Introduction

Laboratory specific environmental controls and activities can result in a great deal of variability in data collected at individual test sites. Therefore, it is of great importance for each site to perform verification studies to supplement other validation activities. Prior to the initiation of verification studies, proper consideration must be given to the design and execution of the study to avoid complications and maximize success. A clear dose response should be considered as a main goal. Additionally, sensitivity of the model should be established using statistical power analysis. For this reason, dose levels that produce subtle changes should be included to provide insight to the range of sensitivity one could expect from a particular study design.

The FDA developed specific guidelines (ICH S7E) to provide strategies for assessing the potential of a test substance to prolong the QT interval. Prolongation of the QT in known to be associated with an increased risk of ventricular tachyarrhythmia and, in some cases, torsade de points (TdP) in humans. TdP is a potentially fatal form of polymorphic ventricular tachycardia associated with a prolonged QT interval.

In accordance with ICH guidelines, we designed this study with the purpose of evaluating the sensitivity and validity of the beagle dog telemetry model at Inotiv to be an effective prescreening predictor of QT prolongation in humans. The canine was selected as it is the most frequently used non-rodent species for studies of pharmaceutical products.

Dofetilide was selected as a positive reference compound as it is a Class III anti-arrhythmic known to cause QT prolongation.

Methods

Eight male beagle dogs that had been previously implanted with digital telemetry devices were used in this study. Digital L11 and L21 devices (Data Sciences International) were implanted onsite at Inotiv. The animals were administered 0.05% methylcellulose in water and doxetilide at doses of 0.001, 0.03 and 0.1 mg/kg using a Latin square design. The route of administration for all doses was oral gavage. There was a washout period of 7 days between doses.

Prior to dosing, all animals were acclimated to the room environment for minimum of 2 days. Inherent variability of the model was assessed with the collection of baseline data on separate occasions. All instances of data collection were interrupted at 24 h in duration. Clinical observations were made using video monitoring equipment in an effort to mitigate room disturbance. Rooms were cleaned and animals were fed each morning prior to data acquisition start.

A Data Sciences Telemetry Recording System with Ponemah (P3) software was used for recording and analysis of the physiologic data. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate, core body temperature (CBT), and lead II ECG were recorded. An ECG waveform morphology assay was completed for each dog at the 0.1 mg/kg dose level using P5 data inputs. Data was collected continuously from at least 2 h prior to dosing through 14 h post dose on each study day. Sampling rates for both ECG and all recording was set to 500 Hz. Derived parameters were automatically calculated by the P3 system.

All telemetry data was organized into phases (3 intervals of 10 minutes) for each 24-hour collection period. Each parameter was analyzed, by phase, with a repeated measure analysis of covariance (RANCOVA). Covariates included baseline data, dosing day, treatment, day after dosing and the interaction of each treatment group and time after dosing. SAS® software was used for the analysis procedure. A Dunnett’s test was used to compare the treated groups with vehicle control. For comparative purposes, this statistical analysis was performed using both a 24-hour baseline period collected prior to dose administration and the 2 hours baseline immediately preceding each dosing day.

Retropective statistical power analysis (RSPA) was conducted on the following parameters: HR, SBP, DBP, MAP, RR, PR, RR interval, QRS interval, QT, QTc, QTcV, QTcVW using different statistical methods. The first method used 8 animals from the study design. The second method involved using only 6 animals from the study design (first 4 animals with L21 implant and first 4 animals with implant L113). The third method involved using only 4 animals from the study design (all animals with L113 implant).

RSPA was conducted, and minimum detectable effect size (changes from control group) were calculated based on observed variance from the analysis model at a power of 0.8 and a significance level of 0.05. A noncentral t distribution was used for the power calculation. For each parameter, the standard deviation was calculated using the error degrees of freedom for the test of treatment effect in the statistical model and the median of standard errors for the difference in least square means between treated groups and the control group in all blocks of study.

Results

A dose dependent increase in QT interval was observed as expected (Figure 1). Control values observed during the course of the study matched those of previous historical data collected at the test site. ECG morphology changes after 0.1 mg/kg doxetilide induced several different conduction disturbances with the most common being a ventricular preexcitation and T-wave morphology changes. A dose response was observed at all 3 dose levels by plotting the relationship between uncorrected QT vs. HR. QT c 4 correlation factors analysed, QSVW and QTcI most appropriately corrected for HR [Figure 2]. Minimum detectable differences were calculated and reported for all parameters as the absolute change that would be detectable with a given statistical power [Table 1]. Power curves were generated to show the relationship between sample size (n=4 vs. n=6 vs. n=4).

As expected, statistical power increase with sample size. In this study, using n=4 animals, we were able to statistically identify an “120 ms change in QT produced with administration of doxetilide (Figure 3).

Conclusions

- Data from the 0.5% methylcellulose group matched previous Inotiv historical control data.
- Dofetilide at all 3 dose levels was associated with a significant increase in the QT.
- Conduction disturbances noted after administration of doxetilide were similar to results from similar studies found in scientific literature.
- QSVW and QTcI provided the most appropriate QT corrections across variable heart rates.

The n=4 Inotiv telemetry beagle dog model exhibits low inherent intra-animal variability and high sensitivity to detect small but significant increases in all parameters including QTcFI (140 ms minimum detectable change).