CARDIAC SAFETY

Use of Continuous ECG for Improvements in Assessing the Standing Response as a Positive Control for QT Prolongation

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Background: Standing invoked change in QT interval has been identified as a promising autonomic maneuver for the assessment of QT/QTc prolongation in patients with underlying heart abnormalities or as a positive control in healthy volunteers for drug studies. Criticism for its more widespread use is the high variability in reported results and the need for a more standardized methodology with defined normal ranges.

Methods: Forty healthy male subjects underwent continuous ECG collection on the day before dosing in a double-blind, placebo-controlled, randomized, single ascending dose trial. A brisk supine to standing (3 minutes) response was conducted at three time points. Results were grouped by treatment cohort or assessed as a pooled group at each time point. Maximum time and median change from baseline ($\Delta T_{\max} \text{QTcF}$, ΔQTcF) were calculated for each individual over sequential 30-second periods staggered by 5 seconds.

Results: Maximum ΔQTcF at all time points and in all groups was significant (i.e., the lower bound of 90% CI was > 5 milliseconds) which is the ICH E14 regulatory requirement for a positive control. Variability of the time to maximum response was also reduced 9-fold by the third time period.

Conclusions: Standing invoked ΔQTcF can be utilized to validate the sensitivity of a study for assessment of the QT interval effect of drugs in early development. The methodology may be used to further improve its diagnostic use of long QT syndromes by reducing the variability and allowing adequate definition of normal limits.

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The QT interval shortens during brief tachycardia but lags behind the change in heart rate (RR interval) causing what is known as hysteresis.¹ Most correction factors do not account for this hysteresis and thus can error in their estimation of the corrected QT value depending on the timing of the measurement during heart rate acceleration or deceleration.^{2–4} This may result in large variability of reported QTc values for even simple autonomic maneuvers that invoke reflex tachycardia,⁵ such as standing.^{6,7} Other factors complicating the assessment of the QT/QTc interval changes include the individual and type of correction factor used,⁸

the disease state of the subject,⁴ the baseline conditions used (fully supine vs semirecumbent),⁹ and changes in the T-wave morphology¹⁰ to name a few.

Recently, standing invoked change in QT interval has been identified as a promising autonomic maneuver for the assessment of QT/QTc prolongation in patients with underlying heart abnormalities or as a positive control in healthy volunteers for drug studies. In 2010, Viskin et al.⁷ showed that QT interval changes induced by brisk standing could aid diagnosis of long QT syndromes. One criticism preventing its

widespread use is a need for a more standardized methodology with defined normal ranges.¹¹ Regulators and drug companies also are interested in this methodology to reduce costs and eliminate the need for separate treatment groups with positive pharmacologic control agents in clinical development. Currently, almost all new chemical entities must undergo a "thorough QT study (TQT)" that includes a positive pharmacological control for assay sensitivity of QT/QTc interval determination. The positive control should have an effect on the mean QT/QTc interval of about 5 milliseconds.¹² A single dose of the antibiotic, moxifloxacin that inhibits the hERG mediated I_{Kr} potassium current is most commonly used as a positive control in healthy volunteers. However, use of moxifloxacin is not feasible in all studies where early QT assessment may be desirable. Attempts have been made to perform near-TQT studies during First-In-Human clinical studies and although excellent sensitivity can be achieved it is recognized that a TQT will still need to be performed as the drug furthers in development.¹²⁻¹⁴

In the following study, we utilized continuous Holter electrocardiogram (ECG) to analyze 30-second sequences of beat-to-beat QT-RR data iteratively every 5 seconds of the recordings to maximize the detection of QTcF changes during initial heart rate acceleration upon standing. The purpose of the analysis was to determine whether adequate assay sensitivity could be achieved for detecting QTcF prolongation in early clinical studies allowing better development decisions before the more expensive TQT study.

METHODS

The data for analysis were collected during a double-blind, placebo-controlled, randomized, single ascending dose phase of a multipart early stage clinical trial with a new chemical entity (NCE), XEN-D0103. Data from 40 healthy male subjects (five cohorts of six subjects receiving active treatment and two receiving placebo) aged 18–45 years, inclusive were obtained. No subject was randomized more than once. Subjects attended a screening visit within 14 days before Day 1 of the study and were admitted to the clinical unit (ICON Development Solutions, Manchester, United Kingdom) on the evening of Day –1 and fasted overnight. Subjects underwent a 12-

lead Holter ECG recording beginning on Day 0, 25 hours before planned dosing on Day 1) and continuing until 24 hours postdose on Day 2. XEN-D0103 capsules or matching placebo for oral administration were given on Day 1 after a second overnight fast.

Subjects met inclusion criteria of body weight between 50 and 100 kg, and body mass index between 18 kg/m² and 32 kg/m², inclusive. Subjects were excluded if they had known heart disease or any of the following ECG findings: QTcF > 450 milliseconds, PR ≥ 210 milliseconds, or QRS ≥ 120 milliseconds, a resting heart rate outside of the range 45–80 bpm, systolic BP < 80 mmHg or > 160 mmHg or a diastolic BP > 90 mmHg or < 45 mmHg or any clinically significant abnormal laboratory test results at screening.

Standing Maneuver and ECG Collection

ECGs were collected during the study via a Mortara Surveyor Telemetry Central system (Milwaukee, WI, USA), used by trained staff in accordance with standard procedure. Stable supine ECGs were collected for safety analysis at specific time points. Before each measurement, subjects were in a stable, fully supine position and refrained from any activity that might change their heart rate. Subjects remained in the same supine position for 15 minutes. During the supine period, subjects did not move or speak, and the surrounding area was quiet (i.e., no television or radio). Subjects were not allowed to sleep during these periods. Subjects were instructed to stand briskly (approximately 2–3 seconds) from the supine position. The start and end times of each supine period were noted.

To assess study sensitivity to detect QTcF changes after exposure to the NCE XEN-D0103, a nonpharmacologic challenge of brisk standing was utilized on Day –1 before treatment at intervals which would encompass both predose and expected peak drug concentrations (4 and 8 hours) later in the study. A 3-minute standing response was conducted in all subjects then grouped by their treatment cohort or assessed as a pooled group.

ECG Analysis

The central ECG laboratory readers at iCardiac Technologies were blinded to treatment allocation

and sequence. ECGs from each single subject were reviewed by the same ECG analyst.

QTcF was calculated at sequential 30-second intervals staggered by 5 seconds (i.e., 0–30 seconds, 5–35 seconds, and so on) so that all beats from an individual at each time point were assessed to determine the largest median QTcF of each 30 sec interval and compared to the prestanding supine baseline value for change from baseline (i.e., ΔQTcF). The maximum median change from the supine baseline immediately before standing was obtained for each individual, at each time point, then averaged for a mean value and compared across predose treatment/pooled placebo groups and time point. The pooled average change in QTcF of all cohorts was determined. The duration of time to maximum ΔQTcF ($\Delta T_{\max}\text{QTcF}$) was also noted for each cohort and pooled response. Both the cohort and total pooled samples ($n = 40$) means were calculated to provide comparison of the mean and variability of a typical study sample size in relation to the larger population response.

Use of Automated High Precision ECG Analyses

Highly automated analysis, using COMPAS software, performed measurements of all ECG parameters of interest in all recorded beats that are deemed “high confidence.”¹⁵ All low confidence beats (as determined by signal/noise, RR, QT, T-wave morphology, and other variability in the ECG parameters) are reviewed and overread by technologists with subsequent QC by Cardiologists in the same manner as in the conventional semiautomated ECG analysis. The beats found acceptable by manual review are included in the analysis. All data entry, analysis and review followed iCardiac standard Quality Control processes.

Statistical Analysis

All statistical analysis was performed using the statistical software R for Windows version 2.11.1.¹⁶ The change-from-baseline (i.e., the supine period immediately before standing) QTcF, QT, HR, RR, and the duration of time to maximum ΔQTcF was calculated using mean and 90% CI based on descriptive statistics.

As this was an exploratory analysis, no formal sample size calculation was made for QTcF. The number of subjects treated in this study is

consistent with other traditional studies of a similar nature and were considered sufficient to allow assessment of the main objectives of the study which were the safety, tolerability, and PK of a NCE such as XEN-D0103.

RESULTS

Baseline ECG parameters for each dose group and pooled placebo are provided in Table 1. Figure 1 shows the mean (90% CI) time course of change in QTcF from baseline (ΔQTcF) during sequential iterations of 30 seconds measurements staggered every 5 seconds over 3 minutes for three episode of standing over 8 hours for the pooled group. The largest mean ΔQTcF occurred at the initial 30-second series of beats (i.e., which is plotted as 15 seconds as an average from 0–30 seconds) of each standing episode and declined rapidly to almost no change in QTcF by 1 minute. After 1 minute, the mean ΔQTcF is no longer positive but can be significantly negative.

Figure 2 shows the maximum ΔQTcF calculated for subjects randomized to their proposed treatment group. The lower bound of 90% CI was > 5 milliseconds for every episode of standing in each treatment group. The magnitude of the response was large (generally > 20 milliseconds), but highly variable initially. The maximum pooled ΔQTcF also increase from a mean of 27.5 milliseconds in the first episode to a mean of 40.5 milliseconds by the third episode. Because these responses represent the mean of the individual maximum response, an analysis of the time for maximum response ($\Delta T_{\max}\text{QTcF}$) was assessed from the 30 seconds iterations performed. Figure 3 shows that the variability of the mean time to maximum response was reduced dramatically from a pooled standard error of 6.4 milliseconds at the first episode of standing to 0.7 milliseconds by the third episode. Examination of individual responses from the first episode of standing revealed that it was typically no more than two individuals from each group that showed a marked difference in $\Delta T_{\max}\text{QTcF}$ that accounted for the variability. This was evident in the placebo group which was the only group that showed almost no variability across each standing episode and had no individuals with marked differences in $\Delta T_{\max}\text{QTcF}$ responses. The mean time to maximum response was consistently between 15 and 18 seconds in treatment groups by

Table 1. Summary of Baseline ECG Parameters (N = 6 Subjects/Treatment and 10 Placebo; N = 40 Pooled)

Group	Mean	SE	90% CI		Mean	SE	90% CI	
			Lower	Upper			Lower	Upper
			HR (bpm)				QTcF (milliseconds)	
Placebo	57.6	2.1	53.8	61.3	391.8	6.1	380.5	403.1
10 mg	57.1	2.2	52.8	61.4	402.6	5.1	392.4	412.8
30 mg	56.0	2.3	51.5	60.6	385.4	5.8	373.6	397.1
60 mg	61.5	2.5	56.6	66.5	401.5	5.6	390.2	412.8
120 mg	52.3	1.4	49.5	55.2	397.2	4.1	388.9	405.5
200 mg	56.0	2.7	50.6	61.5	388.3	6.4	375.4	401.2
Pooled	56.9	0.9	55.3	58.5	394.2	2.5	390.1	398.3
Group			PR (milliseconds)				QRS (milliseconds)	
Placebo	147.5	7.6	133.6	161.5	109.5	1.6	106.5	112.4
10 mg	149.7	9.5	130.7	168.8	106.1	1.7	102.6	109.6
30 mg	137.1	2.4	132.3	141.9	104.6	1.8	101.0	108.2
60 mg	154.1	9.8	134.3	173.9	107.9	2.0	103.8	112.0
120 mg	157.1	8.1	140.8	173.4	108.7	2.1	104.4	113.0
200 mg	154.2	6.6	140.8	167.5	107.3	2.6	102.0	112.5
Pooled	149.7	3.2	144.3	155.1	107.5	0.8	106.2	108.9
Group			QT (milliseconds)				RR (milliseconds)	
Placebo	399.0	8.3	383.9	414.2	1063.5	39.2	991.7	1135.3
10 mg	411.1	9.1	392.7	429.5	1071.4	44.1	982.5	1160.3
30 mg	395.6	7.7	380.2	411.0	1090.1	49.0	991.3	1188.9
60 mg	399.4	7.0	385.2	413.6	992.1	46.2	899.0	1085.2
120 mg	416.6	7.2	402.2	431.0	1159.4	32.1	1094.7	1224.2
200 mg	399.1	8.1	382.7	415.5	1094.6	48.8	996.3	1192.9
Pooled	403.0	3.4	397.4	408.7	1077.0	18.3	1046.2	1107.9

the third episode. The adaptation may be because of varying heart rate change. Heart rate changes during the first episode ranged from increases of 16 to 30 bpm whereas by the third episode, all changes were between 23 and 30 bpm (Fig. 4).

DISCUSSION

This study demonstrated the usage of continuous ECG collection to obtain the peak QTcF change from baseline as a positive control in an early development study where QT prolongation may prove detrimental to the advancement of a NCE, such as XEN-D0103. The study conditions chosen were of a typical Phase 1 setting on the day before ascending single dose escalation in a parallel-designed study of healthy volunteers. Delta QTcF at all time points and in all groups was significantly elevated to where the lower bound of 90% CI was >5 milliseconds which is the ICH E14 regulatory requirement¹² for a positive control. Also evident in this study, was the finding that with repeated usage of this methodology in the same subjects,

variability of the time to maximum response could be substantially reduced to further improve the precision of its usage.

Autonomic maneuvers have not been routinely utilized as positive controls for QT prolongation assessment because of their high variability in the reported responses. This can be caused by a multitude of factors. Some maneuvers are physiologically complex with several neurally distinct components making up a concerted event. For example, the valsalva maneuver, which an individual forces air against a closed glottis, consists of four phases of arterial pressure change that each presumably affect the QT-RR interval relationship very differently. Davidowski and Wolf⁵ studied the effect of several autonomic maneuvers on QTcB and showed changes ranging from -85 ± 52 (SD) milliseconds during dive to 87 ± 21 milliseconds with valsalva. They found, with the exception of exercise, that only a small change in QT or QT variability is associated with heart rate. In 1988, Franz¹ described this lack of QT adaptation to rapid heart rate change in paced hearts as hysteresis which can differ during

Figure 1. Mean change-from-baseline for all subjects (n = 40) after standing QTcF (Δ QTcF) during each 30 seconds iteration staggered every 5 seconds over 3 minutes for three episode of standing over 8 hours. Dashed lines represent upper (UCI) and lower (LCI) 90% confidence intervals.

Figure 2. Mean of the maximum median individual change-from-baseline for standing QTcF (Δ QTcF) across treatment groups and time points as well as the pooled treatment group and time points. n = 6 per treatment, n = 10 per placebo, n = 40 for pooled. Error bars represent upper and lower 90% confidence intervals.

Figure 3. Duration of time to maximum median individual change-from-baseline for standing QTcF ($\Delta T_{\max}$ QTcF) across treatment groups and time points as well as the pooled treatment group and time points. n = 6 for treatment, n = 10 for placebo, n = 40 for pooled. Error bars represent upper and lower 90% confidence intervals.

Figure 4. Mean change-from-baseline for standing HR (Δ HR) across treatment groups and time points as well as the pooled treatment group and time points. n = 6 for treatment, n = 10 for placebo, n = 40 pooled. Error bars represent upper and lower 90% confidence intervals.

rate acceleration versus deceleration. Figure 5 compares the difference in the pooled mean Δ QT and Δ QTcF changes in relationship to the heart rate change at the third episode as it occurs over 3 minutes. The heart rate change is quite consistent

Figure 5. Comparison of mean change-from-baseline HR (Δ HR), QT (Δ QT), and QTcF (Δ QTcF) for the pooled treatment ($n = 40$) during the standing response in the third period.

with a slightly greater effect early on but generally about 25 bpm. In contrast both the Δ QT and Δ QTcF interval changes take over 1 minute to fully adapt to the heart rate change. Thus autonomic maneuvers become dynamically complex QT-RR loops which can be interpreted differently based on timing of the measure during the looping process and the manner in which the measure is compared to a more stable baseline.¹⁷

Recently, two groups have proposed using the standing response as a means for QT interval assessment. In healthy normals, despite the apparent simplicity of the measure during reflex tachycardia, the change in QTcB reported by Williams et al.⁶ was 9.6 ± 9 (95% CI) milliseconds vs 54 ± 11 milliseconds by Viskin et al.⁷ This large discrepancy in results may be explained in the timing of the measurements and whether continuous ECG recordings were used. Williams measured the QT intervals 4 minutes after standing when the QT interval had probably already adapted to the tachycardia and was returning to normal whereas Viskin does not describe the timing but did report that it was at the maximum change in QTcB. Figure 6 illustrates this effect using the beat-to-beat plots from 30-second timeframes of a single subject.³ The beats during the first 45 seconds of standing are above the Fridericia function used and by 120 seconds are almost completely in line with the fit of the correction. However, ECG measures after 150 seconds show hysteresis in the opposite direction

as the heart rate returns toward normal producing a largely negative QTcF value. Williams et al. did report a change after standing at 4 minutes of -8.3 ± 7 milliseconds when using a QTcF correction on the same data which is consistent with our findings (Fig. 1).

One limitation of this method may be that it does not truly represent a positive control test for impaired repolarization, the premise behind the TQT. During a change in heart rate, detection of QT prolongation using any correction derived at a resting baseline for the QT interval in healthy normal volunteers cannot differentiate between impairment of repolarization and normal change in autonomic state.^{18,19} When this methodology is used in healthy normal volunteers, QTcF prolongation is actually the correction error because of normal hysteresis which is different than blockade of hERG (i.e., moxifloxacin). This is different in cardiac disease states where hysteresis is also further impaired by changes in cardiac channels that are responsible for normal adaptation.^{20,21}

In order for brisk standing response to be accepted as a positive control for QT prolongation assessment, the results from study-to-study should be (1) reproducible (2) of similar QTc magnitude, and (3) variability that preferably can meet ICH E14 criteria for exclusion of a 5-millisecond effect or less using the lower 90% CI. Listed below are a few key items that would need to be standardized.

Baseline Resting State

The magnitude of the standing response may be optimized when subjects stand from fully supine versus a semirecumbent position. As we observed, the larger and more consistent the heart rate change, the less variable the data. Also, to avoid fainting even in healthy subjects, water should not be restricted in the hours before study. To obtain low heart rates for larger changes upon standing, the subject should be maintained in a quiet, fully supine position for at least 15 minutes with no talking, radios, or TV in the background.

Correction Factor

With rapid tachycardia, the correction coefficient that produces a flatter QT-RR interval relationship has less chance of detection of QTc prolongation during standing because of hysteresis.

Figure 6. A-D: Temporal change in median QTcF upon standing A: 0–30 seconds, B: 15–45 seconds, C: 90–120 seconds, D: 150–180 seconds from resting supine position (Supine baseline QTcF = 403 milliseconds) in a single subject. Study subject represents similar mean values obtained from larger study (N = 30) at same time intervals after standing (From: Fossa and Zhou³).

This is why, as described above, the Bazett method produced such large results.⁷ A correction factor accounting for hysteresis by averaging heart rate history over a minute or more is obviously not suited for this methodology.²² Use of an individual correction factor would present difficulties for regulators when comparing sensitivity of results from study to study. Therefore, because the Fridericia method is most often used in clinical regulatory studies and provides a magnitude closer to the currently used moxifloxacin, it appears to be a reasonable choice.

Magnitude of the Response

The ICH E14 requirement of 5 milliseconds for a positive control was substantially exceeded with the peak standing response of over 25 milliseconds and lower confidence bounds greater than 20 milliseconds. This may be interpreted as too robust for adequate study sensitivity. However, this may be debatable. As QT measurement technologies improve, many TQT studies with moxifloxacin, have been reported to have peak effects between 10 and 15 milliseconds²³ with

lower bounds approximately 10 milliseconds. An alternative solution may be to raise the standard for a particular positive control response with lower 90% confidence interval bounds approximately 5 milliseconds below the peak mean effect.

When to Perform

In the current parallel-designed study, all standing responses were performed in the baseline period the day before any drug or vehicle treatment. However, if measures are required during study treatment, one should be aware that treatment may affect the standing response and therefore may alter the sensitivity as a positive control measure. In that case, only in a cross-over designed study should measurements be used at time-matched placebo periods. In a parallel-designed study, this would prevent the sensitivity of QT interval from being demonstrated in the same subjects being given the treatment.

CONCLUSIONS

This study showed that brisk standing invoked changes in QTcF using a repetitive iterative analysis of the continuously collected ECG to allow reproducible measures meeting regulatory criteria for a positive control. This methodology can be utilized to validate the sensitivity for assessment of the QT interval with drugs in early development where this may be of particular concern and perhaps if standardized obviate the need for a separate pharmacological control group, such as moxifloxacin. In addition, the methodology may be used to further enhance the use in diagnosis of Long QT syndromes by reducing the variability and allowing adequate definition of normal limits.

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