Telemetry studies in the minipig with reference drugs

Stéphane Loriot, Anne-Marie Bédat, Roy Forster and Alain Simonnard.
CIT, Evreux, France

Introduction
The ICH 57E Guideline requires the use of the most appropriate in vivo species for the assessment of QT prolongation. The distance potential to delay ventricular repolarization. Dogs and monkeys are non-rodent species usually used for these studies. However, minipig is an interesting model for cardiovascular studies due to the similarities of their cardiac anatomy and physiology with humans. The aim of this study was to evaluate whether QT prolongation could be detected in minipigs after treatment with 4 reference compounds.

Materials and Methods
Göttingen minipigs were implanted with internal cardiovascular telemetry devices (TL1M0-210-D700-001 Data Sciences International) in lead II position at least 3 weeks before the beginning of the study.

Details of the treatment schedule is presented in the Table below:

<table>
<thead>
<tr>
<th>Reference item</th>
<th>Dose levels (mg/kg)</th>
<th>Route of administration</th>
<th>Number of animal tested/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dofetilide</td>
<td>0.03, 0.1 and 0.3</td>
<td>Oral</td>
<td>n=5</td>
</tr>
<tr>
<td>Astemizole</td>
<td>10</td>
<td>Oral</td>
<td>n=4</td>
</tr>
<tr>
<td>Sotalol</td>
<td>20</td>
<td>Oral</td>
<td>n=4</td>
</tr>
<tr>
<td>Cipropoxacin</td>
<td>30-100 and 200</td>
<td>Oral Inotropic</td>
<td>n=5</td>
</tr>
</tbody>
</table>

Before the first administration of each reference compound, a single oral dose of the vehicle only (VBR 0.5% methylcellulose aqueous solution) was administered by gavage to each animal. Between dose-levels, a washout period of at least 3 days was respected.

Dofetilide, astemizole and sotalol are known to prolong QT interval. Sotalol is also known to increase PR interval and decrease heart rate. No effects on cardiovascular parameters or ECG recordings had been reported during previous in vivo preclinical studies with cyproxacin by Toyoshima et al. The minipigs were deprived of food overnight before each treatment and were fed no sooner than 7 hours after administration of the test item.

Cardiovascular parameters (mean arterial pressure, diastolic and systolic arterial pressure), ECG parameters (PR, QRS, QT and RR, and heart rate) and body temperature were continuously monitored for 2 hours before administration of the test item and for at least 20 hours after each dose administration using HEM v.4.2 software (Notocord System). QT intervals were corrected according to the formulas of Bazett (QTb), Fridericia (QTf) and Van de Water (QTv) (2, 3, 4), the duration of systole in the electrocardiogram of normal subjects and of patients with heart disease (5), Bazett HC. An analysis of the time-relations of electrocardiograms. Heart. 1920, 7:353-370.

Dofetilide: (Fig 1)
Slight increases in QT and corrected QT were observed at 0.03 mg/kg. Dofetilide at 0.1 and 0.3 mg/kg induced dose-dependent prolongations of QT and corrected QT. After the 0.3 mg/kg administration of Dofetilide, the maximum increase was noted at 30 minutes: + 53 ms (+17%) for QT, + 56 ms (+18%) for QTb, + 84 ms (+28%) for QTf and + 56 ms (+18%) for QTv when compared to the vehicle administration.

No effects on arterial pressure or heart rate were observed after 0.03, 0.1 and 0.3 mg/kg of dofetilide.

Astemizole: (Fig 2)
Astemizole (100 mg/kg) induced significant prolongations of QT and corrected QT, with peak responses approximately 90 minutes post administration: + 46 ms (+15%) for QT, + 76 ms (+22%) for QTb, + 65 ms (+20%) for QTf and + 55 ms (+17%) for QTv when compared to the vehicle administration. No effects on arterial pressure or heart rate were observed after 0.03, 0.1 and 0.3 mg/kg of astemizole.

Sotalol: (Fig 3 to 5)
There was an initial slight increase in heart rate, this was attributed to the stress of manipulation and gavage, since it was also seen in vehicle treated animals (Fig 4). Sotalol induced a decrease in heart rate from 30 to 420 minutes (-25 to -30 %). These results were confirmed by the increase in RR interval (around 40% from 75 to 150 minutes).

PQ interval was also increased after 20 mg/kg of sotalol from 60 to 420 minutes (+10% to +6%). QT, QTf and QTv intervals were increased from around 30 minutes after dose administration, with a peak after 120 minutes (+85 ms (+28%) for QT, + 50 ms (+15%) for QTf, and + 62 ms (+30%) for QTv when compared to the vehicle administration.

No effects were observed on arterial pressure.

Ciprofloxacin
Ciprofloxacin had no effect on any parameter at any of the dose-levels tested (30, 100 and 300 mg/kg).

Dofetilide 0.03 mg/kg
Dofetilide 0.1 mg/kg
Dofetilide 0.3 mg/kg
Vehicle

Reference item

<table>
<thead>
<tr>
<th>Dose levels (mg/kg)</th>
<th>Route of administration</th>
<th>Number of animal tested/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dofetilide 0.03 mg/kg</td>
<td>Oral</td>
<td>n=5</td>
</tr>
<tr>
<td>Dofetilide 0.1 mg/kg</td>
<td>Oral</td>
<td>n=4</td>
</tr>
<tr>
<td>Dofetilide 0.3 mg/kg</td>
<td>Oral</td>
<td>n=4</td>
</tr>
<tr>
<td>Astemizole 10 mg/kg</td>
<td>Oral</td>
<td>n=4</td>
</tr>
<tr>
<td>Sotalol 20 mg/kg</td>
<td>Oral</td>
<td>n=4</td>
</tr>
<tr>
<td>Cipropoxacin 30-100 and 200</td>
<td>Oral Inotropic</td>
<td>n=5</td>
</tr>
</tbody>
</table>

Table 1: Details of the treatment schedule.

Conclusion
The results of this study demonstrated that the circadian cycle, as well as gavage and feeding have a considerable influence on blood pressure and heart rate. This should be taken into consideration when adapting the design of cardiovascular studies to experimental animal models and interpreting results. The timing of oral test item administration should be chosen taking into account the times and half-life of the compound. If possible, the study should be designed to enable the expected effects of the test item to be recorded during the same photoperiod as pre-treatment data. If drug-induced effects are expected rapidly after oral test item administration, minipigs seem to be a favourable choice of animal model, since cardiovascular parameters are less disturbed in minipigs after the gavage procedure than in the other models tested. The results of this study also indicate that animals should be fasted for several hours before the beginning of the recording period.

Bibliography
2- Bazett HC. An analysis of the time-relations of electrocardiograms. Heart. 1920, 7:353-370
5- Bazett HC. An analysis of the time-relations of electrocardiograms. Heart. 1920, 7:353-370
6- Fridericia LS. The duration of systole in the electrocardiogram of normal subjects and of patients with heart disease. Ark Medica Scandinavica. 1900, 50: 439-466