivo techniques in pre-clinical studies to detect cardiac changes have become a subject of discussion within the pharmaceutical industry [7, 15, 16, 25]. The use of unrestrained and free-moving animals as opposed to restrained animals has major advantages for elimin-
ing the increases of heart rate due to restraint-induced stress, because lower heart rates enhance the likelihood of detection of QT prolongation. The traditional technique for electrocardiography (ECG) recording in dogs requires physical restraint and is accompanied by restraint-induced stress [4]. A telemetry technique based on implantation of a transmitter to record physiological data as well as ECG in freely moving animals has been developed [4]. Because of its advantages, the telemetry technique is drawing the attention of pharmaceutical companies worldwide as an alternative technique to traditional techniques. However, the correction method for QT interval using this technique has not yet been sufficiently standardized.

The QT interval is dependent on heart rate [2]. Thus, to account for heart rate-induced changes in the QT interval, various correction formulas have been derived to normalize the QT interval for changes in heart rates (QTc) [12, 16, 26]. Bazett’s formula has been the most frequently used in dogs as well as humans. However, recently, numerous researchers have questioned the adequacy of the formula, because at fast heart rates it over-corrects the QT interval and under-corrects at slow heart rates [8, 12, 15–17, 20, 26–28]. Particularly, in the dog, the adequacy has been more questionable since the dog, unlike human, normally has respiratory sinus arrhythmia and considerable variation in heart rate, depending on its physical and emotional state [27]. In addition, long-term ECG recording does not take into account restraint-induced stress of traditional ECG, and includes the measurements under various conditions: under both steady and non-steady-state cardiac cycles, during different periods of physical activity and rest under various circadian influences [18]. Thus, it can be anticipated that the frequently used and previously proposed formulas are not necessarily adequate for the correction of the QT interval in telemetry ECG. Most of the traditional formulas are derived from the population of QT-RR plots collected from a number of different subjects, but the formula applied to the long-term ECG recording has the advantage that it is possible to derive it from a number of QT-RR plots collected from one subject, which decreases the variation between individuals. As a result, it has not yet been concluded which formula best fits the data from long-term telemetry ECG recording in dogs.

The establishment of a new rate-correction method for the QT interval is presented for long-term telemetry ECG recording in freely moving beagle dogs. First, in order to define the QT-RR relation to be used to derive the formula, the diurnal variations of the QT and RR intervals and the relation between these variations and patterns of behavior of animals were analyzed. In addition, the QT-RR relation of the telemetry ECG was compared with that of the traditional ECG. Then, QT-RR regression coefficient β was estimated for various behavioral conditions: steady and non-steady state of the animal, during light and dark periods, and over 24 h. Finally, the selected rate-correction technique for QT interval was validated based on its capacity to dissociate the effect of heart rate on the QT interval and to detect the QT prolongation with a class III anti-arrhythmic drug.

Materials and Methods

QT-RR relation of the canine telemetry ECG and rate-correction technique

Animals: Ten beagle dogs (strain, Marshall; sex, 5 females, 5 males; age, 15 months) from Marshall Farms USA, Inc. (North Rose, NY 14516, USA) were housed in individual metal pens (Square: W1 × D2 m) in an air conditioned room (Temperature: 22 ± 2°C; relative humidity: 55 ± 10%; light period: 7:00 AM to 7:00 PM). Feeding was conducted between 8:00 and 9:00 a.m. Approximately 300 g of certified dog chow (PMI Certified Canine Diet #5007) was fed daily, and drinking water was provided ad libitum. Animal husbandry procedures during the study were in accordance with the Guideline for Animal Experimentation (Japanese Association for Laboratory Animal Science, 1987), and the experimental protocol and design were approved by the Institutional Animal Care and Use Committee of Banyu Pharmaceutical Co., Ltd., and performed according to the Guidelines for Animal Experimentation of Banyu.

Telemetry system and implantation surgery: The telemetry system included a transmitter, Model#TL11M2-D70-PCT. The data acquisition system consisted of a controller (Dataquest A.R.T. Analog-4), receivers, RL2000, RMC-1, and other devices, RMX-10, DEM, APR-1 (Data Science International Inc., USA). Anesthesia was induced by acepromazine maleate (Fort Dodge Laboratories Inc., USA) and butorphanol tartrate (Bristol-Myer’s Squibb,
Japanese) and maintained by inhalation of 1.5% isoflurane/ oxygen. Dogs were implanted subcutaneously with a telemetry transmitter into the flank. ECG probes were placed between I rib and II rib (−), and between VII rib and VIII rib (+) (Lead II). Dogs were treated with antibiotics and analgesics after the surgery and were allowed to recover for at least 10 days.

**Study 1: Definition study for QT-RR relation**

**ECG recording:** ECG and locomotor activity were measured continuously over 24 h using a DS1 telemetry system and Notocord hem 3.4. The measurements were on four free-moving females in individual pens (W1 × D2 m) in one room, and this procedure was repeated with four males. During the monitoring, access into the room was limited to feeding procedures at 8 a.m. and physical checks at 4 p.m.

**Diurnal variation of QT and RR intervals:** QT, RR, PR and QRS were averaged individually for each dog, over every hour for 24 h during the dark and lighting periods. In addition, the values under steady and non-steady states of the animals were analyzed individually. QT and RR values were averaged over 6 s every 6 min, and were divided into steady and non-steady groups based on whether there was activity of the dog in the pen within 30 s prior to the measurement. The values were then averaged over 24 h of steady state or non-steady states.

**QT-RR log regression coefficient β:** QT-RR data were plotted individually for each animal over 24 h, for the light and dark periods, and for each condition, non-steady and steady states. The associations of QT with RR were analyzed individually by linear regression on a logarithmic scale \[ \log (QT) = \alpha + \beta \log (RR) \], and the coefficient \( \beta \) was estimated over 24 h, for light and dark periods, and for non-steady and steady states. In addition, the coefficient \( \beta \) was compared between the conditions by the paired t-test.

**Study 2: Validation study for rate-correction technique utilizing analysis of covariance**

**Ability to dissociate the effect of heart rate on QT interval:** The QT and RR data recorded in the above mentioned definition study for the QT-RR relation, averaged for 6 s every 6 min over 24 h, and QT-RR pairs were used in this study. The rate-correction utilizing analysis of covariance was performed for individuals according to Spence’s method [27]. First, the association of QT with RR was analyzed by linear regression on a logarithmic scale \[ \log (QT) = \alpha + \beta \log (RR) \] to estimate the coefficient \( \beta \) for each condition: 24 h, steady and non-steady states of the animal. Second, having estimated \( \beta \) the heart rate-adjusted QT interval was then determined from the equation \[ \log (QTc) = \log (QT) - \beta \log (RR) - \log (RRm) \], where RRm is the reference heart rate. QTc was obtained by the inverse log function.

**Analysis of covariance:**

\[ \log (QTc) = \log (QT) - \beta \log (RR) - \log (RRm) \]

\( \beta = \) log regression coefficient

\( RRm = \) reference heart rate

Using the same QT and RR data, rate-correction was calculated by the following formulas:

**Bazett’s formula [2]:**

\[ QTcB = QT / \sqrt{60 / HR} \]

**Van de Water’s formula [28]:**

\[ QTcv = QT - 0.087 \times (RR - 1000) \]

**Matsunaga’s formula [20]:**

\[ QTcm = \log _{600} \times QT / \log RR \]

The ability to dissociate the effect of the heart rate on the QT interval for the rate-correction technique utilizing analysis of covariance was compared with that of Bazett’s, Van de Water’s, and Matsunaga’s formulas using the correlation coefficient \( r \) and p-value between the QTc and RR intervals.

**Ability to detect QT prolongation with dl-sotalol, a class III anti-arrhythmic agent:** Six beagles were assigned randomly into this validation study with drug-treatment. A class III antiarrhythmic agent, dl-sotalol, and propranolol obtained from Sigma Chemical Co. (St. Louis, MO63178, USA) were diluted with 0.9% physiological saline into concentrations of 0.2 mg/ml and 0.8 mg/ml, respectively. The 0.9% physiological saline was used as a control vehicle. First, in pre-test prior to the dosing with saline, ECG was recorded continuously over 24 h and the QT and RR intervals were averaged for 6 s every 6 min over 24 h, and the association of QT with RR was analyzed by linear regression on a logarithmic scale \[ \log (QT) = \alpha + \beta \log (RR) \] to estimate the coefficient \( \beta \) for each animal. Then, saline was first administered intravenously following the same procedures as used for subsequent drug administration. One week after the dosing with saline, propranolol at a dose of 2 mg/kg was administered and subsequently 1
week after the propranolol dosing, dl-sotalol at 4 mg/kg was administered. The dosage level of dl-sotalol was expected to prolong the QT-interval in dogs [9, 14, 29], and propranolol was selected as a reagent which would not prolong the QT-interval [19]. For assessment of QT prolongation, ECG was recorded continuously for 8 h following the administration. QT and RR intervals 1 and 4 h after the dosing were averaged over 30 s, the heart rate-adjusted QT interval was then determined using the rate-correction technique utilizing analysis of covariance, Bazett’s, Van de Water’s and Matsunaga’s formulas [2, 20, 28]. In addition, the coefficient $\beta$ of analysis of covariance used the estimated $\beta$ of the pre-test. Subsequently, change rates (%) of QTc values calculated by each formula were calculated for each dog based on the QTc value of the averaged QT value over 24 h recording in the pre-test. The ability to detect QT prolongation for the rate-correction technique utilizing analysis of covariance was compared with those of Bazett’s, Van de Water’s, and Matsunaga’s formulas based on the p-value with unpaired t-test.

**QT-RR relation of traditional ECG**

QT and RR intervals derived from 122 beagle dogs in traditional ECG historical data were analyzed. All measurements were conducted using a computerized ECG and analytical system (Fukuda ME, Japan). Recordings were made using leads I, II, III, aVR, aVL, aVF, CV5RL, and V_{10} with dogs in right lateral recumbency. QT and RR intervals were averaged over 5 beats from leads II.

In addition, in order to examine the effect of restraint induced stress on QT interval, 8 dogs prepared for telemetry were restrained for 3 min as in the traditional restraining method, and QT and RR values measured under steady, non-steady or restraint by the telemetry technique were compared with those obtained by the traditional technique.

**Statistical analysis**

The QT and RR intervals and the coefficient $\beta$ were compared between light and dark periods, and between steady and non-steady states, and over 24 h by a paired t-test. QT prolongation with dl-sotalol was compared with that 0.9% saline control by an unpaired t-test. Values of p<0.05 were considered significant. Data are expressed as mean ± standard deviation (SD).

**Results**

**QT-RR distribution of the telemetry ECG and the diurnal range**

A representative of the distribution of the QT-RR plots derived from the telemetry ECG is shown in Fig. 1-A. The QT-RR plots were distributed over a wide range of RR intervals and showed a visually positive correlation curve, and the variation was small.

Diurnal ranges of QT and RR intervals under various behavioral conditions are summarized in Table 1. The averaged values of QT and RR intervals over 24 h were 215.7 ± 4.8 msec and 683.4 ± 53.8 msec, respectively. The averaged values of QT and RR intervals over 24 h
under steady state of animals were 224.1 ± 4.6 msec and 769.1 ± 68.5 msec, respectively, and the values under non-steady state were 208.0 ± 8.2 msec and 632.9 ± 79.6 msec, respectively. In addition, the maximum averaged values of QT and RR intervals for one hour were 236.9 ± 7.4 msec and 890.8 ± 87.4 msec in the sleeping period and the minimums were 189.3 ± 11.6 msec and 504.9 ± 58.1 msec in the feeding period, respectively. There was a statistically significant difference between the steady and non-steady values of QT interval (p<0.01), but there was no difference between the light- and dark-period values.

**QT-RR distribution of the traditional ECG and the normal range**

The distribution of the QT-RR plots derived from the traditional ECG are shown in Fig. 1-B. The QT-RR plots are distributed elliptically over a narrow range of RR intervals and the variation is large. The averaged values for QT and RR intervals are 182.5 ± 28.2 and 582.8 ± 19.3 msec, respectively, and the estimated regression coefficient β is 0.307.

There was a significant difference between the value of QT interval in the steady state of the animal using the telemetry system and the value of the traditional method (p<0.01), but there was no difference between the value under restraint using the telemetry system and that of the traditional method (p>0.05). In addition, when the dogs implanted with the telemetry system were restrained by the method of the traditional ECG the QT and RR intervals were shortened from approximately 220 msec and 770 msec in the period of free-moving to approximately 190 msec and 600 msec, respectively. This indicates that QT values ranging from 180 to 190 msec recorded by traditional ECG are influenced by restraint-induced stress.

**Dinurnal rhythm for QT and RR intervals**

Dinurnal rhythms for locomotor activity and QT and RR intervals are shown in Fig. 2. The dinurnal rhythm of the QT interval was synchronized with that of the RR interval over 24 h. Locomotor activity increased markedly at feeding time in the morning and during the physical check in the afternoon, and there was also sporadic activity during the night. Dinurnal changes of QT and RR intervals were consistent with that of locomotor activity. QT and RR intervals were shortened during feeding and during the physical check, and was prolonged during the steady period after the feeding and in the early morning.

**Dinurnal variation of QT-RR regression coefficient β**

Variations of the QT-RR log regression coefficient β over 24 h, day and night, steady and non-steady states are summarized in Table 2. There were significant differences between steady and non-steady states (p<0.01), but there was no significant difference between the dark and light periods (p>0.05). In addition, there was a significant difference between 24 h and the steady state (p<0.01) and between 24 h and the non-steady-state (p<0.05). The coefficient β was lower in value in the steady state than in the non-steady state and was also lower during the dark period than the light period. The averaged value was 0.276 ± 0.52. In addition, the minimum value was 0.153 in the steady state of the animal and the maximum was 0.495 in the non-steady state.

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**Table 1. Diurnal range of QT and RR intervals in the telemetry ECG recording in freely moving beagle dogs**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>24 Hours&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lighting Period&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Condition&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>QT interval (msec)</td>
<td>8</td>
<td>215.7 ± 4.8 (189.3–236.9)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>219.5 ± 4.3</td>
<td>212.5 ± 6.5</td>
</tr>
<tr>
<td>RR interval (msec)</td>
<td>8</td>
<td>683.4 ± 53.8 (504.9–890.8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>721.4 ± 67.6</td>
<td>653.7 ± 70.9</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SD (msec). N: Number of animals. a) Values were averaged, all beats over 24 h. b) Values were averaged, all beats for 24 h during dark- or light periods. c) Values were averaged for 6 s every 6 min and were divided into two groups, steady or non-steady states of animals, and averaged over 24 h. d) Values were averaged for one hour every one hour over 24 h and expressed as minimum - maximum. Values were analyzed statistically with an unpaired t-test. Values of p<0.05 were considered significant. **: p<0.01, Steady vs. Non-Steady.
Fig. 2. Diurnal variations of QT and RR intervals and locomotor activity in freely moving beagle dogs. Continuous recording for ECG and locomotor activity were performed for 24 h using a telemetry system. Measurements for QT and RR intervals were averaged over one hour periods. Measurements for activity sampled for a 10-s period, every 10-s, and averaged over one hour. Each point is expressed as mean ± SD of 8 beagle dogs.

Table 2. Diurnal variation of rate-correction coefficient \( \beta \) derived from QT-RR plots in long-term telemetry ECG in freely moving beagle dogs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>24 Hours</th>
<th>Lighting Period</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>Coefficient ( \beta )</td>
<td>8</td>
<td>0.276 ± 0.052</td>
<td>0.249 ± 0.041</td>
<td>0.311 ± 0.088</td>
</tr>
<tr>
<td>Min–Max</td>
<td>8</td>
<td>0.217–0.384</td>
<td>0.183–0.394</td>
<td>0.227–0.471</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SD (msec) or minimum – maximum. N: Number of animals. The coefficient \( \beta \) was estimated by analysis of linear regression on a logarithmic scale \( \log (QT) = \alpha + \beta \log (RR) \). Values were analyzed statistically with an unpaired t-test. Values of p<0.05 were considered significant. a) Steady vs. Non-Steady, p<0.01. b) Steady vs. 24 h; p<0.01. c) Non-Steady vs. 24 h; p<0.05.

**Ability to dissociate the effect of heart rate**

Table 3 shows correlation between QTc and RR interval over 24 h, steady and non-steady-states based on analysis of covariance, Bazett’s, Van de Water’s, and Matsunaga’s formulas. The correlation coefficient for analysis of covariance was minimum over 24 h, and over steady and non-steady-state, and \( p \)-value was maximum. The correlation coefficient over 24-h and steady-state was lower according to the following ranking: covariance adjustment > Matsunaga > Van de Water < Bazett. The correlation coefficient in the non-steady state was lower according to the following ranking: covariance adjustment < Van de Water < Matsunaga < Bazett. Based on level of discrimination to dissociate the effects of heart rate on the QT interval, the formulas were ranked: covariance adjustment > Matsunaga > Van de Water > Bazett. In addition, Fig. 3 represents a sample of the QT-RR plots. The slope of the QTc-RR linear regression line based on analysis of covariance in both steady and non-steady states was close to horizontal while the slope based on Bazett’s, Van de Water’s and Matsunaga’s formula was steeper. This indicates that QTc changes were in parallel with the RR interval for the analysis of covariance method/formula.

**Ability to detect QT prolongation with dl-sotalol**

Comparison between analysis of covariance and
Table 3. Comparison of the ability to dissociate the effect of heart rate on QT interval under steady and non-steady states of animals among four rate-correction formulas: Analysis of Covariance, Bazett, Van de Water and Matsunaga.

<table>
<thead>
<tr>
<th></th>
<th>24 Hours</th>
<th>Steady</th>
<th>Non-Steady</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
<td>Rank</td>
</tr>
<tr>
<td>Covariance</td>
<td>0.08 ± 0.04</td>
<td>0.29 ± 0.22</td>
<td>1.1</td>
</tr>
<tr>
<td>Bazett</td>
<td>0.68 ± 0.14</td>
<td>&lt; 0.001</td>
<td>4.0</td>
</tr>
<tr>
<td>Van de Water</td>
<td>0.39 ± 0.24</td>
<td>0.09 ± 0.39</td>
<td>2.5</td>
</tr>
<tr>
<td>Matsunaga</td>
<td>0.36 ± 0.12</td>
<td>&lt; 0.001</td>
<td>2.4</td>
</tr>
</tbody>
</table>

The correlation coefficient $r$ and $p$-value were estimated between QTc and RR interval. The four rate-correction methods were ranked based on the $r$ and $p$-value. Values for $r$ and $p$-value are expressed as mean ± SD (N=8). Values for Rank are expressed as mean (N=8). $r$: correlation coefficient. QTc values were calculated using four formulas: Covariance Adjustment, Bazett, Van de Water, Matsunaga.

A. Analysis of Covariance vs. Bazett

B. Analysis of Covariance vs. Van de Water

C. Analysis of Covariance vs. Matsunaga

Fig. 3. A representative of comparison of the ability to dissociate the effect of heart rate on the QT interval between analysis of covariance and Bazett (A), Van de Water (B) and Matsunaga (C) under two behavioral condition: steady (a) and non-steady (b). ○: Analysis of Covariance. ■: Bazett. ◆: Van de Water. ▲: Matsunaga.
Bazett’s, Van de Water’s, and Matsunaga’s formulas based on capacity to detect QT prolongation with dl-sotalol are shown in Fig. 4. Analysis of Covariance and Van de Water’s formula detected significant prolongation of QT prolongation at both 1 and 4 h (p<0.01). Matsunaga’s formula also detected the prolongation and the p-value at 4 h was under 0.05. Bazett’s formula could not detect the prolongation at 1 h (p>0.05).

**Discussion**

The present investigation attempted to establish a new rate-correction method for the QT interval in long-term telemetry ECG recording in freely moving beagle dogs. The present study demonstrated clearly that the distribution of the telemetry QT-RR plots was different from that of the traditional ECG. The QT-RR plots were distributed over wide range of RR intervals and showed a visually positive correlation curve, and the variation was small. On the other hand, the QT-RR plots of traditional ECG were distributed elliptically over a narrow range of RR intervals and the variation was large, and the QT and RR values were influenced by restraint-induced stress. In addition, the averaged QT value of traditional ECG in the present study was consistent with the values of previous reports, but that of telemetry ECG was approximately 15% higher. In general, normal levels for QT intervals have been between 180 and 190 msec [10, 13, 23, 24]. Numerous correction formulas have been derived utilizing the QT-RR relation of the traditional ECG and have been validated based on that relation [12, 16]. However, the results of the present study indicate that the correction formula derived from the traditional ECG and the validation based on the QT-RR plots are not necessarily adequate for the telemetry ECG.

The first investigation of the present study also demonstrated that the diurnal rhythm of the QT interval is synchronized with that of the RR interval over 24 h. QT and RR intervals were shortened during feeding and during physical checks, and was prolonged during the steady state after feeding and in the early morning. It is well known that in humans QT and RR intervals are prolonged during sleeping but shorten during exercise [6, 11], and this phenomenon is associated with the change in autonomic nervous activity [1, 3, 5, 22].
In the present study, the diurnal rhythms of QT and RR intervals in beagle dogs were different from those of humans. However, this can be explained by the characteristic natural behavior of dogs which have repeated short-term wake-sleep cycles in a day [21]. Thus, the results of the first investigation revealed that the diurnal changes of QT and RR intervals are dependent on the physical and emotional states of the animals, a relationship which is similar to that found in humans.

In addition, the present study revealed the relation between the distribution of QT-RR plots area and the state of the animal. Levels of QT and RR intervals under the steady state condition had not been established sufficiently for beagle dogs. The present study established their levels by separating into groups, QT-RR plots based on the condition of the animal. The maximum averaged values of QT and RR intervals for one hour were 236.9 ± 7.4 msec and 890.8 ± 87.4 msec in the sleeping period and the minimums were 189.3 ± 11.6 msec and 504.9 ± 58.1 msec in the feeding period, respectively. The averaged values over 24 h under the steady state of animals were 224.1 ± 4.6 msec and 769.1 ± 68.5 msec, respectively, and the values under the non-steady state were 208.0 ± 8.2 msec and 632.9 ± 79.6 msec, respectively. These results show clearly that the values of QT and RR derived from the telemetry ECG had considerable diurnal variation compared to those of the traditional ECG and that the distribution of QT-RR plots changes, depending on the physical and emotional states of the animal.

In the second investigation of the present study, the estimated coefficient \( \beta \) was 0.276 ± 0.104 (Mean ± 2SD) in 24-h QT-RR plots of 8 dogs. The regression coefficient \( \beta \) also indicates the degree of rate correction; for example, in Bazett’s formula it is 0.5, Fridericia it is 0.3, and in Kawataki it is 0.25 [2, 17, 26]. Thus, the results of the present study indicate that an adequate correction coefficient for the QT interval of telemetry ECG is between the coefficient of Fridericia and the coefficient of Kawataki. In addition, diurnal variation of the coefficient \( \beta \) ranged from 0.153 to 0.495 (minimum to maximum), and the coefficients for \( \beta \) under steady state and non-steady-states were 0.224 ± 0.052 and 0.332 ± 0.089 in 24-h QT-RR plots of 8 dogs, respectively. The coefficient \( \beta \) of QT-RR plots and the diurnal variation had previously not been defined sufficiently. The present study revealed that the coefficient \( \beta \) has considerable variation during the day and has unexpected changes which are dependent on the physical and emotional states of the animal. Thus, the ideal correction method for long-term telemetry ECG requires the selection of a coefficient of \( \beta \) which are chosen according to the conditions of measurement.

Analysis of covariance is often used to adjust a continuous variable such as QT for a covariate, in this case HR [27]. First, the association of QT with HR (in pre-test or control data) is analyzed by linear regression on a logarithmic scale \[ \log (\text{QT}) = \alpha + \beta \log (\text{HR}) \] to estimate the coefficient \( \beta \). Then, having estimated \( \beta \) the heart rate-adjusted QT interval is then determined from the equation \[ \log (\text{QT}_{\text{ca}}) = \log \text{QT} - \beta \log (\text{HR}) - \log (\text{HR}_{\text{m}}) \], where \( \text{HR}_{\text{m}} \) is the reference heart rate. Analysis of covariance is a flexible rate technique and use of a covariance-adjusted QT has the major advantage that it is derived from data of the same dogs used for assessment of treatment effects under identical experimental conditions and measurement protocols [27]. Analysis of covariance as a correction technique for QT interval was validated by comparison with Bazett’s, Van de Water’s, and Matsunaga’s formulas based on its ability to dissociate the effects of heart rate on the QT interval and to detect the QT prolongation with dl-sotalol, a class III anti-arrhythmic drug. Spence et al. showed a covariance adjustment under a typical toxicity study; they estimated \( \beta \) using the pre-test data from 198 dogs [27]. Our method used the individual coefficient \( \beta \) which was estimated from 24 h telemetry recording from each dog prior to the study, and the QT interval for the RR interval was corrected using an individually derived formula for each dog. In the present study, in order to validate the rate-correction technique utilizing analysis of covariance, three previously proposed formulas were selected. Bazett’s formula was selected because it is the most frequently used and is a standard technique worldwide. Matsunaga’s formula was selected because it is derived from QT-RR plots of the Holter ECG recording [20], and the condition of measurement is similar to that of telemetry. Van de Water’s formula was selected because it was reported that the relationship between HR and the QT interval was established by increase of HR by pacing in 10 anesthetized mongrel dogs [28], and the QT-RR relation in the steady state condition in the present study was linear, a similar result to that reported by Van de
Water.

An ideal correction formula for QT should eliminate completely the effect of HR in the corrected QT value. The correction between corrected QT and HR should be zero apart from sampling variation and the regression line should be horizontal [27]. In the present study, based on elimination of effects of heart rate on the QT interval, the formulas were ranked: covariance adjustment > Matsunaga > Van de Water > Bazett. Bazett’s formula showed the highest value for the correlation coefficient, particularly under the steady-state condition, and could not detect a significant difference in QT prolongation at 1 h after dosing. This result supports previous reports that Bazett’s formula overcorrects under the non-steady state and undercorrects under the steady state condition and can not detect QT prolongation. Matsunaga’s formula was derived from the QT-RR plots of Holter ECG. The QT-RR plots were collected over a long time from one freely moving beagle dog. Therefore, the collecting procedure for the QT-RR plots of Matsunaga’s formula is similar to that of the telemetry ECG of the present study. The difference between Matsunaga’s formula and the rate-correction technique utilizing analysis of covariance used in the present study is whether the rate-correction coefficient is fixed or flexible. The result of the present study showed that rate-correction by analysis of covariance was superior to that of Matsunaga’s formula. Matsunaga’s formula showed slightly higher values under the non-steady state than under the steady state, and detected significant differences of QT prolongation at 1 h and the p-value at 4 h was p<0.05. These results lend support to the idea that the ideal correction method for long-term telemetry ECG requires selection of a flexible coefficient $\beta$ adjusted to the condition of the measurements. In addition, this validation study showed that the rate-correction technique utilizing analysis of covariance was the method best fitting all measurements, over 24 h, steady and non-steady state conditions, and based on detection of QT prolongation of $d$-sotalol, the formulas were ranked: covariance adjustment > Van de Water > Matsunaga > Bazett.

In conclusion, the present study revealed that the QT-RR regression coefficient $\beta$ has considerable variation during the day, depending on the physical and emotional states of the animal. Thus, the ideal rate-correction technique for the QT interval in long-term telemetry ECG recording requires selection of a flexible coefficient $\beta$ which is chosen according to the conditions of measurement, and the rate-correction method utilizing analysis of covariance estimating the coefficient $\beta$ for each dog is an acceptable solution for data from telemetry ECG recordings.

References