PRACTICAL APPLICATION OF GUINEA PIG TELEMETRY SYSTEM FOR QT EVALUATION

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ABSTRACT — The purpose of this study was to evaluate a telemetry system for examining QT evaluation in the conscious free-moving guinea pig using 10 reference compounds whose effects on human QT interval are well established: 8 positive references (bepridil, terfenadine, cisapride, haloperidol, pimozide, quinidine, E-4031 and thioridazine), and 2 negative references (propranolol and nifedipine). Pharmacokinetic experiments were also performed for the 8 positive references. Telemetry transmitters were implanted subcutaneously in male Hartley guinea pigs, and the RR and QT intervals were measured. All 8 positive references prolonged QTc (QTc = k × QT/RR1/2) 10% or more during the 60 min observation period. When the values of the QTc changes were plotted against the serum concentrations, the resulting curves exhibited an anticlockwise hysteresis loop for all 8 references. In guinea pigs treated with haloperidol, changes of the T-wave shape from positive to flat were observed. The 2 negative references did not prolong the QTc. These findings suggest that the present telemetry guinea pig model is useful for QT evaluation in the early stages of drug development, because of the small body size of guinea pigs and their action potential configuration, which is similar to that of humans.

KEY WORDS: Telemetry system, Guinea pig, QT interval, QTc, In vivo

INTRODUCTION

QT interval prolongation caused by non-antiarrhythmic drugs is presently a matter of serious concern in the development of drugs because of the accompanying proarrhythmic potentials (Woosley et al., 1993; De Abajo and Rodriguez, 1999; Hill et al., 1998; Ohmura et al., 1999; Metzger and Friedman, 1993; Shah, 2002); thus QT assessment in the early stages of drug development is indispensable for preclinical drug evaluation (De Clerck et al., 2002; Picard and Lacroix, 2003). QT assessment can be examined in the early stages of drug development using cloned hERG channels, Purkinje fibers, papillary muscles, ventricular myocytes or Langendorff preparations. However, integrated effects of drugs on Purkinje fibers, M cells, endocardium, epicardium, channels, etc., can be examined only in in vivo animal models.

Rats, rabbits, dogs and monkeys are widely used for in vivo QT evaluation (Harada et al., 2002; Yoshida et al., 2002; Farkas et al., 2002; Horii et al., 2002). Guinea pigs are also used for QT evaluation in vivo.
under anesthesia (Kii et al., 2001; Chiang et al., 2002; Ohtani et al., 2001; Minematsu et al., 2001), because of their small body size, ease in handling and their action potential configuration, which is similar to that of humans (Nerbonne, 2000). An ideal in vivo animal model for QT evaluation should satisfy the following requisites; QT should not be influenced by anesthesia, the dose required should be small, animals should be easily obtained, and the ion channels of the animal heart should be similar to those of humans. Considering that using radio-telemetry one can monitor the electrocardiogram (ECG) in awake and free-moving animals, guinea pigs with a telemetry ECG system seem to satisfy all those requisites. However, there are few reports on drug-induced QT interval prolongation investigated in guinea pigs employing a telemetry ECG system (Gras, et al., 1996), and no precise investigation of a telemetry guinea pig model for in vivo QT evaluation has been published as yet.

The purpose of this study was to estimate the feasibility of a telemetry guinea pig model for in vivo QT evaluation using positive/negative reference compounds that are well known to/not to induce QT interval prolongation in humans. The reference compounds were selected from each of several categories based on their mechanism of QT prolongation in humans; bepridil (class IV anti-arrhythmic drug, Ca** blocker), terfenadine (anti-histaminic), cisapride (gastrokinetic drugs), haloperidol, pimozide, thioridazine (psychiatric drugs), quinidine (class Ia anti-arrhythmic drug), E-4031 (IKr blocker), propranolol (class II anti-arrhythmic drug, β-blocker) and nifedipine (Ca** blocker). We also investigated the relationships between drug concentration in blood and QT interval prolongation in guinea pigs.

**MATERIALS AND METHODS**

**Animals**

Male guinea pigs (Crj:Hartley, Charles River Japan Inc., Kanagawa, Japan) were used in this study. Animals were maintained on a 12-hr light/dark cycle (light on at 7:00) and kept in individual cages. All procedures involving animals were approved by the Animal Care and Use Committee of Pfizer Global Research & Development Nagoya Laboratories.

**Transmitter implantation**

A transmitter (TA11CA-F40, Data Sciences International Inc., St. Paul, MN, U.S.A.) was implanted into a subcutaneous pocket made in the right or left flank under isoflurane anesthesia. Bipolar electrodes for ECG were implanted to record lead Apex-Base ECG; one lead (negative pole) was placed between the scapulas, and the other (positive pole) was positioned around the sternum to obtain a clear T-wave. All animals were subcutaneously administered antibiotics (Mycillin solution, 10 mg/kg, Meiji Seika Kaisha, Ltd., Yokohama, Japan) once after the operation, and allowed to recover for 7 days before administration of the test compound.

**Reference Compounds**

Bepridil (10 mg/kg, Sigma Chemical Co., St. Louis, MO, U.S.A.), terfenadine (4 mg/kg, ICN Biomedicals Inc., Costa Mesa, CA, U.S.A.), cisapride (3 mg/kg, synthesized by Pfizer Inc., Kent, U.K.), haloperidol (2 mg/kg, Sigma Chemical Co.), pimozide (5 mg/kg, Sigma Chemical Co.), quinidine (30 mg/kg, Sigma Chemical Co.), E-4031 (0.1 mg/kg, Sigma Chemical Co.) and thioridazine (6 mg/kg, ICN Biomedicals Inc.) were used as positive references in this study. Propranolol (3 mg/kg, AstraZeneca, Osaka, Japan) and nifedipine (0.2 mg/kg, Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as negative references. The dose levels of the reference compounds were referred to the doses used in dog experiments to assess QT prolongation, which were relevant to the exposure levels in humans (Tashibu, 2004; Toyoshima, 2004). Saline was administered (i.v.) as control. For intravenous bolus administration (n=6), E-4031 and thioridazine were dissolved in distilled water, and the others were dissolved in distilled water containing 0.5-10% lactic acid (Kanto Chemical Co., Inc., Tokyo, Japan). Each animal was used to test up to 3 compounds, at intervals of 3-5 days.

**Data Collection**

ECG data were collected continuously using the Dataquest ART data acquisition system (Data Sciences International Inc.), and the RR and QT intervals were automatically analyzed using the HEM ver. 3.4 (NOTOCORD SYSTEMS, Croissy-sur-Seine, France). Signals were sampled at 1 kHz, and noises centered at 50 Hz (HAM) were removed. ECG was recorded 24 hr prior to compound administration, because Akita et al. (2001) reported circadian rhythms in some guinea pigs, and 5, 10, 20, 30, 40 and 60 min post-dose. The RR and QT intervals were calculated as the average value from ECGs recorded during 1 min. The Bazett formula QTc = QT/RR1/2 is widely used to correct the QT interval for the variation of the RR
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interval, but we used QTc = k × QT/RR$^{0.5}$, which was best fitted for a telemetry guinea pig model (data not shown).

Pharmacokinetic experiments
Pharmacokinetic experiments of the 8 positive references (bepridil, terfenadine, cisapride, haloperidol, pimozide, quinidine, E-4031 and thioridazine) were performed using other sham-operated animals to avoid possible effects of blood sampling on the heart rate (n=3). Blood samples (approximately 0.3 mL) were collected at 5, 30 and 60 min after intravenous administration, and centrifuged to obtain serum samples. Serum samples of all test compounds except for saline were mixed with methanol and buspirone (Sigma Chemical Co.) as the internal standard. Serum concentrations were analyzed by high-pressure liquid chromatography using YMC J'sphere ODS L-80 column (35 mm × 2.0 mm, YMC Co., Ltd., Kyoto, Japan) and determined by tandem mass spectrometric detection operating in positive ion mode using a model API3000 mass spectrometer (MDS SCIEX, Concord, Canada).

Statistical analysis
The mean of QTc changes in each test compound was compared with that in saline using Student’s t-test (JIS, 1965). A p<0.05 was regarded as statistically significant. When no homogeneity of variance was obtained by F test (JIS, 1965), the data was analyzed by Welch’s t-test (Welch, 1938).

RESULTS

ECG morphology in guinea pigs
A typical example of normal ECG and drug-induced QT prolongation recorded by the telemetry system is shown in Fig. 1. The ECG recorded with lead Apex-Base always showed a clear and positive T-wave. Even if the RR interval decreased and/or the QT interval was prolonged, the endpoint of one T-wave did not fuse into the next P-wave, and the electrical baselines between the T-wave and the next P-wave were distinguishable regardless of the reference compound.

Effects of the test compounds on QTc
All 8 positive references prolonged the QTc, and except for haloperidol the prolongation was statistically significant (Fig. 2). Of these positive references, bepridil, pimozide, terfenadine and quinidine transiently decreased the QTc 5 min after the administra-

tion. The maximal QTc prolongation was observed at 10 min after the E-4031 treatment, and at 30-40 min after treatment with either bepridil, cisapride, haloperidol, quinidine or thioridazine. Terfenadine and pimozide gradually increased the QTc until 60 min after the administration. The 2 negative references, propranolol and nifedipine, tended to reduce QTc 5-10 min after administration and then the QTc recovered gradually until the 60 min time-point. Saline induced a minimal QTc change until 60 min post-dose.

As for the effect of the drugs in each animal (Fig. 3), all compounds, except for saline, propranolol and nifedipine, induced 10% or more maximum QTc prolongation at the dose levels used in the present study, and all 8 positive references, except for haloperidol, induced a statistically significant QTc prolongation. The number of animals showing 10% or more maximum QTc prolongation was 6 in the quinidine treatment, 5 each in the bepridil, terfenadine and cisapride treatment, 4 in the pimozide treatment, 3 each in the E-4031 and thioridazine treatment, and 2 in the haloperidol treatment. The maximum QTc prolongation was 0-6% in the saline, propranolol and nifedipine treatments. One animal each in the terfenadine and cisapride treatments showed only a slightly prolonged

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**Fig. 1.** Typical example of normal ECG waveforms and drug-induced QT prolongation recorded by the telemetry system. The administration of bepridil (10 mg/kg) changed the values of QT interval from 118 (QTc; 243 msec) to 143 (QTc; 292 msec) 60 min after administration. Voltage calibration denotes 1 mV. Scale bar: 100 msec. Dotted line: the end of T-wave at pre-dose. Solid line: the end of T-wave at post-dose.
Fig. 2. Plots of means plus S.D. of RR interval (△) and QTc (▽) versus time after the dosing of saline and test compounds (n=6): bepridil (10 mg/kg), terfenadine (4 mg/kg), cisapride (3 mg/kg), haloperidol (2 mg/kg), pimozide (5 mg/kg), quinidine (30 mg/kg), E-4031 (0.1 mg/kg), thioridazine (6 mg/kg), propranolol (3 mg/kg) and nifedipine (0.2 mg/kg). *p<0.05, **p<0.01 compared with the values of QTc changes of the saline treatment at the same time point. The P values for pimozide at 5 min, propranolol at 10 min and nifedipine at 5 min after administration were significant, because QTc was shortened rather than lengthened.
Effects on RR interval

E-4031, terfenadine, haloperidol, thioridazine, nifedipine and saline tended to reduce the RR interval 5 min after administration (Fig. 2). On the other hand, bepridil, cisapride and pimozide tended to induce prolongations of the RR interval. The dispersion of the RR interval was obviously larger than that of the QTc in all treatments including saline.

Arrhythmia induction

In the haloperidol treatment, changes of the T-wave from positive to flat were observed around 10-20 min after administration (Fig. 4). No other obvious changes, such as ventricular premature contraction or Torsades de Pointes, were detected with any compound, except for convulsions observed in 1-4 of 6 animals immediately after administration of bepridil, pimozide and quinidine.

Pharmacokinetic analysis

The serum concentration of each of the 8 positive compounds decreased during the observation period of 60 min after administration, and the order of drug elimination rate was as follows: E-4031 > quinidine, haloperidol > terfenadine, cisapride > pimozide > thioridazine > bepridil (Table 1). When the values of the QTc changes at 5, 30 and 60 min were plotted against the serum concentrations, the resulting curves exhibited an anticlockwise hysteresis loop: this was observed with all positive compounds (Fig. 5). In the bepridil treatment, the values at 30 min were not obtained because the retraction of the blood vessel prevented blood sampling.

DISCUSSION

Guinea pigs have attracted a great deal of attention for in vivo QT assessment of newly discovered drugs at the pre-clinical stage because of their small size and their action potential configuration, which is similar to that of humans. Indeed, De Clerck et al. (2002) proposed that the anesthetized guinea pig model was a useful tool for first line in vivo investigation of the effects of a new chemical entity on cardiac electrophysiology. The purpose of this study was to estimate the feasibility of a telemetry guinea pig model for in vivo QT evaluation using 10 reference compounds: 8 positive references that are well known to prolong and 2 negative references that are thought not to prolong the QT interval in humans. The dose levels of the reference compounds were referred to the doses used in dog experiments to assess QT prolongation, which were relevant to the exposure levels in humans (Tashibu, 2004; Toyoshima, 2004).
Since changes in QT interval are known to inversely correlate with changes of the heart rate in guinea pigs (Hayes et al., 1994; Hamlin, 2003), we have compared various QT correction formulas with RR interval such as RR$^{1/2}$ (Bazett’s), RR$^{1/3}$ (Fridericia’s) and logRR (Matsunaga’s) (Bazett, 1920; Fridericia, 1920; Matsunaga et al., 1997), and have used the best fitted one, QTc = k × QT/RR$^{1/2}$ (data not shown), for this telemetry guinea pig model. Saline, used as control, showed less than 3% QTc change 5 min after administration, although the RR interval transiently decreased by 20% or more, and we could reconfirm the adequacy of our QT correction formulas.

The 8 positive references induced a QTc prolongation; however, the patterns of QTc prolongation were not the same. A shortening of the QTc was observed 5 min after the administration of 4 positive compounds (bepridil, terfenadine, pimozide and quinidine) and of the negative references (propranolol and nifedipine), but the reason for it was unclear. With the 4 positive references, the QTc was prolonged after 5 min and significant QTc prolongation was observed. Although with 6 of the 8 positive references the maximal QTc prolongation was observed at 10-40 min followed by a

Table 1. Serum concentrations of test compounds after the dosing of 8 positive references (n=3). Values are the mean ± S.D. The elimination rates were calculated by dividing the mean values at 60 min by those at 5 min. In the bepridil treatment, the value at 30 min was not obtained because retraction of the blood vessel prevented blood sampling.

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>Serum concentration (ng/mL)</th>
<th>Elimination rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Bepridil (10 mg/kg)</td>
<td>1907 ± 187</td>
<td>NA$^a$</td>
</tr>
<tr>
<td>Terfenadine (4 mg/kg)</td>
<td>224 ± 44</td>
<td>131 ± 43</td>
</tr>
<tr>
<td>Cisapride (3 mg/kg)</td>
<td>331 ± 72</td>
<td>195 ± 66</td>
</tr>
<tr>
<td>Haloperidol (2 mg/kg)</td>
<td>271 ± 39</td>
<td>129 ± 33</td>
</tr>
<tr>
<td>Pimozide (5 mg/kg)</td>
<td>130 ± 21</td>
<td>113 ± 13</td>
</tr>
<tr>
<td>Quinidine (30 mg/kg)</td>
<td>5513 ± 750</td>
<td>3387 ± 378</td>
</tr>
<tr>
<td>E-4031 (0.1 mg/kg)</td>
<td>16.6 ± 6.09</td>
<td>6.34 ± 1.24</td>
</tr>
<tr>
<td>Thioridazine (6 mg/kg)</td>
<td>1028 ± 128</td>
<td>821 ± 152</td>
</tr>
</tbody>
</table>

$^a$ NA=Not applicable

Fig. 5. Relationships between the values of QTc changes of 8 positive references and drug concentrations in serum. Values are the mean of QTc changes (n=6) and that of drug concentrations in serum (n=3). The values of QTc changes were plotted against the serum concentrations. Each arrow indicates an elapse time.
Telemetry system for QT evaluation in guinea pigs.

recovery, terfenadine and pimozide induced the maximal QTc prolongation at 60 min. These findings suggested that in some cases it may not be appropriate to evaluate the effects of drugs on the QT interval using the telemetry guinea pig model shortly after administration of a test compound.

Drug-induced QTc prolongation was statistically significant in all positive reference treatments, except for haloperidol, at the dose used in this study (Fig. 2). Moreover, a nearly 10% QTc change seems to be a criterion for detecting drug-induced QTc prolongation. Using this criterion, the effect of haloperidol on QTc prolongation could be detected in 2 animals (Fig. 3). Thus, we conclude that it is useful for QT evaluation of drugs to investigate both the time course of mean QTc values and the maximum QTc prolongation in each animal.

Changes of the T-wave shape from positive to flat were observed in the haloperidol treatment. This finding coincides with the effect, called “T-wave flattening” or “flattening of T-wave”, observed after intravenous administration of haloperidol in clinical cases. There are several clinical reports that T-wave flattening accompanied by QTc prolongation may indicate the possibility of developing Torsades de Pointes (Hunt and Stern, 1995; Hassaballa and Balk, 2003; Tisdale et al., 2001; Hatta et al., 2001; Douglas and Block, 2000; Metzger and Friedman, 1993). Therefore, this telemetry guinea pig model may be useful for a morphological evaluation of the T-wave as well as QT interval prolongation.

In this study, all test compounds were administered intravenously to reduce the amount of compound used on the assumption that in the early stages of drug development there is not too much available; however, we detected QTc prolongation with all 8 positive references from the results of time course of mean QTc values or the maximum effects on QTc in each animal, even with those intended for p.o. administration. When the values of QTc changes were plotted against the serum concentrations, the resulting curves exhibited an anticlockwise hysteresis loop for all the test compounds. This may be because of the delayed distribution of compounds into the ventricle. In fact, Minematsu et al. (2001) detected a delayed distribution of tacrolimus (FK506), an immunosuppressant, to the ventricle in guinea pigs, and showed that the observed QTc prolongation paralleled the actual ventricular concentrations with no lag-time. The present results suggest that although the blood concentration rapidly decreased after intravenous administration, as a result of the delayed distribution, a QTc prolongation comparatively similar to that observed after p.o. administration was detected in this telemetry guinea pig model.

In conclusion, we evaluated a telemetry system for its usefulness regarding QT evaluation in conscious free-moving guinea pigs, using positive/negative drugs that are well known to/not to induce QT interval prolongation in humans. The results suggested that the present telemetry guinea pig model is useful to evaluate the QT interval in the early stages of drug development, because of the small size of guinea pigs and their action potential configuration, which is similar to that of humans.

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