ORIGINAL ARTICLE

Stability of the Effect of a Standardized Meal on QTc

Jörg Täubel, M.D., F.F.P.M.,* Sara Fernandes, Ph.D.,* and Georg Ferber, Ph.D.†

From the *Richmond Pharmacology Ltd., St George's University of London, London, United Kingdom; and

†Statistik Georg Ferber GmbH, Riehen, Switzerland

Background: The assessment of QTc changes after the intake of a standardized meal has been proposed as an alternative approach to prove assay sensitivity when the proarrhythmic potential of a drug is to be excluded in either TQT or intensive Phase I QT studies.

Methods: In this article, an analysis of the food effect at baseline across periods in two different studies is presented to support the robustness of the method.

Results: The results show that the time-effect attributed to food is stable over different study periods demonstrating consistency of the physiological response triggered by food.

Conclusions: Stability and reproducibility of the effect is comparable with moxifloxacin.

Ann Noninvasive Electrocardiol 2017;22(1):e12371, DOI: 10.1111/anec.12371

QTc; interval; food effect; assay sensitivity

Experience with concentration-response (CR) analysis presents an alternative approach to cardiac safety assessments. QTc data obtained in single- and multiple-dose First-In-Human (FIH) trials and other Phase I trials such as drug-drug interaction studies can be used to characterize QTc effects during drug development.¹ The results from the IQ-CSRC study have provided additional support for the transition from TQT to early QT assessments with smaller sample sizes than those typically used in standard TQT studies.² Concerns surrounding the validation of early-phase clinical QT assessment are focused on their ability to detect small effects of regulatory concern, and the inclusion of a positive control to show assay sensitivity providing reliable protection against false negatives. The adoption of food effect as the positive control has been successfully demonstrated to improve the confidence in QT assessment in different dedicated early-phase studies.^{3–7}

Preceding studies have described the effects of food on the QT interval and QTcB prolongation for 1 hour after meal ingestion has been reported.⁸ Later, the use of the same correction method confirmed a temporary increase in QTcB with a subsequent shortening returning to baseline after 2 hours of meal intake with a maximum shortening at 4 hours. In addition to the QTcB

biphasic pattern, this same study showed a maximum QTcF and QTcI shortening at 2 and 3 hours, respectively, indicating that for a complete analysis of the food effect on QT interval the period of analysis after a meal must be extended.³

In a dedicated study, concentration-response analysis for insulin, glucose, and C-peptide showed that insulin has no effect on QTc while glucose and C-peptide were found to have positive and negative effects, respectively.⁹ These findings are in line with previous studies where administration of C-peptide to type 1 diabetes patients shortened the QT interval¹⁰, whereas glucose was shown to significantly prolong the QT interval in C-peptide-deficient patients and healthy subjects treated with somatostatin.¹¹ Hence, QTc shortening was proposed to be a net effect of the antagonistic effects exerted by C-peptide and glucose. The greatest effect is observed between 3 and 4 hours after the start of an insulin release stimulating meal (IRSM) and thus falls outside the postprandial period.

All this suggests that a carbohydrate-rich meal has a significant and reproducible physiological effect on cardiac repolarization, that is, a true effect which can be measured. It has been suggested that this effect can be used to show assay sensitivity in TQT or intensive QT (iQT)

Address for correspondence: Jörg Täubel, M.D., F.F.P.M., Richmond Pharmacology Ltd, St George's University of London, Cranmer Terrace, SW17 0RE London, UK. Tel: +44(0)20 8664 5200; Fax: +44(0)20 8664 5201; E-mail: j.taubel@richmondpharmacology.com

© 2016 Wiley Periodicals, Inc.
DOI: 10.1111/anec.12371

studies, in particular in situations, where a pharmacological positive control either cannot be used³ or would represent a significant additional burden, as is usually the case if Phase I studies are used to confirm the absence of QT prolongation for a medicine. An essential prerequisite for such a use is stability and reproducibility of the effect. In this communication, we report results on the stability and reproducibility of this effect seen across the four periods of one TQT study¹² and a Phase I study⁶ which represents a real-life example of an iQT study. In both studies the effect of meal on the ECG was studied on four replicate days within the same subjects.

MATERIAL AND METHODS

This work was based on baseline day ECG data from two four-period crossover studies in healthy volunteers. Data from subjects with available ECG and PK data from all four treatment periods were used. Study 1 (iQT) was a double-blind, randomized, placebo-controlled, four-way cross-over single ascending dose Phase I study that included 32 subjects.⁶ Study 2 (TQT) was a randomized, double-blind, placebo and positive-controlled, four-way crossover TQT study with 40 subjects.¹² In both studies, each period consisted of a placebo baseline ECG day (Day-1) preceding the respective treatment days. ECG recordings were made at the following time points in Study 1: predose—15, 30, and 45 minutes; 1, 1.25, 1.5, 1.75, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 hours postdose of each period. In Study 2, ECGs were taken at predose—2, 8 and 30 minutes; 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 hours postdose at Day -1 (baseline) of each period. In both studies, ECGs were recorded as heart rate-controlled triplicates using MAC1200 ECG recorders. Before any ECG recording, the subjects maintained an undisturbed supine resting position for at least 10 minutes and avoided postural changes during the ECG recordings. At each time point, the ECGs were recorded in triplicate at 1-minute intervals during 3 minutes. Each ECG lasted 10 seconds. Automatic ECG analysis was performed by the Marquette 12SL ECG Analysis Program (MEAP). All ECGs and their associated automated interval measurements were subsequently reviewed and manually adjudicated by qualified cardiologists specialized in the assessment of QT studies under

blinded conditions before data analysis. If manual adjustments of the automated measurement became necessary, a second cardiologist confirmed the assessment. Any disagreement between the first and second reader was adjudicated by a third and most senior cardiologist. Details of this process have been described by Täubel et. al.¹³ For further analysis, the mean across the triplicates was used. Both studies utilized the same ECG core lab facility, equipment, and processes: electronically stored 12-lead ECGs were automatically and continuously transferred to the MUSE CV information system (GE Healthcare, Chalfont St Giles, Buckinghamshire, United Kingdom).

Standardized meals identical in all four periods with similar nutritional value were served as follows at the baseline days of Study 1 (iQT): lunch, dinner, and an evening snack at 5, 9, and 13 hours postdose. Lunch (which is the reference meal in this study) was required to be completed within 20 minutes. Lunch contained 335 kcal of carbohydrates and had an approximated ratio of 58% carbohydrate to 22% fat to 23% protein; 575 kcal in total. Subjects participating in Study 2 (TQT) were served breakfast (reference meal) before dosing, lunch and dinner at approximately 4 and 12 hours postdose, respectively. Subsequent meals were served over 4 hours after the reference meal and therefore not affecting the window of food effect assessment in this study. On baseline and treatment days, breakfasts were identical across all periods delivering 475 kcal as carbohydrates with an approximated ratio of 75% carbohydrate, 15% fat, 10% protein; 650 kcal in total. Breakfast was served one hour before and was completed 30 minutes prior to the dosing time.

From the QTcF values of the baseline day of each period, only the predose value and the two values in the time interval 1–4 hours after the meal were utilized for this analysis. For Study 1 (iQT), where lunch was used as reference meal, these are the time points 6 and 8 hours after the time corresponding to placebo administration, while for Study 2 (TQT), where breakfast was given 1 hour before placebo administration, we used the time points 2 min and 2 hours after the time corresponding to placebo administration. The time points chosen from both studies therefore correspond to 1 and 3 hours after the start of the meal. The 1 and 3 hours time points were

chosen as data was available at these time points for both studies.

To assess the stability of the effect of the meal, we fitted a linear mixed model to the data of each study, with $QTcF$ as a dependent variable, and period and time point and their interaction as factors, and a random intercept per subject. Based on this model, an analysis of variance was performed. If the food effect was stable across periods, the interaction term in this analysis should be small and statistically not significant. We further present the model-based estimates for the effect at each period and time point, that is, the contrast of the value at the respective time point minus the effect at the predose value.

In a recent publication, Hnatkova et al.,⁵ investigated the stability of the food effect in one of their studies by looking at the number of subjects that showed a prolongation after food intake. We therefore also investigated the number and percentage of subjects not showing a shortening effect as well as the intraindividual variability of the change from predose baseline of $QTcF$ across the four periods.

RESULTS

In Figure 1 the time course of $QTcF$ at the baseline day is displayed using mean values and two-sided 90% confidence intervals. The shortening effect of the meal can be clearly seen for both studies and across all periods presenting a similar time course, but greater $QTcF$ shortening effect

in the TQT study. The results of the analysis of variance for the two studies are shown in Table 1. Consistently, both the time and the period effect are significant, while the interaction between the two is not. The absence of a significant interaction suggests that the effect of food does not change from one period to another. The results show a change over time within each period, which represents the effect of the meal and an effect of period, that is, QTc values differ from one period to another. However, there is no indication that changes over time, that is, the food effect itself is different from one period to the other—which would result in a significant interaction term. The intercept reflects the mean $QTcF$ seen, which trivially is different from zero. The model-based estimates of the food effect, that is, change from time 0 to the respective time point are given in Table 2. The shortening is between 5 and 8 ms in Study 1 (iQT) and between 8 and 12 ms in Study 2 (TQT), with all two-sided 90% confidence intervals being consistently negative and excluding zero confirming a statistically significant and stable effect.

Individual $QTcF$ changes ($\Delta QTcF$) for the four periods for both studies are represented in Figure 2 and the mean number of subjects with QT prolongation is presented in Table 2. These findings support the reproducibility of the food effect within each subject. Some subjects may present a prolongation effect in one or more periods, but the mean food effect on QT is consistently present and most distinct at the time point 3 hours

Figure 1. Time course of $QTcF$ at the baseline day by study period. $\Delta QTcF$ at different times of day is presented as a mean value and the two-sided 90% confidence intervals are illustrated for each time point.

Table 1. Analysis of Variance

Effect	df Num	df Den	F	P	Effect	df Num	df Den	F	P
Study 1					Study 2				
Intercept	1	324	24993.8	<0.001	Intercept	1	414	29529.8	<0.001
Time point	2	324	34.5	<0.001	Time point	2	414	110.2	<0.001
Period	3	324	4.9	0.002	Period	3	414	14.3	<0.001
Interaction	6	324	0.5	0.792	Interaction	6	414	1.7	0.117

after a meal in a typical TQT study (Study 2). The intraperiod ranges of $\Delta Q_{Tc}F$, that is, the largest value minus the smallest value of a subject have typical values around 12–16 ms in both studies, with a maximum of 47 ms occurring in one subject of Study 1 (iQT) 1 hour after the start of the meal.

DISCUSSION

In December 2015, the ICH E14 guideline has been revised to generate guidance on how

exposure-response modeling can be used to characterize the potential for a drug to affect cardiac repolarization and to modulate the Q_{Tc} interval.¹⁴ This revised guidance states that a separate positive control would not be necessary if sufficiently high exposure levels are achieved in the study.

In many cases, due to their small sample size, Phase I studies can fail to demonstrate a significant result in particular as their focus is not centered on ECG assessments. Cardiac safety is one of several secondary objectives and not the primary objective

Figure 2. Individual Q_{Tc} changes from baseline grouped by ascending median across the four periods at 1 and 3 hours after a standardized meal for Study 1 (iQT) and Study 2 (TQT). Each column presents the data for one subject with one value per period.

Table 2. Model Estimates of the Food Effect and Intrasubject Variability

Table 2. Model Estimates of the Food Effect and Intrasubject Variability						
Time	Period	Change From Time 0				Mean No of Subjects with $\Delta QTcF > 0$ Across Periods
		Mean	SE	90% CI		
Study 1						
1 hour from start of meal	P1	−5.1	1.7	−8.0	−2.2	7.25 (22.7%)
	P2	−7.8	1.8	−10.7	−4.8	
	P3	−5.1	1.7	−8.0	−2.2	
	P4	−7.6	1.7	−10.4	−4.7	
3 hours from start of meal	P1	−6.8	1.8	−9.7	−3.9	6 (18.8%)
	P2	−6.6	1.8	−9.6	−3.7	
	P3	−4.7	1.7	−7.5	−1.8	
	P4	−7.1	1.7	−10.0	−4.2	
Study 2						
1 hour from start of meal	P1	−6.5	1.31	−8.6	−4.3	7.25 (18.1%)
	P2	−6.5	1.32	−8.6	−4.3	
	P3	−4.7	1.34	−6.9	−2.5	
	P4	−6.5	1.34	−8.7	−4.3	
3 hours from start of meal	P1	−8.4	1.31	−10.6	−6.3	1.75 (4.4%)
	P2	−8.2	1.32	−10.4	−6.0	
	P3	−10.4	1.34	−12.6	−8.2	
	P4	−12.1	1.34	−14.3	−9.9	

which differentiates Phase I studies from TQT studies and the CSRC study.^{1,2} Typically, and unlike a TQT study, a FIH study does not include a positive control arm to confirm ECG assay sensitivity. Proving assay sensitivity can have significant implications when demonstrating that even small QT effects can be reliably excluded in FIH and other Phase I studies.

The effect of a meal on the QTc interval is a reliable and ubiquitously available alternative method to prove assay sensitivity. The additional analysis necessary can easily be incorporated into the design of almost any type of clinical trial including specialist oncology and pediatric trials by analyzing the premeal baseline compared to one or two time points after the first meal of the study day. This approach does not require a separate arm/period to determine assay sensitivity or any changes to a Phase I study design as only an appropriate dietary plan with adequate meal supervision, two to three ECG time points after a meal, and subsequent analysis are required to demonstrate the food effect on QTc interval. The statistical power is greater than in instances where a separate positive control arm is included because the effect of a meal can be assessed in every subject and on any of the observation days.

In this retrospect analysis using baseline data of two studies where a standardized meal has

either been given after a placebo dose (Study 1) or before placebo administration (Study 2), the shortening of QTcF could be shown consistently. The F-values in Table 1 clearly show a hierarchy of the effects with the food effect dominating the period effect and the F-values for the interaction term in turn being only 10% of those of the period effect, the interaction not being significant any more. It is important to note that even though both studies consisted of four periods, timing of the meals differ between studies. At the time of Study 1 we were unaware of the decrease in QTcF induced by a meal. Therefore, appropriate carbohydrate content was not intended for the dietary plan of Study 1. The carbohydrate content may be of particular importance if the effect is to be consistently reproduced as it may influence the production of insulin and consequently C-peptide which is released in equimolar concentrations. The relationship between the ratio of glucose and C-peptide concentration and the resulting shortening of QTc was demonstrated and the plasma concentration of these analytes was shown to be lower if the meal composition is altered toward a higher fat to carbohydrate ratio.⁹

Study 2 (TQT) included 20% more subjects than Study 1 (iQT) and was a formally designed TQT study where meal intake was carefully controlled and an undisturbed environment was

created in order to optimize the study assessment of ECG effects by avoiding signal noise due to autonomic effects. This is reflected by the much lower variability (SE) of the data. In contrast, Study 1 (iQT) was conducted in a Phase I setting typical for single ascending dose trials where subjects were frequently disturbed and mobilized for the performance of various study assessments including a battery of cognitive tests.

A greater effect on QTcF is observed in Study 2 (TQT) maybe as a result of a higher carbohydrate content of the meals given and a more strictly standardized study protocol focusing on the measurements of ECG data. The effect of food 3 hours after start of the meal is more reproducible in Study 2 and the number of subjects not showing QTcF shortening in all periods is much smaller than in Study 1 (iQT). The greater intrasubject variability observed 1 hour after the start of a meal may be due to the fact that the placebo administered intravenously was given around this time point causing autonomic noise and or that glucose plasma levels from the meal one hour earlier may still have been high outbalancing the effects of C-peptide. These findings support previous studies where more pronounced QTc shortening is observed during the second half of the 4-hour period after a standardized meal.

In a recent publication, Hnatkova et al.⁵ report on a retrospect investigation of the food effect in a large two-period study with a total of four baselines per subject. They investigated the average of three QTc values measured between 1 and 3 hours after start of lunch and compared them to the average over six time points in the morning, obtained under fasting conditions. A modest decrease in QTc after lunch was found, with mean values of only 2.83 ms and 1.24 ms in females and males, respectively. The composition of the meal given in this study is unknown and in part, this small value can also be explained by the inclusion of a value measured only 1 hour after lunch intake, where the shortening effect is still expected to be very small. In order to assess the reproducibility of the effect, the number of subjects without shortening was used, which is about 1% in their data. This compares favorably with our numbers, which are between 5% and 20%. However, this apparent discrepancy is easily explained by the fact that the numbers of Hnatkova et al. are based on averages over three

postprandial and six preprandial time points (averaging three time points were shortening events are captured while at the same time magnitude of effect is reduced), while ours are individually counted for each difference of the pre- and one postprandial time point. Since our studies were designed to use a full baseline day, only one pre-dose time point was available for both ECG assessment days. In addition, Hnatkova et al. report pair wise normalized repeatability errors (difference/mean) between 0.87 and 1.23. Again, similar values across all periods calculated in our studies (medians range from 1.4 to 2.1) are somewhat higher, since we did not average over time points and took the extremes over all four periods.

Individual data investigating the intrasubject variability in studies using moxifloxacin as a positive control in different study days are scarce and more analysis of the individual moxifloxacin effect on QTc would help to substantiate a comparison between the two approaches to confirm assay sensitivity. Two studies reported to the FDA evaluated the effect of moxifloxacin in different days.^{15,16} The first study evaluating the time course effect of moxifloxacin in 42 subjects in three different days had shown a small variation of 1.9 ms in the mean peak $\Delta\Delta\text{QTcI}$ at 3 hours for moxifloxacin between Day 9 (11.6 ms) and Day 12 (9.7 ms).¹⁵ The second study assessed the effect of moxifloxacin on the QT interval in 62 subjects in four occasions and revealed a maximum variation of 4.1 ms in the mean peak $\Delta\Delta\text{QTcI}$ at 4 hours between Days 6 (12.4 ms) and 12 (8.3 ms).¹⁶ In our studies, variation in the point estimate of the mean of the food effects between periods is lower as the greatest variation of 3.9 ms is observed 3 hours after the meal start in Study 2, the largest in Study 1 is 2.4 ms, also seen at 3 hours. The variation between periods one hour after the start of a meal is smaller even, 2.7 ms in Study 1 and 1.8 ms in Study 2.

Recently, moxifloxacin was used as a positive control in a SAD and a TQT study and the results from these studies were compared.¹⁷ The moxifloxacin Exposure-Response (ER) slope value from the SAD study was twice the value in the TQT study and the 90% CI were 30–40% greater in the SAD study indicating that the moxifloxacin effect on QTc is also susceptible to higher variability in a Phase I environment. The authors of the reported study accept and endorse the need

for a positive control in Phase I iQT studies showing that similar to our studies, a greater variability in data is derived from these studies, as opposed to the TQT studies due to several factors most notable the sample size.

The results of the present analysis indicate that the time-effect attributed to food is stable over periods and suggest that meal-induced changes on QTc are reproducible and can perform at least as well as moxifloxacin in a TQT setting and still very adequately in a SAD or MAD environment; the main advantage over the inclusion of a moxifloxacin arm being that the effects can be assessed in all subjects in all parts of the study thereby providing a much larger sample size in an iQT study than a moxifloxacin arm ever could.

The effect of a meal on the QTc interval represents a true effect on cardiac repolarization and therefore cannot be ignored. It follows that the intake of food and sugar-containing drinks in QTc studies must be rigorously controlled as they may become a confounding factor. Data to assess the meal effect on QTc should be readily available in most studies given that volunteers have to be fed during a study and given that many studies will utilize continuous 12-lead Holter monitoring and/or have dedicated ECG time points during the 1–3 hours following the meal. Therefore, the analysis of the effect will considerably enhance the validity of the data without creating the extra burden of utilizing other methods.

REFERENCES

1. Darpo B, Sarapa N, Garnett C, et al. The IQ-CSRC prospective clinical phase I study: Can early QT assessment using exposure response analysis replace the thorough QT study? *Ann Noninvasive Electrocardiol* 2014;19:70–81.
2. Darpo B, Benson C, Dota C, et al. Results from the IQ-CSRC prospective study support replacement of the thorough QT study by QT assessment in the early clinical phase. *Clin Pharmacol Ther* 2015;97:326–335.
3. Taubel J, Wong AH, Naseem A, et al. Shortening of the QT interval after food can be used to demonstrate assay sensitivity in thorough QT studies. *J Clin Pharmacol* 2012;52:1222–1239.
4. Hnatkova K, Kowalski D, Keirns JJ, et al. QTc changes after meal intake: Sex differences and correlates. *J Electrocardiol* 2014;47:856–862.
5. Hnatkova K, Kowalski D, Keirns JJ, et al. Reproducibility of QTc interval changes after meal intake. *J Electrocardiol* 2015;48:194–202.
6. Taubel J, Ferber G, Lorch U, et al. Single doses up to 800 mg of E-52862 do not prolong the QTc interval – A retrospective validation by pharmacokinetic-pharmacodynamic modelling of electrocardiography data utilising the effects of a meal on QTc to demonstrate ECG assay sensitivity. *PLoS ONE* 2015;10:e0136369.
7. Taubel J, Ferber G, Izquierdo I. Confirmation of the cardiac safety of rupatadine in a single ascending dose and multiple ascending dose study in Japanese Healthy Subjects using intensive ECG assessments. DOI: 10.1002/cpdd.216/abstract Conference: 2015 Annual Meeting, At San Francisco, CA, Volume: Clin Pharmacol Drug Dev 4; (S1):1–60.
8. Nagy D, DeMeersman R, Gallager D, et al. QTc interval (cardiac repolarization): Lengthening after meals. *Obes Res* 1997;5:531–537.
9. Taubel J, Lorch U, Ferber G, et al. Insulin at normal physiological levels does not prolong QTc interval in thorough QT studies performed in healthy volunteers. *Br J Clin Pharmacol* 2013;75:392–403.
10. Wahren J, Ekbery K, Jornvall H. C-peptide and neuropathy in type 1 diabetes. *Immunol Endor Metab Agents Med Chem* 2007;7:69–77.
11. Gordin D, Forsblom C, Ronnback M, et al. Acute hyperglycaemia disturbs cardiac repolarization in type 1 diabetes. *Diabet Med* 2008;25:101–105.
12. Taubel J, Fox G, Ferber G, et al. Investigating the effect of intravenous APD421 (Amisulpride) and the ethnic differences between Japanese and Caucasians on cardiac conduction. The 36th Annual Meeting of the Japanese Society of Clinical Pharmacology and Therapeutics 2015.
13. Taubel J, Ferber G, Lorch U, et al. Thorough QT study of the effect of oral moxifloxacin on QTc interval in the fed and fasted state in healthy Japanese and Caucasian subjects. *Br J Clin Pharmacol* 2013;77:170–179.
14. E14 Implementation Working Group. ICH E14 Guideline: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. Questions & Answers (R3): 2015: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Q_As_R3_Step4.pdf. Accessed 10 January 2016
15. FDA application number 206627Orig1s000. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/206627Orig1s000OtherR.pdf. Accessed: 10 January 2016.
16. FDA application number 022567Orig1s000. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/022567Orig1s000OtherR.pdf. Accessed: 10 January 2016
17. Shah RR, Maison-Blanche P, Duvauchelle T, et al. Establishing assay sensitivity in QT studies: Experience with the use of moxifloxacin in an early phase clinical pharmacology study and comparison with its effect in a thorough QT study. *Eur J Clin Pharmacol* 2015;71:1451–1459.