Introduction

QT interval prolongation constitutes a critical issue in the development of a new drug-candidate (Hanson et al, 2006). A recent methodological refinement in non-clinical safety pharmacology telemetry studies designed to detect drug-induced QT prolongation is the probabilistic method (Holzgrefe et al, 2007). This is the method that we use routinely in our laboratory in association with the QT shift method in large animals such as dogs and Cynomolgus monkeys (Champerouex et al, 2009).

In the present study, we have developed this methodology in conscious Guinea pigs. The principle is based on the probabilistic determination of individual QT/RR relationships from a beat-to-beat ECG analysis over a 24-hour treatment-free period. The QT shift method consists of a direct reading of the drug-induced QT interval shift in relation to the normal individual QT/RR relationship. Accuracy and reliability of this method were tested by using several reference drugs such as sotalol, thioridazine, doxifilitide, terfenadine, haloperidol, cisapride and mofloxicacin.

Material and methods

Animals: 8 Guinea pigs (3 males and 5 females) (Charles River Laboratories, France), weighing between 704 and 800 g on the first day of the study.

Instrumentation: Animals were instrumented at least 3 weeks before the beginning of treatments. The instrumentation was performed under aseptic conditions using Ketamine 40 mg/kg and Dexametadomidine 0.5 mg/kg i.m. At the end of the surgery anesthesia was reversed with Atipemazole 0.5 mg/Kg. A TL11M2C50PXT telemetric transmitter (DSI, USA) was placed in the peritoneal cavity. The electrodes of transmitters were placed in Lead II using subcutaneous electrodes.

Housing and dosing: During the telemetry recording periods, animals were housed in individual cages of standard dimensions, in air conditioned animal house, 20-24°C, 12:12 hours light cycle (light on at 7.30 a.m.), with solid diet and drinking water provided ad libitum. Treatments with drugs were performed between 3.00 p.m. and 5.31 p.m.

Telemetry ECG data acquisition and analysis: ECG waveforms were continuously recorded at a sampling rate of 500 Hz using the ART™ acquisition software release 4.2 (DSI, USA). Cardiac conduction times including QT interval were calculated from a beat-to-beat analysis using an internal software in RPL (RS/1 programming language, RS/1 release 6.3, Applied Materials), GLP validated.

The individual control QT/RR relationship was built from data recorded during a 24-hour treatment free period. All collected QT/RR pairs were sorted by RR values into successive 10 ms increments. Reduced mean values of QT and RR intervals were calculated for each 10 ms RR increment. A minimum of 250 QT/RR pairs were computed for each 10 ms RR increment. The QT/RR relationship was well defined within the range of 200 ms – 300 ms for RR values.

QT shift calculation: The QT shift values were calculated from the difference between the post-dosing value of QT (QTpd) (1) and the control QT value (Q) derived from the control individual QT/RR relationship for the same post-dosing RR value. The difference (QTpd-QTcontrol) is named QT shift.

Statistical analysis: The effects of reference substances were compared with those of the vehicle by analysis of variance for repeated measurements with a multiple comparison test i.e. Least Significant Difference’s test (P≤0.05) (Winer, 1971).

Depending on the reference substance assessed, the study involved n=5 or n=6 animals for the statistical analysis.

Results

Effect of sotalol 30 mg/kg (1) vs vehicle (1)

Effect of terfenadine 100 mg/kg (1) vs vehicle (1)

Effect of cisapride 6 mg/kg (1) vs vehicle (1)

Effect of thioridazine 20 mg/kg (1) vs vehicle (1)

Effect of haloperidol 10 mg/kg (1) vs vehicle (1)

Effect of doxifilitide 3 mg/kg (1) vs vehicle (1)

Effect of mofloxicacin 90 mg/kg (1) vs vehicle (1)

• A very good correlation between results of QT shift calculations and QT corrected data according to the Holzgrefe formula was observed
• The results showed a very high sensitivity of the QT shift method in the Guinea pig.

The statistical sensitivity threshold for detection of QT prolongation according to a standard study design including 6 Guinea pigs, 3 males and 3 females is excellent (3 – 4 ms): Parameters Units Statistical sensitivity

| QTc (Holzgrefe formula) | ms | 3.7 |
| QT shift | ms | 3.2 |
| QT interval | ms | 6.0 |
| PQ interval | ms | 1.4 |
| PR interval | ms | 1.6 |
| QRS complex duration | ms | 0.3 |
| Heart rate | bpm | 11.8 |
| Body temperature | °C | 6.2 |

Statistical sensitivity: smallest change being statistically significant derived from the LSD’s test, when compared to control group

• Very low sensitivity thresholds were also found for the other cardiac conduction times in the Guinea pig

Conclusion

The results showed a very high sensitivity of the QT shift method in Guinea pigs. The QT shift method applied in this species, in combination with the probabilistic method described by Holzgrefe, provides a reliable and precise assessment of drug-induced QT interval prolongation that can be particularly helpful in the early phase of development of a new drug-candidate.

References