Triplicate ECGs are Sufficient in Obtaining Precise Estimates of QTcF

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BACKGROUND

Recently, there has been a trend towards intensive ECG collection in First-in-Human (FIH) studies to evaluate proarrhythmic liability. In this regard, there was an update in the ICH E14 Guidance Questions and Answers document in December of 2015[1] that specifically addressed intensive ECG collection and concentration-effect modelling in FIH studies as an alternative to performing a thorough QT (TQT) study. However, what was not delineated was the number of ECG replicates which should be obtained to optimize pharmacokinetic/pharmacodynamic analysis.

The impact of replicates on QTc variability has significant implications on both study design and cost.[2, 3, 4] Control of intrinsic and extrinsic factors such as circadian rhythm, food effect, postural changes, activity levels, and the conduct environment are critical for minimizing QTc variability and thus study costs.[5, 6, 7] The study population demographics and on-treatment effect can also influence QTc variance.[4] Increased variability due to any of these factors can lead to an increase in sample size for TQT studies which is not feasible for FIH studies. An alternative could be to increase the number of ECG replicates in order to reduce the QTc variability. Although this may allow for reduced sample size, increasing the number of replicates acquired does increase the ECG acquisition and analysis cost. As such, the purpose of this investigation was to determine the optimal number of ECG replicates required to obtain precise values of QTc in order to assess QTc prolongation risk of new compounds.

METHODOLOGY

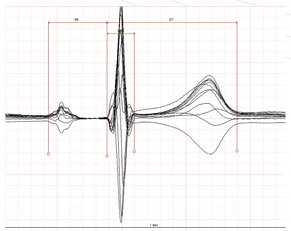
Thirty-six (36) healthy subjects were enrolled and 33 completed a double-blind, randomized 2-way crossover study comparing the effects of moxifloxacin (single dose of Avelox® 400 mg tablet) to matching placebo on QT corrected for heart rate using Fridericia's correction (QTcF). Subjects were stratified by gender and by ethnicity.

The study drug was administered after a 10 hour (h) fast. On the day of dosing, lunch was scheduled at 4.3 h postdose and had to be completed at least 1 h prior to the 6 h postdose ECG extraction. Dinner was scheduled at 9.5 h postdose and had to be completed at least 1 h prior to the 12 h postdose ECG extraction. Water was not permitted from 1 h before dosing until 1 h after dosing, with the exception of water administered with study drug.

Holter monitors were used to collect continuous 12-lead ECG data. At 10 pre-specified time points, 1 through 10 replicates of 10-second, 12-lead ECG recordings were extracted from the Holter data during a 5 minute window employing Antares® software. The arithmetic mean of the replicate values was used as the value for that time point. Baseline was the average of 3 separate predose time points, each with (n) replicate ECGs.

Celerion's ECG core laboratory uses a validated, highly automated method in which cardiologist review and adjudication is limited to ECGs demonstrating qualitative characteristics or quantitative parameters that may be associated with inaccurate automated measurement. All extracted ECG recordings were automatically measured by CAL ECG software from AMPS, LLC. The quality and interval values of the ECG recordings were assessed by the AMPS automated algorithm (FAT-QT) and ECG recordings meeting pre-configured criteria thresholds were directly entered into the database without cardiologist review. All ECG recordings not meeting the pre-configured criteria thresholds were assigned to a single board-certified cardiologist experienced in the interpretation and adjudication of pharmaceutical study ECGs. The cardiologist was blinded to subject, time, and treatment. All baseline and on-treatment ECG recordings were analyzed using a superimposed representative-complex method in which automatic calculation of a representative median beat, comprising all the raw beats considered as normal from each individual lead, was constructed. Subsequently, the individual lead median beats were then superimposed to generate a composite representative beat for all leads (Figure 1).

Figure 1. Superimposed Representative-Complex Method



For each postdose scheduled time point of ECG collection, the change from baseline in QTcF interval (ΔQTcF) was analyzed by a mixed-model, analysis of covariance (ANCOVA). The fixed terms in the model were treatment, sequence, period, and gender. Each subject's baseline value (average of 3 predose time points, each being the average of n replicates) was included as a covariate. The random term was subject nested within sequence. The statistical analysis was performed using SAS® PROC MIXED. Placebo-corrected, change-from-baseline in QTcF (ΔΔQTcF) was calculated as the difference between least-squares means (LSM) of moxifloxacin and placebo.

RESULTS

Results of "Standard" Triplicate ECGs

The magnitude and time course of ΔΔQTcF was typical for moxifloxacin. The estimates are shown for n=3 replicates in Table 1.

Table 1. Results of Analysis Based on 3 Replicates

h	Between-subject SD	Within-subject SD	ΔΔQTcF Estimate (ms)	SE of Estimate	One-sided 90% LCL	One-sided 90% UCL
0.5	1.12	5.22	9.65	1.27	7.50	11.80
1	0.99	4.22	10.61	1.03	8.87	12.35
2	2.63	4.17	11.87	1.02	10.14	13.60
2.5	3.59	3.39	12.68	0.84	11.25	14.10
3	3.14	4.61	12.84	1.13	10.93	14.76
3.5	3.53	3.64	13.06	0.90	11.53	14.58
6	5.59	7.69	9.27	1.89	6.06	12.48
7	6.27	4.54	8.79	1.13	6.87	10.71
12	4.92	5.80	8.57	1.43	6.14	11.01
24	3.04	4.49	5.54	1.10	3.67	7.40

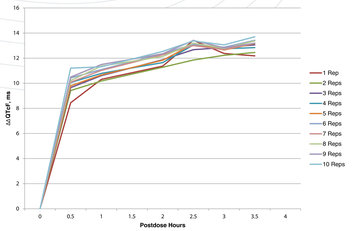
SD = standard deviation, QTcF = QT corrected for heart rate using Fridericia's correction, ΔΔQTcF = placebo-corrected change from baseline in QTcF, SE = standard error, LCL = lower confidence limit, UCL = upper confidence limit.

Maximum effect of moxifloxacin ΔΔQTcF (13.06 milliseconds (ms); 90% CI: 11.53 – 14.58 ms) occurred at 3.5 h postdose.

Effect of Number of Replicates on Moxifloxacin ΔΔQTcF

The effect of number of replicates on moxifloxacin ΔΔQTcF is shown in Figure 2. The maximum moxifloxacin ΔΔQTcF occurred at either 2.5 h (Replicates [Reps] 1, 4, and 9) or at 3.5 h (Reps 2, 3, 5, 6, 7, 8, and 10) with a median (range) magnitude of 13.29 ms (12.45 – 13.70 ms).

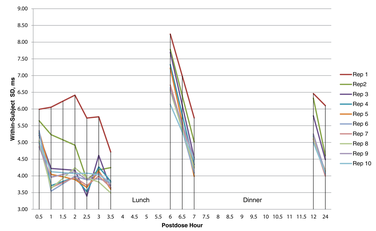
Figure 2. The Effect of Number of Replicates on Moxifloxacin ΔΔQTcF During Time Interval of Peak Effect



Effect of Number of Replicates on the Within-Subject Standard Deviation (SD) of ΔQTcF

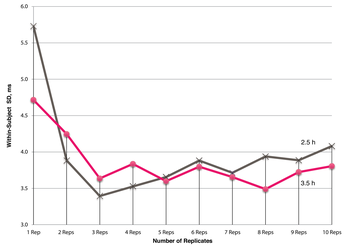
The effect of number of replicates on the within-subject variability of ΔQTcF is shown in Figure 3. During the first 3.5 h, where moxifloxacin has its peak effect on ΔQTcF, the within-subject SD was similar for number of replicates ≥3. There did not seem to be an advantage in terms of within-subject SD to increase the number of replicates above 3.

Figure 3. The Effect of Number of Replicates on the Within-Subject SD of ΔQTcF



Focusing on the 2 time points where maximum moxifloxacin ΔΔQTcF occurred (2.5 and 3.5 h postdose), the benefit of using up to 3 replicates was evident, with no advantage in higher number of replicates (Figure 4).

Figure 4. The Effect of Number of Replicates on the Within-Subject SD of ΔQTcF at 2.5 and 3.5 h Postdose



Possible Effect of Food Ingestion on Within-Subject SD of ΔQTcF

In order to control for factors that are known to increase the intrinsic variability of the QT interval, the study drug was administered in the fasted state and the first meal was scheduled after the anticipated peak effect of moxifloxacin. The within-subject variability of ΔQTcF at 6 h postdose may have been related to subjects having ingested lunch between 4.3 – 5 h postdose. Both placebo and moxifloxacin subjects had increased variability in ΔQTcF at 6 h postdose.

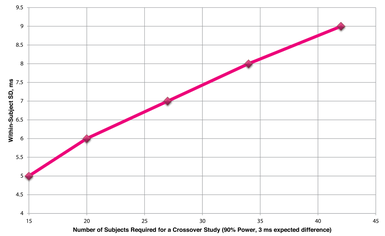
Translating Precision into Sample Size

The sample size for a TQT study is determined based on:[8]

- Within-subject variability (SD)
- Expected QTc prolongation of the drug
- Power of the test
- Number of time points at which the drug is expected to have a QT prolongation effect

Figure 5 shows how variability affects the number of subjects required in a crossover study, with an expected QTc prolongation effect of 3 ms, 90% power, and the assumption that the effect of 3 ms will only be manifested at 3 time points (e.g., around t_{max}).

Figure 5. The Effect of Within-Subject SD of ΔΔQTcF on Sample Size for a Crossover Study



DISCUSSION

The optimal number of ECG replicates required in QT intensive studies has been a matter of discussion for many years. While the FDA does not have any official position on the number of replicates, it suggests performing at least 3 over the course of 5 minutes at each nominal time point as part of study conduct particularly when doing PK/PD regression analysis. The results of this investigation lend support to this recommendation.

- Confirmation of moxifloxacin assay sensitivity was not affected by the number of replicates recorded. The peak ΔΔQTcF ranged from 12 to 14 ms and was observed between 2.5 to 3.5 h postdose.
- At the peak effect of moxifloxacin, the within-subject SD was lowest and under 5 ms when 3 or more ECG replicates were obtained. This is below the industry standard of approximately 8 ms for crossover studies.
- The greatest decremental change in within-subject SD was noted at approximately 6 and 12 h independent of the number of replicates acquired. These time points followed intake of meals.
- QT variability is affected by intrinsic and extrinsic factors and the low variability observed in this study may have resulted from rigorous control of key parameters including food intake, activity levels, and subject positioning during ECG acquisition.
- The variability estimates decreased rapidly as the number of replicates increased, underscoring that there is only a modest incremental reduction in QTc variability beyond 3 replicate ECGs.
- Low QT variability influences the power calculations in crossover studies and enables the recruitment of a smaller number of subjects for TQT studies.
- The use of a highly automated approach to QT measurement appears to provide the optimal balance between cost and precision regarding the number of replicates required in ECG intensive studies.

CONCLUSIONS

There is only a modest incremental reduction in QTc variability beyond the recording of 3 replicate ECGs and the magnitude of benefit would not seem to justify the additional resource expenditure to obtain a higher number of replicates. Acquiring additional replicates beyond 3 confers little increase in precision of the estimated QTcF value as the SD of QTc decreases rapidly as the number of replicates increases with little change after 3 replicates. This in turn has a direct effect on sample size as decreasing variability reduces the number of subjects needed for evaluating QTc prolongation risk and increasing feasibility of adding this assessment to FIH studies. As such, triplicate ECGs are sufficient to obtain precise estimates of ΔΔQTcF with acceptably low data variability in association with reduced cost of ECG acquisition. Finally, it appears that there is a constellation of intrinsic and environmental factors, such as the impact of meals, which may modulate QT variability and should be considered during drug development and study design.

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