Introduction

During the last decade, great attention has been given to early safety pharmacology assessment and to the use of small animal models for the early evaluation of effects on cardiovascular function during the pharmaceutical development process. Among these models, the guinea-pig is now well established as one of the most predictive for the assessment of QT lengthening and arrhythmia inducing potentials of drug candidates, with the following advantages:

• Intra-animal variability in terms of ECG parameters (QT, RR) in the guinea-pig is much lower than in the dog or monkey.

• The respiration signal was amplified (EMKA Technologies) and continuously recorded (IOX, EMKA Technologies, France) in the case of statistically significant treatment/time interaction.

• A minimal amount of compound is needed for guinea-pig treatment, making them a convenient choice for early safety pharmacology programmes.

The guinea-pig is a well established model for the evaluation of respiratory function. ECG recording in anaesthetized the guinea-pig was proposed by De Clerck et al. in 2002 for the in vitro screening of effects of new chemical entities on cardiac electrophysiology. Guinea-pigs are also easy to handle as a non-anesthetized model using implanted telemetry, but chronic implantation of telemetry devices in these animals requires surgical intervention that may be tedious, time-consuming and expensive. Therefore, the purpose of the present study was to evaluate the use of a jacketed external telemetry system, involving no surgery and no anaesthesia, for the assessment of potential effects on ECG and respiratory function in freely moving guinea-pigs.

Materials and methods

Animal model

Eight female guinea pigs (SFP Dunkin Hartley) weighing between 400 and 600g were used in the present study. Animals were fasted prior to the days of treatment, but were not deprived of water. Electrodes of a DSI transmitter, TL11M2-C50-PXT were placed on the shaved skin of the animal in lead II position. The transmitter was then placed in a pocket of a small external jacket worn by the animal. A habituation period of at least 30 minutes was allowed before treatments.

Test items

To evaluate the sensitivity of the model, low doses of sotalol (10 mg/kg) and clonidine (5 mg/kg) were tested in the present study. Test items and vehicle (0.5% methylcellulose) were administered by the oral route. A washout period of at least 4 days was observed between each administration.

ECG recordings

ECG signals were recorded continuously on-line at a rate of 200 Hz using the program ART Analóg (Data Sciences International), then stored and analyzed using a second program (HEM, Natocord). Heart rate (HR, beats/min) and ECG parameters (PR, QRS, QT and QTc interval durations (ms)) were calculated from 10 beats in the minute preceding the time points of 0, 30, 60, 120, 180 and 240 minutes after dosing. QTc values were obtained by correction of QT with Bazett’s formula.

Recording of Respiratory parameters

Measurements of respiratory parameters were performed by whole body plethysmography. Each animal was placed in a recording chamber and the respiration signal was recorded for at least 1 hour before drug administration to obtain baseline values. After stabilization of its respiratory rate, the test item or vehicle was administered to the animal, which was then returned to the recording chamber. A habituation period of at least 30 minutes was allowed before treatments.

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• Respiratory parameters (breaths per minute, BPM), peak inspiratory and expiratory flows (PIF and PEF, mL/s), respiratory rate (breaths per minute, BPM).

• Tidal and minute volumes (TV and MV, mL). Statistical analyses

Statistical analyses

Statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc.). Two-way ANOVA analysis of variance (ANOVA) for repeated measurements was performed using SAS PROC MIXED. One-way ANOVA followed by a Dunnett test (if one way ANOVA p-value < 0.05) was implemented at each time-point in the case of statistically significant treatment/time interaction.

Results

Effect of sotalol (10 mg/kg) on ECG parameters

Sotalol induced a significant decrease in heart rate, although a smaller decrease was also observed for this parameter for up to 2 hours after treatment with the vehicle. Increases in PR interval with no noticeable effect on QT were observed (Figure 1). Sotalol induced increases in QT and QTc to a lesser extent in GTC, with the maximum difference from controls observed at 1 hour after treatment.

Effect of clonidine (5 mg/kg) on cardio-respiratory parameters

Alterations in respiratory parameters were observed for at least 30 minutes after administration of the vehicle (figure 2). These alterations were attributed to the handling stress produced in these animals by the treatment procedure.

When compared to the vehicle, clonidine induced significant increases in respiratory rate at 60 and 90 minutes after administration. Significant decreases in tidal volume, minute volume, peak inspiratory and expiratory flows were also observed for up to 45 or 60 minutes after administration of clonidine, followed by statistically significant transient increases in these parameters at 90 minutes.

Clonidine at 5 mg/kg had no noticeable effect on ECG parameters, recorded simultaneously in the plethysmograph. Similar results were observed after administration of theophylline (data not shown).

Conclusion and discussion

Jacketed external telemetry (JET) provides a reliable approach for continuous ECG recording in conscious freely moving small animals. In this model significant increases in QT and QTc (Bazett) were observed after oral administration of a low dose of sotalol (10 mg/kg).

Simultaneous recording of ECG and respiratory parameters are easily achieved by placing jacketed external telemetry devices in plethysmographs. Under these conditions, treatment with clonidine resulted in well established changes in respiratory parameters, without disturbance of the ECG. These results support the utility of jacketed external telemetry for the investigation of cardio-respiratory functions in the conscious guinea-pig in early safety pharmacology screening programs.

This model does not require surgery of anaesthesia and is cost efficient with a fast turnover time, since the same group of animals can be treated several times after an appropriate washout period, respecting the 3R’s principles.

References


• Leodres, B. et al., Poster presentation at 5PS Annual Meeting, Strasbourg, France, September 2009.