Abstract. In safety pharmacology studies, the effect of a test compound on the electrocardiogram is routinely examined by using conscious dogs. However, the results may be widely variable. The monkey, on the other hand, has scarcely been used for such studies; and as yet, there have not been reported studies on monkeys conducted at several facilities with a standard protocol. In the present study, we examined inter-facility variabilities in electrocardiographic and hemodynamic parameters as described below. We analyzed the data from 8 facilities (9 test groups) on dogs and 5 facilities (7 test groups) on monkeys. This data was obtained from the studies employing the following standard protocol: dl-Sotalol or a vehicle (0.5 w/v% methylcellulose solution) was given to animals; and the PR interval, QRS duration, QT interval, heart rate, and mean blood pressure were determined time-sequentially before and after administration of the vehicle or dl-sotalol in each test group. dl-Sotalol produced a prolongation of the maximum mean QTcF interval in conscious dogs and QTcB interval in conscious monkeys by more than 10% in every test group. No difference in the corrected QT interval among the test groups was observed in dogs, but a difference was observed in monkeys.

Keywords: conscious dog, conscious monkey, QTc interval, inter-facility variability, dl-sotalol

Introduction

The prolongation of QT interval is associated with various pathological states such as hypocalcemia, hypokalemia, central nervous system disorders, intraventricular conduction defects, changes in autonomic balance, and accidental poisoning (1, 2). This prolongation indicates delayed cardiac repolarization and is a known clinical risk factor for the development of severe, life-threatening ventricular arrhythmias including Torsade de Pointes (TdP) (3). Thus, it is important to evaluate the potential risk of TdP by a compound early in the drug discovery program. Since the appearance of the article by Ackerman, there has been much debate within the pharmaceutical industry as to the relative merits of various in vitro and in vivo techniques for detecting this effect preclinically (4 – 6).

Recently, conscious animals chronically implanted with telemetry devices have been widely used for preclinical in vivo electrocardiographic (ECG) evaluation of drugs in safety pharmacological studies (7). However, the usefulness of an in vivo QT assay using conscious dogs and monkeys or the usefulness of other systems for evaluating the risk of drug-induced QT interval prolongation has not been documented systematically, and little background data is available for such a telemetry study. Therefore, in order to construct a database that can be useful for the development of new chemical entities and to support the application of the ICH S7B guideline (8), a project called “QT Interval Prolongation Project for Database Construction (QT PRODACT)” was organized by the Japan Pharmaceutical Manu-
facturers Association. The present report describes the results of our study on inter-facility variability performed following a standard protocol using the dog and monkey telemetry assay.

Materials and Methods

The experiments using dogs were conducted at 8 facilities (9 test groups) and those using monkeys conducted at 5 facilities (7 test groups) in accordance with the common protocol described by Toyoshima et al. (9) and Ando et al. (10).

Test compounds and vehicle

dl-Sotalol (SOTACOR® Tablets, 40 mg; Bristol Pharmaceuticals K.K., Tokyo) was suspended in a 0.5 w/v% methylcellulose (Kanto Chemical Co., Inc., Tokyo; Shin-Etsu Chemical Co., Ltd., Tokyo; Wako Pure Chemical Industries, Ltd., Osaka) solution at 2 mg/mL concentration for dogs and 1 mg/mL concentration for monkeys just before use.

Animals

The experimental protocol and design were approved by the Animal Care and Use Committee at each facility, and the study was performed in accordance with the Guidelines for Animal Experimentation of the institution.

Dogs

Nine test groups of 4 male beagle dogs each were prepared in 8 facilities. The dogs weighing 7.8 – 14.8 kg, 8 – 23 months of age (from Covance Research Products, Cumberland, VA, USA; Kitayama Labes Co., Ltd., Minowa, Nagano; and Marshall Farms USA, Inc., North Rose, NY, USA). were housed in individual metal pens or cages in an air-conditioned room at each facility. The lighting period was 12 h /day at all facilities. Approximately 250 – 350 g of certified dog chow (DS-A: Oriental Yeast Co., Ltd., Tokyo; PMI Certified Laboratory Canine Diet #5007: PMI Nutrition International, Inc., Co., Henderson, CO, USA; or TC-1: Maruha Petfood Co., Ltd., Tokyo) was fed to each animal daily, and drinking water was provided ad libitum (Appendices 1-1 and 1-2).

Monkeys

Seven test groups of 4 male monkeys each were prepared in 5 facilities. The monkeys weighing 2.8 – 6.3 kg, 3 – 7 years of age (from Nafovanny, Dong Nai, Vietnam; China National Scientific Instruments & Materials, Hangzhou, China; Covance Research Products, Inc., Denver, PA, USA; and Siconbrec, Inc., Manila, Philippines) were housed in individual metal pens or cages in an air-conditioned room at each facility. The lighting period was 12 h/day at all facilities. Approximately 100 g of solid diet (Certified Primate Diet 5048: PMI Nutrition International, Inc., Co.; CMK-2: CLEA Japan, Inc., Co., Tokyo; Monkey bit: Nosan Corporation, Kanagawa; or PS: Oriental Yeast Co., Ltd.) was fed to each animal daily, and drinking water was provided ad libitum (Appendices 2-1 and 2-2).

Telemetry system

The telemetry system consisted of a transmitter (TL11M2-D70-PCT; Data Science International, Arden Hills, MN, USA), a receiver (RMC-1 or RLA2000, Data Science International), a data acquisition system (Dataquest A.R.T. Analog, Open A.R.T. or UA-10; Data Science International) and data analysis software (HEM ver. 3.4 or 3.5; NOTOCORD Systems SAS, Crossy sur Seine, France).

Surgical implantation of a telemetry transmitter

Surgical implantation of a telemetry transmitter was properly performed under anesthesia according to basic veterinary surgical procedures. The telemetry transmitter was implanted in the left lateroabdominal subcutaneous area for dogs and in the abdominal cavity for monkeys. A catheter for measurement of blood pressure was passed subcutaneously to the area of the left femoral artery and then 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 (approximate lead II or Base-Apex position) through a subcutaneous tunnel. Dogs and monkeys were treated with antibiotics and allowed to recover for at least 2 weeks after surgery.

Experiments

dl-Sotalol at 10 mg/kg in dogs and 5 mg/kg in monkeys and the vehicle were orally given to animals that recovered well from the surgical procedure or to those animals that were allowed an adequate recovery period, which was sufficient for the disappearance of any effects of previously used drugs. Treatment with the vehicle or dl-sotalol was made at intervals of at least one week. Five mL/kg volume of dl-sotalol or the same volume of the vehicle was administered orally at around 10:00 AM. Animals were fasted for 16 h before dosing. On the day of administration, diet was given 4 h after dosing. In order that the test is carried out under the same condition as in Toyoshima et al. (9) and Ando et al. (10), blood was collected but discarded without an assay for drug concentration. During the monitoring, access into the room was limited to the feeding procedures,
Analysis procedures
The mean blood pressure (MBP), heart rate (HR), and ECG were analyzed before and at 1, 2, 4, 8, and 24 h after dosing of dl-sotalol or vehicle in dogs; because the time required for the influence of collecting blood to disappear differed between dogs and monkeys, these parameters were analyzed before and at 1, 2, 3, 4, 5, 8, and/or 24 h after dl-sotalol or vehicle was given to monkeys. The telemetry signals over 30 s were analyzed for MBP and HR. Electrocardiographic parameters (PR interval, QRS duration, and QT interval) were analyzed using the averages of 10 consecutive beats at each assay point. If the ECG waveform at a time point was judged to be unanalyzable because of noise, another 10 serial beats nearest to the point were analyzed. The analysis results were reviewed and corrected manually on a computer display by software (XLECg ver. 2.0.4, NOTOCORD Systems SAS). The QT interval was corrected for HR by Fridericia’s formula (QTcF) for dogs and by Bazett’s formula (QTcB) for monkeys.

Evaluation of the effect of dl-sotalol
The effect of dl-sotalol was evaluated as follows: Firstly, the vehicle average baseline (VAB) value was calculated individually for all values obtained from the animals treated with the vehicle. Secondly, percentage changes from the VAB value were calculated individually for all assay points. The mean percentage changes from the VAB value were calculated at each assay point and the values were compared statistically between the vehicle and dl-sotalol. In addition, differences in percentage changes between the vehicle and dl-sotalol were calculated individually. The maximum differences in the mean percent changes of the QTcF or QTcB interval in each test group were used for the evaluation of the effect of dl-sotalol.

Statistical analyses
Inter-facility variability of VAB value: The inter-facility variability of the VAB value was calculated using a linear nested model that has the animal and test groups as a random effect. Animal is nested in the test group. The linear nested model can divide variability into the inter-facility variability and the intra-facility variability (11).

Effect of dl-sotalol: The effect of dl-sotalol was examined using the linear model for each test group that has the drug, time, and animal as a fixed effect. Time is nested in the interaction term of drug and animal.

Inter-facility variability in the effect of dl-sotalol: The inter-facility variability was examined on the QTcF and QTcB interval in the post-dosing period of dl-sotalol and vehicle by the use of the linear nested model that has the test group and animal as a random effect. Animal is nested in the test group.

Influences of breeder on the effect of dl-sotalol: The influences of breeder for all the parameters were examined at pre-dosing and post-dosing of dl-sotalol using the linear model. This model has the drug, time, consideration factor, and interaction term of those factors as a fixed effect. At first, the drug × time × consideration factor as the highest interaction term was examined. If this was not significant, the model was reduced. Namely the drug × consideration factor as the second highest factor was examined, and so on (12).

Results

Vehicle average baseline value
Inter-facility variability: Dog: For MBP, the variance between the test groups was larger than that between the animals. For other parameters, however, the variance between the animals was larger than that between the test groups (Table 1).

Monkey: For HR, QRS duration, and QT interval, the variance between the test groups was larger than that between the animals. For other parameters, however, the variance between the animals was larger than that between the test groups (Table 2).

Effect of dl-sotalol: Dog: dl-Sotalol produced a prolongation of the maximum mean QT and QTcF intervals by more than 10% (12%–18%), with a statistically significant prolongation of QTcF interval, in every test group. The change was observed at 1 or 2 h after oral administration of dl-sotalol (Fig. 1, Appendix 3). A significant prolongation of PR interval was observed in 7 of 9 test groups, and dl-sotalol showed PR interval prolongation with the maximum mean difference of more than 10% in all test groups. No significant changes were observed in QRS duration for any test groups. Although a weak reduction of HR and MBP was observed in a few test groups, there were no marked effects on MBP or HR after dl-sotalol treatment. No significant inter-facility variability was found in QTcF prolongation (Appendices 4-1 to 4-6).

Monkey: Although dl-sotalol produced a prolongation of the maximum mean QTcB interval by more than 10% (11%–23%) in every test group, a statistically significant prolongation of QTcB was observed only in 2 of 7 test groups. A prolongation of the maximum mean QTcB was observed at 1, 2, or 8 h after administration (Fig. 2, Appendix 5). Variance in QTcB between the
Vehicles average baseline values of mean blood pressure, heart rate, PR interval, QRS duration, QT interval, and QTcF interval in each test group in conscious dogs

<table>
<thead>
<tr>
<th>Test group</th>
<th>No. of animals</th>
<th>MBP (mmHg)</th>
<th>HR (beats/min)</th>
<th>PR interval (ms)</th>
<th>QRS duration (ms)</th>
<th>QT interval (ms)</th>
<th>QTcF interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>83 ± 2</td>
<td>78 ± 5</td>
<td>123 ± 18</td>
<td>48 ± 2</td>
<td>229 ± 6</td>
<td>247 ± 8</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>105 ± 1</td>
<td>80 ± 2</td>
<td>118 ± 14</td>
<td>47 ± 1</td>
<td>228 ± 10</td>
<td>248 ± 12</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>102 ± 8</td>
<td>77 ± 4</td>
<td>106 ± 12</td>
<td>41 ± 3</td>
<td>217 ± 8</td>
<td>235 ± 12</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>87 ± 5</td>
<td>59 ± 5</td>
<td>125 ± 9</td>
<td>35 ± 3</td>
<td>229 ± 9</td>
<td>227 ± 6</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
<td>125 ± 4</td>
<td>97 ± 20</td>
<td>95 ± 9</td>
<td>42 ± 9</td>
<td>202 ± 17</td>
<td>232 ± 10</td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>94 ± 6</td>
<td>61 ± 4</td>
<td>106 ± 6</td>
<td>38 ± 2</td>
<td>230 ± 7</td>
<td>231 ± 6</td>
</tr>
<tr>
<td>G</td>
<td>4</td>
<td>97 ± 10</td>
<td>88 ± 7</td>
<td>109 ± 6</td>
<td>39 ± 5</td>
<td>215 ± 11</td>
<td>243 ± 8</td>
</tr>
<tr>
<td>H</td>
<td>4</td>
<td>90 ± 16</td>
<td>81 ± 18</td>
<td>104 ± 13</td>
<td>43 ± 2</td>
<td>219 ± 18</td>
<td>238 ± 11</td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>102 ± 10</td>
<td>76 ± 15</td>
<td>118 ± 11</td>
<td>35 ± 1</td>
<td>215 ± 12</td>
<td>229 ± 8</td>
</tr>
<tr>
<td>Mean</td>
<td>36</td>
<td>99 ± 14</td>
<td>77 ± 15</td>
<td>112 ± 14</td>
<td>41 ± 6</td>
<td>221 ± 14</td>
<td>237 ± 11</td>
</tr>
</tbody>
</table>

Variance Between test groups 139.8 112.8 60.2 16.5 57 38.3
Variance Between animals 60.8 121.1 133.3 17 134.8 87.3

MBP: Mean blood pressure, HR: Heart rate, Mean: Mean value for all animals. Each value represents means ± S.D. of 4 or 28 dogs.

Table 2. Vehicle average baseline values of mean blood pressure, heart rate, PR interval, QRS duration, QT interval, and QTcF interval in each test group in conscious monkeys

<table>
<thead>
<tr>
<th>Test group</th>
<th>No. of animals</th>
<th>MBP (mmHg)</th>
<th>HR (beats/min)</th>
<th>PR interval (ms)</th>
<th>QRS duration (ms)</th>
<th>QT interval (ms)</th>
<th>QTcB interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>4</td>
<td>101 ± 10</td>
<td>123 ± 9</td>
<td>75 ± 7</td>
<td>39 ± 4</td>
<td>237 ± 14</td>
<td>337 ± 16</td>
</tr>
<tr>
<td>K</td>
<td>4</td>
<td>87 ± 11</td>
<td>125 ± 16</td>
<td>77 ± 11</td>
<td>28 ± 2</td>
<td>242 ± 19</td>
<td>345 ± 19</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
<td>73 ± 9</td>
<td>129 ± 25</td>
<td>88 ± 6</td>
<td>43 ± 1</td>
<td>222 ± 14</td>
<td>321 ± 10</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>83 ± 14</td>
<td>100 ± 6</td>
<td>76 ± 8</td>
<td>34 ± 2</td>
<td>256 ± 15</td>
<td>328 ± 17</td>
</tr>
<tr>
<td>N</td>
<td>4</td>
<td>80 ± 6</td>
<td>145 ± 10</td>
<td>84 ± 8</td>
<td>29 ± 3</td>
<td>221 ± 9</td>
<td>337 ± 11</td>
</tr>
<tr>
<td>O</td>
<td>4</td>
<td>77 ± 8</td>
<td>160 ± 16</td>
<td>79 ± 5</td>
<td>42 ± 5</td>
<td>200 ± 10</td>
<td>323 ± 12</td>
</tr>
<tr>
<td>P</td>
<td>4</td>
<td>76 ± 4</td>
<td>170 ± 7</td>
<td>78 ± 4</td>
<td>36 ± 2</td>
<td>254 ± 24</td>
<td>354 ± 16</td>
</tr>
<tr>
<td>Mean</td>
<td>28</td>
<td>82 ± 12</td>
<td>136 ± 26</td>
<td>80 ± 8</td>
<td>36 ± 6</td>
<td>233 ± 23</td>
<td>335 ± 17</td>
</tr>
</tbody>
</table>

Variance Between test groups 62.4 524.9 10.4 31.9 337.5 92.7
Variance Between animals 86.6 199.7 52.9 8.7 252.6 212.9

MBP: Mean blood pressure, HR: Heart rate, Mean: Mean value for all animals. Each value represents means ± S.D. of 4 or 28 monkeys.

test groups was larger than that between the animals. There were no marked effects on other parameters after dl-sotalol treatment (Appendices 6-1 to 6-6).

Influence of breeder, animal age, or body weight in dogs

As the breeder, animal age, or body weight range in animals varied among the facilities, the influence of these factors was examined in the present study.

Breeders: For 9 studies, dogs were supplied from 3 different breeders: X (5 test groups), Y (3 test groups), and Z (1 test group).

Statistically significant differences among the 3 breeders were observed in the VAB value of each parameter (Table 3).

dl-Sotalol produced a prolongation of the maximum mean QTcF interval by more than 10% (11% – 18%) (Fig. 3, Appendix 7), and a significant PR, QT, and QTcF interval prolongation induced by dl-sotalol was observed in dogs from every breeder. However, statistically significant differences among the 3 breeders were observed in HR and QT interval, but not in the other parameters including MBP, PR interval, QRS duration, or QTcF interval (Appendices 8-1 and 8-2).

Animal age: A correlation between each parameter in the VAB value and the animal age and also a correlation between effect of dl-sotalol on each parameter and the animal age were not observed (Appendix 9).

Body weights: A correlation between each parameter in the VAB value and the body weight and also a correlation between the effect of dl-sotalol on each
parameter and the body weight were not observed (Appendix 9).

Influence of country of origin, animal age, or body weight in monkeys

Country of origin: For 7 studies, monkeys were supplied from 3 different countries: China (1 test group), Philippines (2 test groups), and Vietnam (4 test groups).

Although a difference among the 3 countries of origin was observed in the VAB values of MBP and QRS duration, there was no difference among the 3 countries of origin in the other parameters (Table 4).

dl-Sotalol produced a prolongation of the maximum mean QTcF interval by more than 10% (13% – 19%)

Table 3. Vehicle average baseline values of mean blood pressure, heart rate, PR interval, QRS duration, QT interval, and QTcF interval for 3 breeders in conscious dogs

<table>
<thead>
<tr>
<th>Breeder</th>
<th>No. of test groups</th>
<th>MBP (mmHg)</th>
<th>HR (beats/min)</th>
<th>PR interval (ms)</th>
<th>QRS duration (ms)</th>
<th>QT interval (ms)</th>
<th>QTc interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>5 (20)</td>
<td>96 ± 11</td>
<td>81 ± 9</td>
<td>112 ± 14</td>
<td>43 ± 5</td>
<td>222 ± 12</td>
<td>242 ± 11</td>
</tr>
<tr>
<td>Y</td>
<td>3 (12)</td>
<td>94 ± 9</td>
<td>65 ± 12</td>
<td>116 ± 11</td>
<td>36 ± 3</td>
<td>225 ± 11</td>
<td>229 ± 6</td>
</tr>
<tr>
<td>Z</td>
<td>1 (4)</td>
<td>125 ± 5</td>
<td>97 ± 20</td>
<td>96 ± 9</td>
<td>42 ± 9</td>
<td>202 ± 17</td>
<td>232 ± 10</td>
</tr>
</tbody>
</table>

ANOVA  

P<0.001  P<0.001  P = 0.0261  P = 0.008  P = 0.0083  P = 0.0014

* Numbers in parentheses are the number of animals. MBP: Mean blood pressure, HR: Heart rate.
There was no statistically significant difference among the 3 countries of origin in the QTcB interval (Appendices 11-1 and 11-2).

**Animal age:** Although there was a weak relationship between the animal age and the VAB value of HR ($R^2 = 0.3248$), no relationship was observed between the animal age and any other parameter (MBP, PR interval, QRS duration, QT interval, and QTcB interval) (Appendix 12).

**Body weights:** There was a weak relationship between the body weight and the VAB value of MBP ($R^2 = 0.2277$). However, there was no relationship between the body weight and any other parameter (HR, PR interval, QRS duration, QT interval, and QTcB interval) (Appendix 12).

### Table 4
Vehicle average baseline values of mean blood pressure, heart rate, PR interval, QRS duration, QT interval, and QTcB interval in conscious monkeys from 3 countries of origin

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>No. of test groups</th>
<th>MBP (mmHg)</th>
<th>HR (beats/min)</th>
<th>PR interval (ms)</th>
<th>QRS duration (ms)</th>
<th>QT interval (ms)</th>
<th>QTcB interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>1 (4)</td>
<td>101 ± 10</td>
<td>123 ± 9</td>
<td>75 ± 7</td>
<td>39 ± 4</td>
<td>237 ± 14</td>
<td>337 ± 16</td>
</tr>
<tr>
<td>Philippines</td>
<td>2 (8)</td>
<td>75 ± 7</td>
<td>149 ± 28</td>
<td>83 ± 7</td>
<td>39 ± 4</td>
<td>238 ± 25</td>
<td>338 ± 22</td>
</tr>
<tr>
<td>Vietnam</td>
<td>4 (16)</td>
<td>82 ± 10</td>
<td>132 ± 26</td>
<td>79 ± 8</td>
<td>33 ± 6</td>
<td>230 ± 25</td>
<td>333 ± 16</td>
</tr>
</tbody>
</table>

ANOVA

|                | $P = 0.0004$       | $P = 0.1746$  | $P = 0.2144$  | $P = 0.0298$  | $P = 0.7284$  | $P = 0.8080$  |

*Numbers in parentheses are the number of animals. MBP: Mean blood pressure, HR: Heart rate.
Discussion

Effect of dl-sotalol

This study using dl-sotalol as a common positive compound was designed to demonstrate whether each facility had a sufficient system to do accurate testing. In 1965, dl-sotalol was described as a non-cardioselective β-blocker lacking intrinsic sympathomimetic effect or local anesthetic properties (13). Since then, the pharmacokinetics and pharmacodynamics properties of dl-sotalol have been documented in numerous clinical and non-clinical reports (14 – 40). For these reasons, we thought that dl-sotalol is suited to serve as a common positive compound.

Dogs: Because an oral administration of 10 mg/5 mL/kg of dl-sotalol caused a prolongation of the QTcF interval in conscious dogs in the preliminary studies, where the maximum plasma concentrations (C_max) reached approximately 4 – 6 µg/mL (1 – 2 times the C_max values in humans given a therapeutic dose of dl-sotalol, data not shown) at 1 h after dosing, the same dosage (10 mg/5 mL per kg) of dl-sotalol was employed in the present study using dogs. As a result, dl-sotalol produced a statistically significant prolongation of the QTcF interval in comparison with the time-matched vehicle group. A prolongation of the maximum mean QTcF interval by more than 10% was observed at 1 or 2 h after oral administration of dl-sotalol in each test group in dogs. A significant difference among the test groups was not observed in QT or QTcF interval after dosing of dl-sotalol. These results indicate that each facility sufficient ability to run an accurate test. From the point of view of the breeder difference, a significant QTcF interval prolongation induced by dl-sotalol was observed in dogs from every breeder, and there was no breeder difference among the 3 breeders. On the other hand, although it was reported that the corrected QT interval prolongation after dosing of dl-sotalol was observed more significantly in neonates (8 – 15 days of age, body weight of 930 – 1400 g) than adult dogs (40) and that the QT interval was prolonged more in neonates with a smaller body surface area. There was no correlation between the age and QTcF interval prolongation and also a no correlation between the body weight and QTcF interval prolongation in the ranges of age or body weight used in the present study. These results suggest that the QTcF interval prolongation after dosing of dl-sotalol was not affected by the breeder difference or a certain extent of difference in animal age or body weight.

As for other parameters, a significant difference among 3 breeders was observed in HR after dosing of dl-sotalol. As previously mentioned, dl-sotalol causes a reduction in HR associated with the class III action potential. It is also known that more than 80% of an oral dose of dl-sotalol is absorbed (29) and dl-sotalol is not metabolized and only weakly bound to plasma proteins (30 – 35). Although the plasma concentration of dl-sotalol was not measured in the present study, preliminary studies indicated that the plasma concentration of dl-sotalol varied little in animals administered the same dose of the drug. Therefore, a difference in the reduction of HR induced by dl-sotalol apparently seemed to be influenced by the usual state of HR. It was reported that the HR in dogs taken using the telemetry system and Holter recorder ranged from approximately 70 to 120 beats/min (41 – 45). In the present study, the mean VAB value of HR in all test groups was 77 ± 15 beats/min. When the test groups were compared, HR was slightly higher in one test group (97 ± 20 beats/min in test group E) and slightly lower in two test groups (59 ± 5 beats/min in test group D and 61 ± 4 beats/min in test group F), although a significant difference among test groups was not observed in the VAB value of HR. Dogs in test group E were 13 months of age, 7.8 – 11.7 kg in body weight, and supplied from Breeder Z; dogs in test group D were 13 – 14 months of age, 10.4 – 11.0 kg in body weight, and supplied from Breeder Y; and dogs in test group F were 19 months of age, 13.2 – 14.8 kg in body weight, and supplied from Breeder Y. There was no relationship between HR and the animal age or between HR and the body weight. However, a significant difference among the 3 breeders was observed in the VAB value of HR. Therefore, the difference in HR seemed to be brought about by the difference in the breeders.

Monkeys: Although the maximum mean QTcB interval prolongation by more than 10% was observed in every test group, a statistically significant prolongation of QTcB interval produced by dl-sotalol in comparison with the time-matched vehicle group was observed only in 2 of 7 test groups. We thought the reason for this was that the time (1, 2, 3, or 8 h after administration) when the maximum mean QTcB interval prolongation was observed was different among the test groups. Although the variance in QTcB interval between the test groups was larger than that between the animals, both the variances were large and there no great difference in the variance between the test group and the animal. These results seemed to suggest that the variability between the test groups was due to individual variation. As an oral administration of 10 mg/5 mL per kg of dl-sotalol caused TdP in conscious monkeys in the preliminary studies, monkeys were administered 5 mg/5 mL per kg of dl-sotalol in the present study. The above-
mentioned result in the preliminary studies seemed to suggest that conscious monkeys are more sensitive to dl-sotalol than conscious dogs and that it is easy to yield a higher variance in monkeys. Although monkeys may have a great sensitivity to dl-sotalol, the maximum plasma concentration of dl-sotalol in monkeys reached approximately one third of that in the dogs (in-house data, data not shown). In addition, Omata et al. reported that although the reason is not clear, monkeys may be more sensitive to arrhythmogenic compounds or \( I_{K_c} \) blockers than dogs, because \( EC_{10} \) values (effective concentrations for prolonging QTc interval by 10%) of 10 positive compounds with potential proarrhythmic effects, except for thioridazine, were lower in conscious monkeys than in conscious dogs, and the ventricular arrhythmias after dosing of some positive compounds were observed in conscious monkeys but not in conscious dogs (46). As for dl-sotalol, there are a number of reports about the pharmacokinetics of dl-sotalol in dogs and humans, stating that the bioavailability of dl-sotalol in dogs and humans was approximately 100% (29 – 39), but there has been no report about this matter in monkeys. Therefore, although further investigations may be necessary, the present results seem to suggest that a metabolic pathway to dl-sotalol in monkeys is different from that in dogs and humans, and the differences in sensitivity to arrhythmogenic compounds or \( I_{K_c} \) blockers may be due to a species difference.

**Vehicle average baseline value**

**Dogs:** In general, a normal level of QT interval in dogs measured by a traditional method has been known to be between 180 and 190 ms (47 – 49). Miyazaki et al., however, reported that the normal level of QT interval on telemetry ECG was approximately 15% higher (50). Our results that the VAB value of QT interval was 221 ± 14 ms were consistent with the report by Miyazaki et al. For the other parameters also, our results were consistent with those reported in the literature (41, 42, 45, 46, 51).

As for the difference in VAB value among the test groups and breeders, there was a significant difference among the 3 breeders for every parameter, with only a small difference among the test groups. However, there was no significant difference in the QTcF interval prolongation induced by dl-sotalol among the test groups and also among the 3 breeders. These results seem to suggest that a slight difference is present in the electrocardiographic and hemodynamic parameters in a stational state among the breeders, but it did not influence reactivity to the compound. It is well known that in humans, QT and RR intervals are prolonged during sleeping but shortened during exercise (52, 53), and this phenomenon is associated with changes in the autonomic nervous activity (54 – 58). It was also reported that they were similarly affected by the physical and emotional states of the animal. In the present study, as we measured RR and QT interval for 24 h before dosing of the vehicle or test compound, QT and RR intervals were prolonged in the early morning, shortened around 9:00 AM that was the feeding time, prolonged thereafter, and then returned to a steady state around 3:00 PM. There seems to be no great variation in this rhythm, although animals were fasted for 16 h from the day before administration to the day of administration of vehicle or dl-sotalol and given food 4 h after dosing. Although a significant difference among every group studied was not observed in QTcF interval prolongation, we haven’t examined the relationship between the circadian rhythm and the fasting or the time of feeding in the present study. Therefore, further investigations may be necessary regarding this point.

**Monkeys:** In the VAB value of HR, QRS duration, and QT interval, the variance between the test groups was larger than that between the animals. Of these, the variance in the QT interval seemed to be related to the dispersion of the HR, as the variance in the corrected QT interval between the test groups was small. As for HR, a significant difference was observed among the age groups, and there was a weak correlation between the animal age and HR. In conscious monkeys, however, it was reported that the HR was not different between the young (5.9-year-old) and the old (19.8-year-old) (59). Therefore, the difference in the HR among the test groups seemed to be due to not the age difference, but due to individual difference. Moreover, the MBP of monkeys from China was slightly high and there was a weak correlation between MBP and the body weight, but it was reported that MBP was not correlated with sex, age, or body weight in conscious monkeys (60). Therefore, these results seemed to suggest that there was a slight difference in MBP of monkeys from 3 different countries.

**Conclusion**

It is conceivable that the inter-facility differences in ECG parameters and hemodynamic parameters are yielded by numerous factors including the animal breeder, body weight, age, sex, environment of measurement, breeding, telemetry systems (transmitter receiver and data analysis software), animal character, and individuals who measured the ECG. The inter-facility difference in corrected QT interval may also be due to the correction methods used. In the present study, although a significant difference among the test groups
was not observed in the QTcF interval after dosing of dl-sotalol, significant differences among the breeders were observed in the VAB values of every parameter in the conscious dogs. However, there was no significant difference in QT and QTcF interval prolongation after dosing of dl-sotalol among the breeders. These results seem to suggest that the response to the compound was not affected by the breeder difference, although there were slight differences in hemodynamic and electrocardiogram parameters in the steady state among the breeders. Additionally in conscious monkeys, although dl-sotalol produced a prolongation of the maximum mean QTcB interval with more than 10% in every test group, the variance in QTcB between the test groups was larger than that between the animals. However, the influence of the individual difference seemed to be undeniable, as the variance in QTcB between animals was large. The in vivo QT assay using conscious dogs and monkeys with the standard protocol applicable to routine safety pharmacology studies is considered to be a sensitive and useful model for an assessment of the drug potential to prolong QT interval in humans. The plasma concentration of a test compound or its metabolites should be carefully taken into consideration for this purpose because, as for the monkeys, the individual difference is extremely large.

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