Diurnal Profile of the QTc Interval Following Moxifloxacin Administration

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Abstract

Understanding the physiological fluctuations in the corrected QT (QTc) interval is important to accurately interpret the variations in drug-induced prolongation. The present study aimed to define the time course of the effect of moxifloxacin on the QT interval to understand the duration of the responses to moxifloxacin. This retrospective analysis was performed on data taken from a thorough QT 4-way crossover study with 40 subjects. Each period consisted of a baseline electrocardiogram (ECG) day (day −1) and a treatment day (day 1). On both days, ECGs were recorded simultaneously using 2 different systems operating in parallel: a bedside ECG and a continuous Holter recording. The subjects were randomized to 1 of 4 treatments: 5 mg and 40 mg of intravenous amisulpride, a single oral dose of moxifloxacin (400 mg), or placebo. Standardized meals, identical in all 4 periods, with similar nutritional value were served. Bedside ECG results confirmed that the moxifloxacin peak effect was delayed in the fed state and showed that the Fridericia corrected QT prolongation induced by moxifloxacin persisted until the end of the 24-hour measurement period. The use of continuous Holter monitoring provided further insight, as it revealed that the moxifloxacin effect on QTc was influenced by diurnal and nocturnal environmental factors, and hysteresis effects were noticeable. The findings suggested that moxifloxacin prolongs QTc beyond its elimination from the blood circulation. This is of relevance to current concentration-effect modeling approaches, which presume the absence of hysteresis effects.

Keywords

Holter, moxifloxacin, QTc interval, time-course profile

The effect of drugs on cardiac repolarization—through assessment of the QT interval of the electrocardiogram (ECG)—is an important tool in the assessment of the propensity of novel medical products to induce arrhythmias, which has been a major cause of drug withdrawals from the market or clinical development.[1–4] Clinical and pharmacological studies have indicated that the vulnerability to induced arrhythmias varies throughout the day.[5,6]

Regulation of cardiac function by diurnal factors enables efficient coupling of physiological response to anticipated environmental demand.[7] Biorhythms approximating to a 24-hour cycle are found in several electrophysiological parameters such as QT interval, QRS duration, and heart rate (HR) variability,[8] while cardiac cells themselves exhibit endogenous circadian activity cycles[9,10] that can be entrained by environmental features.[11] Diurnal changes in ion channel function regulate these rhythms.

The modulation of the QT interval by diurnal factors and its dependence on time of day may also have implications on the measurement of repolarization speed in cardiotoxicity studies of investigative medical products. It has been suggested that the magnitude of the effect of a drug on the corrected QT (QTc) interval may depend on the time of day.[12] A shortening of the Fridericia corrected QT (QTcF) after a standardized meal has also been well documented.[13,14] The effect of a standardized meal is reflected in the estimates of the "spontaneous" diurnal changes that need to be included in the concentration-response model if individual placebo-corrected changes from the baseline of QTcF values ($\Delta\Delta$QTcF) are not available.[15] For these reasons, taking diurnal temporal changes into consideration may better delineate the ability of a drug to delay cardiac repolarization.

Moxifloxacin is a broad-spectrum fluoroquinolone antibiotic with a well-documented and consistent QT prolongation effect and is widely used in thorough QT (TQT) studies as positive control to demonstrate the

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sensitivity of the assay.[16,17] The importance of circadian modulators has been recognized in the quantification of the moxifloxacin drug-response relationship through pharmacokinetic (PK) QT modeling.[18] Moxifloxacin binds to and inhibits the human ether-a-go-go-related gene (hERG) I_{Kr} α subunit and thereby prolongs the cardiac repolarization interval. Patch-clamp studies indicate that moxifloxacin can bind with high affinity to the open I_{Kr} and block the conductance.[19]

Even though the direct inhibition of the hERG channel is the best characterized mechanism by which drugs can inhibit cardiac repolarization,[20] other inhibitory mechanisms include the I_{Kr} interference with hERG-protein intracellular trafficking[21–23] or promoting the degradation of this protein[24] and action on the autonomic nervous system[25,26] or the sinoatrial node.[27] Stereochemical modeling indicates several different mechanisms by which drugs can inhibit this ion conductance, some of which would trap the drug in the inactivated channel, thereby extending the dissociation of the drug from the protein well beyond the plasma exposure.[20] This suggests that in cardiotoxicity studies the modulation of repolarization should be followed for a period well beyond the plasma exposure to pharmacologically effective concentrations. Such a delay in returning to baseline values can be interpreted as a form of hysteresis, that is, a delay between PK and pharmacodynamic effects. This is particularly important when single/multiple ascending dose intensive ECG monitoring is used, usually employing concentration-effect modeling.[28]

The aim of the present study was to describe the diurnal changes in cardiac repolarization, assess the impact of standardized meals on the QT interval adjusted by the Fridericia correction, and characterize the variation in cardiac repolarization duration over 24 hours following 400-mg moxifloxacin administration with periodic bedside ECG combined with continuous Holter ECG data in order to elucidate the true time course of the QTc prolongation attributable to moxifloxacin.

Methods

The study was approved by an authorized Research Ethics Committee (NRES Committee South Central–Berkshire B; ref: 13/SC/0496), approved by the Medicines and Healthcare Products Regulatory Authority, and registered with EudraCT (ref: 2013-002-669-20) and ClinicalTrials.gov (ref: NCT02661594). Its conduct was in accordance with the principles of the Declaration of Helsinki, current UK law, and good clinical practice guidelines. Each subject received verbal and written information followed by signing of the informed consent form prior to any procedures taking place. A post hoc analysis was performed on the 12-lead Holter data and 12-lead ECG data obtained during a TQT study published by Täubel et al,[29] which was part of a single-center, randomized, double-blind, placebo- and positive-controlled, 4-way crossover study of a novel intravenous formulation of amisulpride.

Forty eligible white and Japanese subjects (5 Japanese women, 12 Japanese men, 12 white women, and 11 white men) were randomized to 1 of 4 treatment sequences and received single doses of oral moxifloxacin 400 mg or intravenous amisulpride 5 mg (infused over 2 minutes) or intravenous amisulpride 40 mg (infused over 8 minutes) or placebo. Intravenous placebo consisted of a 2.5-mL normal saline solution infused over 2 minutes, and 20 mL of normal saline infused over 8 minutes, in parallel syringe drivers starting at the same time. The study was not blinded with respect to moxifloxacin administration. Moxifloxacin was provided as single 400-mg tablet preceded on day –1 by a single, inactive moxifloxacin-placebo tablet. These treatments were part of a 4-period crossover design following a set of Williams squares.

Each period consisted of a placebo baseline ECG day (day –1) and a treatment day (day 1). The ECGs on the treatment day were taken at the corresponding time points as on the baseline day. There was at least a 7-day washout interval between study drug administrations (day 1) in periods 1 through 4. The ECG was recorded simultaneously using 2 different systems operating in parallel: a bedside ECG and an ambulatory recording. Twelve-lead, 10-second triplicate ECGs were used for the confirmatory TQT analysis, whereas the 12-lead Holter data acquired was stored for backup and the methodology research presented here.

While in the clinic, subjects were served breakfast 1 hour before dosing, and lunch and dinner at approximately 6 hours and 12 hours after dose, respectively. A snack was served 16 hours after dose. On baseline and treatment days, the same breakfasts were given to both men and women and races across all periods delivering 652.8 kcal with an approximate ratio of 73% carbohydrate, 16% fat, and 11% protein. The same standardized lunches and dinners were served throughout the study; however, they were portion controlled to achieve the sex-specific daily calorie allocation of approximately 2300 and 1700 kcal daily intake for men and women, respectively.

ECG Recording

Twelve-lead bedside ECGs were recorded using a MAC1200® ECG recorder (GE Healthcare, Buckinghamshire, UK) and stored electronically on the Medical MUSE® information system (GE Healthcare). Bedside

MAC1200 device used 12SL algorithm where the QT interval was measured from a median complex reducing the influence of noise, and also measured from global fiducial points from all 12 simultaneous leads. Bedside ECG recordings were made at the following time points: predose; 2, 8, and 30 minutes; and 1, 1.5, 2, 3, 4, 5, 6, 12, and 24 hours after dose of each treatment period. Before any bedside 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 to reduce variance and improve the precision of measurement. Each bedside ECG recording lasted 10 seconds. The successive triplicates were performed at 1-minute intervals over 3 minutes. The QT interval, RR interval and HR, PR interval and QRS duration, the presence or absence of U-waves, and quantitative and qualitative ECG variations were assessed by cardiologists with extensive experience in manual on-screen overreading with electronic calipers using the commercially available MUSE® in its latest version to correct any implausible readings presented by the automated process.

Continuous 12-lead Holter recordings were obtained using a Getemed Holter ECG device (GE Healthcare, Boston, Massachusetts) with a CardioMem® CM 3000 digital recorder on the four baseline and the four study days. Getemed Holter utilized beat-to-beat QT measurement where the Q_{begin}, J_{point}, and T_{end} were found using a threshold base method in the squared first derivative of the ECG. Obtaining measurements from dual electrodes ensured that bedside and Holter recordings were of parallel physiological signals. The Holter extraction module allowed averaging between 3 and 61 beats, where the QRS completes were superimposed to measure Q_{start}, J_{point}, and T_{end}, subsequently allowing export of QT, RR, and QTcF values.

All parameters (heart rate, QT, QTcF) were extracted by averaging 3 consecutive beats generating approximately 29,000 values per 24-hour Holter record. Each mean value was compared to an average of the 22 subsequent values (moving average) in order to exclude outlier values. If the difference between the value and the averaged value was greater than 5%, then it was assumed that the value was implausible and therefore was excluded from analysis. Random checks of the excluded points were performed to confirm that all excluded values were artefacts. Outlier value exclusion was carried out to exclude movement artefacts, as Holter data measurements are performed continuously including periods of ambulation and study-related procedures. This may result in noise and incorrect measurement of ECG parameters. The 24-hour period was then divided into 144 intervals of 10 minutes. The values were averaged over the 10-minute periods.

For each subject, period and parameter, the mean across all 144 time points was calculated and subtracted from the data for each time point. The mean value across subjects and its two-sided 90% confidence interval (CI) were then calculated for each time point, parameter, and period.

Results

A total of 40 subjects were enrolled in the study. Thirty-eight of the 40 eligible subjects completed the study, 2 subjects being withdrawn. One subject received 5 and 40 mg amisulpride before withdrawal due to noncompliance with the protocol, and one received moxifloxacin before choosing to withdraw.

As reported in Täubel et al,[29] data from a bedside ECG showed that following administration of moxifloxacin, the ΔQTcF profile peaked at 6 hours, with a difference of 14.2 milliseconds from baseline (90%CI, 12.6, 15.8 milliseconds). The increase in ΔQTcF observed with moxifloxacin at 6 hours was still present at 24 hours (11.7; 90%CI, 9.9, 13.5 milliseconds) (Figure 1). The ΔΔQTcF profile demonstrated a QTcF peak at 4 hours above the threshold for regulatory concern (12.3 milliseconds; 90%CI, 10.1, 14.6 milliseconds). At 12 hours, the ΔΔQTcF value was 11.1 milliseconds (90%CI, 9.3, 13.0 milliseconds), which is only a few milliseconds less than the peak effect, and at 24 hours was still raised to 6.7 milliseconds (90%CI, 4.5, 9 milliseconds) (Figure 1 and Table 1).

As the distribution of the bedside ECG sampling time points may fail to reveal existing fluctuations of the QTc effect during the hours between ECGs record, continuous Holter measurements were retrospectively analyzed. Of the 10,145,242 values read, 1,205,803 (11.9%) outlier values were excluded from analysis.

Holter records were shown to be a valuable tool when reporting circadian patterns in cardiac repolarization.[30,31] In this study, the baseline Holter QTcF measurements reproduced the well-established diurnal profile. The QTc interval was longer during hours of sleep. A transient increase in the QTc interval is seen during the first hour after awakening and before the first meal of the day. During the day, significant decreases in QTcF were observed consistently after breakfast and lunch, with a less pronounced shortening after dinner, which is also shorter in duration. The maximum QTc effect occurred 3 to 4 hours after the start of each meal (Figure 2A). A steep increase in mean HR was observed following each meal (Figure 2B).

The Holter QTcF measurements after moxifloxacin administration followed the pattern observed in the conventional analysis of the bedside ECG-derived QTcF values. The results across all 4 periods were reproducible. The 24-hour profile of moxifloxacin displayed

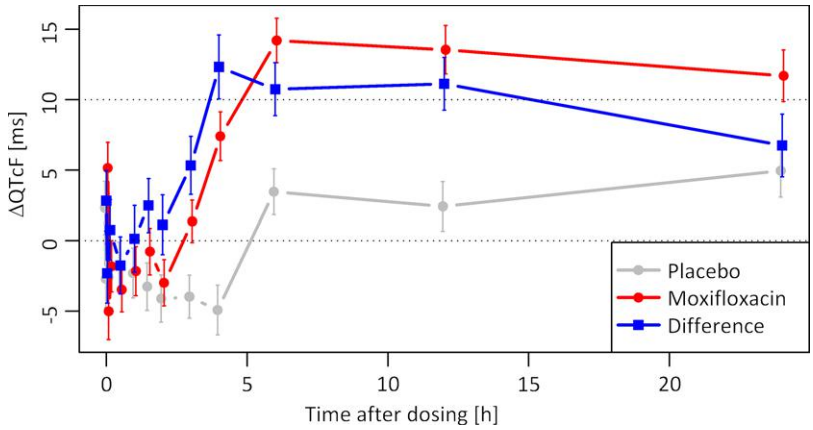

Figure 1. Placebo and moxifloxacin mean change from average baseline (ΔQTcF) and moxifloxacin time matched placebo corrected change from baseline (ΔΔQTcF). The 90%CIs are shown as vertical lines and the threshold of 10 milliseconds is shown as a horizontal dotted line.

Table 1. Difference to Time-Matched Placebo of Change in QTcF From Average Baseline (ΔΔQTc)

				90% Confidence Interval	
Treatment	Time	Mean	SE	Lower	Upper
Moxifloxacin 400 mg	00:00	2.8	1.29	0.7	5.0
	00:02	−2.3	1.27	−4.4	−0.2
	00:08	0.8	1.29	−1.4	2.9
	00:30	−1.8	1.22	−3.8	0.3
	01:00	0.1	1.42	−2.2	2.5
	01:30	2.5	1.15	0.6	4.4
	02:00	1.1	1.28	−1.0	3.2
	03:00	5.3	1.24	3.3	7.4
	04:00	12.3	1.37	10.1	14.6
	06:00	10.7	1.13	8.9	12.6
	12:00	11.1	1.13	9.3	13.0
	24:00	6.7	1.34	4.5	9.0

a persisting QTcF prolonging effect at the end of the Holter recordings, 24 hours after dose administration. This effect was well above the threshold of concern (Figure 3A). The plot of the time course of ΔQTcF (Figure 3A) adjusted for baseline (ΔQTcF) showed a rapid increase in QTc corresponding to expected peak moxifloxacin concentrations in the first 6 hours after the dose (9.7 milliseconds; 90%CI, 7.2, 12.2 milliseconds), followed by a second increase in QTc at around 12 hours (9.2 milliseconds; 90%CI, 7.4, 10.9 milliseconds) and a third increase from 18 to 24 hours (9.6-millisecond peak registered at 21.5 hours; 90%CI, 6.5, 12.7 milliseconds). The transient decrease in the change in QTc from baseline at 10 and 15 hours was similar in both moxifloxacin and placebo treatment groups and can be associated with the meal effects. When compared with the baseline day, similar day-night differences as well as after-meal patterns were observed on the treatment day (day 1) after administration of placebo (Figure 3). When adjusted for predose baseline, meals shortened ΔQTcF by 5 to 10 milliseconds, while sleep prolonged repolarization.

To clearly establish the effect attributable to moxifloxacin, the effects of meals and sleep on QTcF were removed by subtracting the baseline data and the placebo data to calculate the double difference (ΔΔQTcF) (Figure 4). By doing so, the ΔQTcF shortening effect after lunch previously seen in Figure 3A is attenuated. However, the effect seen starting at 10 hours after dose is still present and beyond what could be expected from the corresponding plasma concentration at this time of day.

The mean QTcF values derived at equivalent time points did not differ systematically from the bedside ECG results. The peak effect of moxifloxacin exposure is seen after a delay of 4 hours after dosing with a point estimate (90%CI) of 11.6 milliseconds (9.4, 13.8 milliseconds), which is due to the moxifloxacin dose being administered after breakfast, leading to a delay in absorption and additionally to a direct reduction of the moxifloxacin PK and QTc effect.[33]

Discussion

The QTc prolonging effects of the antibacterial fluoroquinolone moxifloxacin are well characterized through its use in many TQT studies to demonstrate the sensitivity of the assay.[16] The PK and QT response of moxifloxacin has been so well characterized that regulatory authorities expect the studies to demonstrate the characteristic extent of prolongation as well as the time course of the drug effect. In this TQT study, the effect of moxifloxacin on QTcF was used to confirm the sensitivity of the study to detect a relevant increase in QTcF, that is, the lower bound of the 90% CI of time-matched placebo-corrected increase in QTc interval from baseline (ΔΔQTc) was greater than

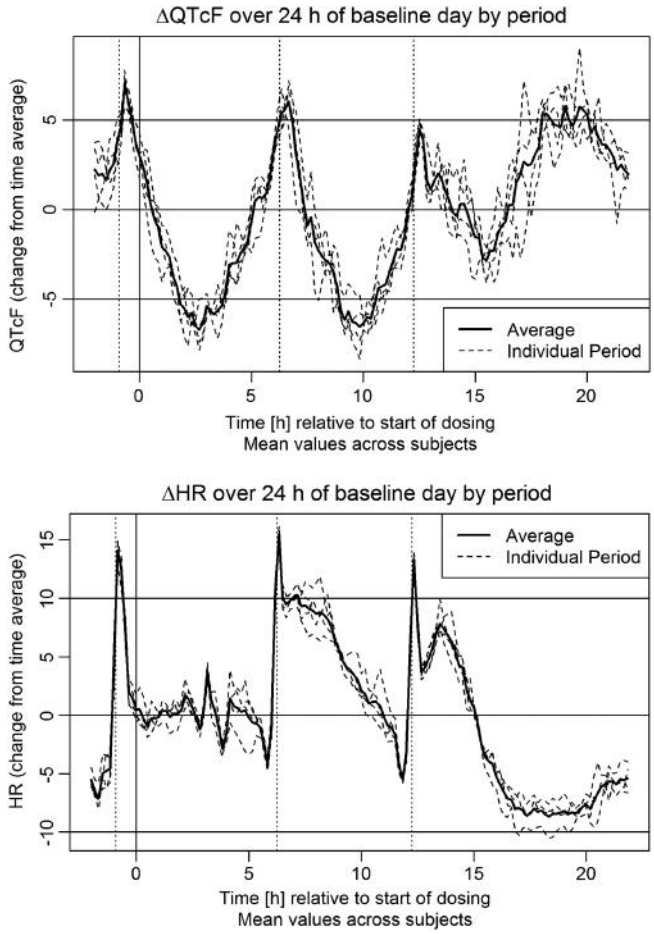

Figure 2. Time course (change from individual average baseline) of QTcF and HR on day −1 by period. For each subject the average over the 24-hour period was subtracted from the value of each time point. Mean values across all subjects are presented per period and averaged across all 4 periods. Times of moxifloxacin administration and meals are indicated by a black line and dotted lines, respectively.

5 milliseconds following a single 400-mg oral dose.[29] The moxifloxacin profile was consistent with previous studies in which moxifloxacin was administered in the fed state, whereby the peak of the QTc effect is delayed if oral moxifloxacin is administered after a meal.[32] Of note was the observed QTc prolongation at the 12- and 24-hour time points. Even though this is a common observation,[33,34] it is rarely discussed in the literature.

In the present report, we used continuous analysis based on Holter recordings to investigate a potential relationship between diurnal variations in QTc interval and the magnitude of the effect of moxifloxacin. The baseline data were also analyzed to give further insight into the validity of the data before the assessment of drug effects. We will refer to diurnal rhythms rather than circadian rhythms, as these must continue under constant conditions, while diurnal rhythms are synchronized with the day/night cycle and may be endogenously generated, or it may simply be a response to environmental factors.

Consideration of diurnal rhythm of the QT interval has been shown to be important when assessing the potential QT prolongation effect of a drug. The trends of the diurnal rhythmicity in the QT interval have been widely studied, and sex, HR, and autonomic influences have been identified as different modulators of variability.[31,35,36] The magnitude of the HR QTc 24-hour variations was shown to be inconsistent in previous studies,[31,36–38] as were the methodologies for data acquisition, ECG analysis, and HR correction used. Discrepancies have been shown to be dependent on the HR correction formula used, and QTc values obtained with the Bazett formula were shown not to produce a clear diurnal pattern.[39] Nevertheless, the QT interval was consistently shown to be longer during sleep than during the awake state[36,40] due to increased vagal tone and nocturnal decline in sympathetic nerve activity.[41,42]

Our baseline results showed a distinct diurnal rhythm, with a significant difference between day and night. This finding is consistent with that reported by Browne et al,[40] whereby prolongation of the QT interval is observed during sleep. The effect of meals on QTcF was seen in the time-course plots following

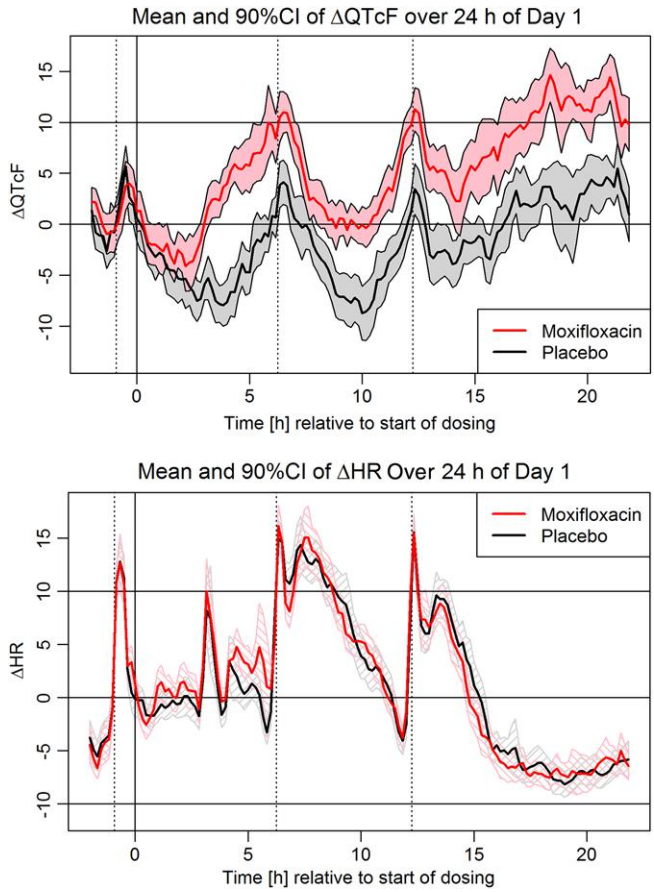

Figure 3. Time course of QTcF and HR on day 1. For each subject the average over the 24-hour period was subtracted from the value of each time point. Mean values across all 4 periods across all subjects are presented by treatment, and the 2-sided 90%CIs are represented by the shaded areas. Times of moxifloxacin, placebo administration, and meals are indicated by a black line and dotted lines, respectively.

placebo administration. QTcF profile was consistent within different meals, suggesting satisfactory heart rate correction using the Fridericia formula. A study by Smetana et al[39] also reported that QTc values obtained with the Fridericia formula showed a distinct diurnal rhythm.

The time effect attributed to food was reproducible over different study periods, with a marked effect after breakfast and lunch and less obvious effect after dinner probably due to the fact that after dinner, subjects retire to bed and may fall asleep. The QTc shortening after a meal was previously proposed to be a result of the net effect of the antagonistic effects exerted by C-peptide and glucose.[43] This and other published studies confirm that the QT interval shortening in response to standardized meals is reproducible and independent of time of day.[44] The marked effect on HR and the inverse relation between HR and QTcF has been previously reported after food intake.[13]

Of note was the short-lasting peak in HR values during the 3 to 4 hours after dose, which corresponds to the end of bed rest required for after-dose study assessments (Figure 3B) and most volunteers would have left their beds for physical mobilization. These Holter results emphasize the importance of activity and feeding effects in defining the diurnal variation in QT interval. They also highlight the importance of the understanding and control of environmental temporal fluctuations in QTc when conducting QT studies of cardiotoxicity.

Graphic displays of the QTc effect following moxifloxacin administration show the effects of meals and sleep on cardiac repolarization in the ΔQTcF plot (Figure 3), which are canceled out in the ΔΔQTcF plot (Figure 4). The change from average baseline and from placebo plot (double delta) thus represents the true action of moxifloxacin administration on QTcF. Notably, the QTcF prolongation above the threshold of concern, when adjusted for meal and sleep effects—by subtracting placebo and baseline effects—persists to the end of the monitoring period and probably beyond. This observation has been published by others, and the present study adds evidence that this is a drug effect and not a random effect due to other unknown factors.

The moxifloxacin effect on QT was previously satisfactorily described with a direct and proportional

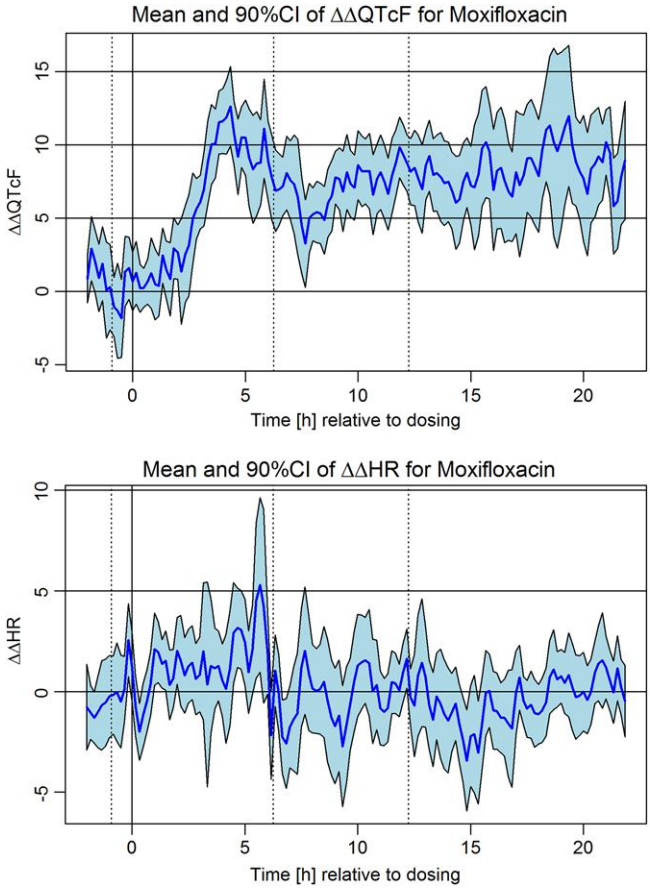

Figure 4. Time course of ΔΔQTcF (change from average baseline and from placebo) and HR on day 1. Mean values are presented, and the 90%CI is represented by the light blue shading. Times of moxifloxacin administration and meals are indicated by a black line and dotted lines, respectively.

concentration effect,[45] but the results presented suggest that the relationship between moxifloxacin concentration and the effect being measured is not a simple direct relationship. The PK of moxifloxacin following oral administration are well characterized,[46,47] and therefore samples for the measurement of moxifloxacin concentration were not collected in this study. However, studies conducted at the same site[48] have reproduced the well-established PK profile supporting the fact that the administered moxifloxacin will have largely disappeared from the systemic circulation at the end of ECG recording.

In a study by Bloomfield et al,[34] a momentary decrease in the change in QTc from baseline 5 and 6 hours after moxifloxacin and placebo administration was observed, suggesting that food may be responsible for attenuating the QTcF prolongation caused by moxifloxacin. Our most recent studies suggest that this may be the most plausible explanation (data not shown). In the same study by Bloomfield et al, the QTc interval remained elevated above the predose baseline value for up to 48 hours after the dose, with values above the 10-millisecond threshold at 18 hours. Holzgrefe et al[33] highlighted the need to further express the data as the time-matched change from placebo (double delta) to clearly ascertain the treatment effect and showed a similar raised QT interval up to 24 hours following treatment with moxifloxacin, despite the excellent correlation between the QTc double delta and moxifloxacin plasma levels ($r^2 = 0.83$).

A retrospective analysis of pooled data from 20 TQT studies with moxifloxacin given as a single 400-mg dose reported only a modest hysteresis between moxifloxacin plasma concentrations and QTc, and including hysteresis did not significantly alter the model slope.[16] However, mean plasma concentrations of moxifloxacin and mean ΔΔQTcF at each time point recorded in a study conducted by Kumagai et al[49] demonstrated a hysteresis effect. The authors attributed the hysteresis effects to environmental factors. Some potential causes for hysteresis include distribution delay between the plasma and effect site, response delay, sensitization of receptors or slow receptor kinetics, regulation of receptors after ongoing exposure, and the formation and subsequent accumulation of active metabolites through drug metabolism, as well as delayed or modified activity.[50] Patch-clamp studies have shown that the binding of moxifloxacin to hERG is reversible

and use dependent[19]; thus, the long-term effect on repolarization may be exerted through another long-term mechanism, such as the production of the pharmacologically active moxifloxacin glucuronide metabolite[51] or the inhibition of expression or trafficking of hERG to the cardiomyocyte plasma membrane.[22] Moxifloxacin is mainly metabolized to glucuronide and sulfate conjugates. Exposure to the metabolites was shown to be lower,[52] while apparent half-lives were similar to that of moxifloxacin,[53] which makes the metabolites an unlikely cause of such delay in returning to baseline.

As previous PK-QTc analyses point to a linear relationship between the plasma concentration of moxifloxacin and the increase in QTc interval, moxifloxacin has been used in concentration-effect modeling (CEM), wherein models assumed the absence of hysteresis.[18,54–57]

Concentration-effect modeling analyses for moxifloxacin have performed well in TQT studies to establish assay sensitivity and produced very consistent results in agreement with those of the time point analysis. Nevertheless, reliable methods to verify the absence or presence of hysteresis are essential to allow drawing valid conclusions from concentration-effect modeling to assess QT liability, yet little is known about QTc effects beyond the intensive PK and ECG sampling normally carried out up to 6 or 8 hours after dose, with very sparse sampling beyond typically 12 and 24 hours. The capture of the time course of the "recovery" of cardiac repolarization speed from moxifloxacin-induced prolongation reported here indicates the hysteresis effect results in a delayed return of the QTc levels to baseline rather than a delay in the in QTc increase following moxifloxacin administration. Therefore, assessing the extent of hysteresis in the relationship between exposure to moxifloxacin and changes in cardiac repolarization speed would require intensive assessment of the ECG, covering a wide enough time window that is best accomplished using continuous ambulatory monitoring. Although the analysis of continuous Holter ECG measurements is a less well-established technique,[58] it provided a continuous quasi beat-by-beat data acquisition for the present retrospective analysis that correlated well with the gold-standard bedside ECG data. Bedside and Holter estimates of QTcF and HR showed a reasonable correlation with the continuous ECG recording, providing a more accurate reflection of ECG changes over a time period. Confirmation of sparsely placed single time points against a continuous Holter can be useful quality measure. The reproducibility of the data across all 4 study periods supported the appropriateness of this Holter-based approach in the evaluation of drug effects, including the moxifloxacin time-course effect. This study also added to the understanding of diurnal variation in the ECG effects and how much bias can potentially be introduced by meals if not properly controlled.

Declaration of Conflicting Interests

Jörg Täubel and Sara Fernandes are employees of Richmond Pharmacology Ltd. Georg Ferber is an employee of Statistik Georg Ferber GmbH.

Data Sharing

Requests for access to data should be addressed to the corresponding author.

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